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Étude des effets de la kétamine dans un modèle de dépression résistante aux traitements : le rat Wistar-Kyoto soumis au stress léger chronique

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- Stay hard

Abréviations

Note au lecteur : afin d'éviter toute disparité entre les abréviations employées dans les chapitres du manuscrit rédigées en français et les articles de recherches, rédigés en anglais, toutes les abréviations utilisées au travers de ce manuscrit de thèse sont au format anglophone. Voici la liste des abréviations les plus employées.

5-HT : Sérotonine (Serotonin / 5-HydroxyTryptamine)

ACC : Cortex cingulaire antérieur (Anterior Cingulate Cortex)

aCSF : Liquide céphalo-rachidien artificiel (Artificial Cerebro-Spinal Fluid)

ADNc : ADN complémentaire (Complementary DNA / cDNA)

AMPA-R : Récepteurs α-amino-3-hydroxy-5-méthylisoxazol-4-propionate (α-Amino-3-hydroxy-5-Methyl-4-isoxazolePropionic Acid Receptors)

AMY : Amygdale (Amygdala)

ARNm : ARN messager (Messenger RNA / mRNA)

BDNF : Facteur neurotrophique dérivé du cerveau (Brain-Derived Neurotrophic Factor)

BLA : Amygdale basolatérale (Basolateral Amygdala)

CMS : Stress léger chronique (Chronic Mild Stress)

DA : Dopamine (Dopamine)

dHPC : Hippocampe dorsal (Dorsal Hippocampus)

dPFC : Cortex préfrontal dorsal (Dorsal Prefrontal Cortex)

DSM : Manuel diagnostique et statistique des troubles mentaux (Diagnostic and Statistical Manual of Mental Disorders)

ECoG : Électrocorticogramme (Electrocorticogram)

EPM : Test du labyrinthe en croix surélevé (Elevated Plus Maze)

fEPSP : Potentiel de champ post-synaptique excitateur (Field Excitatory PostSynaptic Potential)

FST : Test de la nage forcée (Forced Swim Test)

fMRI : Imagerie par résonance magnétique fonctionnelle (Functional Magnetic Resonance Imaging)

GABA : Acide γ-aminobutyrique (γ-AminoButyric Acid)

Glu : Glutamate (Glutamate)

GR : Récepteurs aux glucocorticoïdes (Glucocorticoid Receptors)

HPA : Axe hypothalamo-hypophyso-surrénalien (Hypothalamic-Pituitary-Adrenal Axis)

HPC : Hippocampe (Hippocampus)

ip : Intrapéritonéal (Intraperitoneal)

Ket : Kétamine (Ketamine)

LFP : Potentiel de champ local (Local Field Potential)

LHb : Habénula latérale (Lateral Habenula)

LTD : Dépression à long terme (Long Term Depression)

LTP : Potentialisation à long terme (Long Term Potentiation)

mPFC : Cortex préfrontal médian (Medial Prefrontal Cortex)

MRI : Imagerie par résonance magnétique (Magnetic Resonance Imaging)

NA : Noradrénaline (Noradrenaline)

NaCl : Chlorure de sodium (Sodium Chloride)

NMDA-R : Récepteurs N-méthyl-D-aspartate (N-Methyl-D-Aspartate Receptors)

NREM : Sommeil lent (Non-Rapid Eye Movement Sleep)

NS : Non stressé (Non-Stressed)

OFT : Test du champ ouvert (Open Field Test)

PET-scan : Tomographie par émission de positons (Positron Emission Tomography Scan)

PFC : Cortex préfrontal (Prefrontal Cortex)

PRL : Partie prélimbique du cortex préfrontal médian (Prelimbic Cortex)

REM : Sommeil paradoxal (Rapid Eye Movement Sleep)

RT-qPCR : Réaction en chaîne par polymérase quantitative après transcription inverse (Reverse Transcription Quantitative Polymerase Chain Reaction)

sc : Sous-cutané (Subcutaneous)

SPT : Test de préférence au saccharose (Sucrose Preference Test)

SSRI : Inhibiteurs sélectifs de la recapture de la sérotonine (Selective Serotonin Reuptake Inhibitors)

TRD : Dépression résistante aux traitements (Treatment-Resistant Depression)

WKY : Wistar-Kyoto (rat) (Wistar-Kyoto Rat)

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Avant-Propos

Si cette thèse concerne exclusivement des travaux réalisés sur un modèle animal, il semble opportun d'en évoquer le contexte initial. Les présents travaux ont débuté dans le cadre d'un projet de recherche translationnel, le consortium européen ERA-NET NEURON Neuromarket (Neuroimaging and Blood Markers as Indicators of Ketamine Efficacy in Treatment Resistant Depression). Ce projet visait à optimiser la prise en charge thérapeutique de la dépression résistante aux traitements par la kétamine, *via* l'identification de biomarqueurs périphériques et centraux. Il comprenait à la fois des études cliniques et des études mécanistiques menées en parallèle chez le rat Wistar-Kyoto, ces dernières constituant le point de départ de la présente thèse. Bien que celle-ci ait ensuite évolué de manière relativement indépendante, ce cadre a orienté la définition globale des travaux. Des analyses complémentaires étaient prévues dans le cadre d'une éventuelle prolongation de la thèse. Néanmoins, malgré l'absence de celle-ci, cinq différentes études ont pu être réalisées.

Afin de faciliter la lecture de cette thèse, un court préambule figure avant chaque partie, en résumant le contenu et le contextualisant.

INTRODUCTION GÉNÉRALE

Étant donné le contexte dans lequel ont débuté les travaux de cette thèse, mon introduction générale est structurée de manière à proposer une progression cohérente allant de la compréhension de la pathologie dépressive chez l'humain à la mise en œuvre du modèle animal utilisé pour les approches expérimentales employées. Dans une première partie, je présente donc la dépression chez l'humain, en abordant les principaux mécanismes physiopathologiques, ainsi que les approches thérapeutiques actuelles, dont la kétamine, en soulignant leurs limites respectives. La deuxième partie est consacrée à la modélisation animale de la dépression par le paradigme du stress léger chronique (chronic mild stress, CMS). J'y décris ses principales caractéristiques, puis je développe plus spécifiquement les paramètres comportementaux, physiologiques et neurobiologiques qui ont été évalués dans le cadre de cette thèse. Cette partie se conclut par une présentation du modèle constitué par le rat Wistar-Kyoto exposé au CMS, qui constitue le cœur expérimental des présents travaux. Enfin, la troisième partie expose les objectifs principaux de cette thèse et la démarche scientifique adoptée.

Par souci de concision, j'ai fait le choix de ne pas développer les autres modèles animaux de dépression, bien qu'ils soient nombreux et présentent divers intérêts spécifiques. Le lecteur désireux d'approfondir cette question pourra notamment consulter les revues « The future of rodent models in depression research » (Gururajan et al., 2019) et « Animal Models of Depression: What Can They Teach Us about the Human Disease? » (Becker et al., 2021).

I) La dépression

A) Description et caractéristiques générales

La dépression est une pathologie psychiatrique avec une prévalence en constante augmentation. Les troubles dépressifs affectent environ 322 millions de personnes (Rong et al., 2025) et sont depuis plusieurs années considérés par l'Organisation mondiale de la Santé comme la principale cause d'invalidité dans le monde (Friedrich, 2017). Une méta-analyse épidémiologique globale a estimé, parmi la population générale, une prévalence ponctuelle de la dépression d'environ 12,9 %, de 7,2% au cours d'une année, et de 10.8% sur la vie entière (Lim et al., 2018). Cette prévalence a connu une croissance marquée et soutenue dans le monde entre 1990 et 2021, avec une augmentation estimée à 88,5 % (Chen et al., 2025). Il est également important de noter une disparité selon le sexe : plus de femmes sont diagnostiquées, avec un ratio femme:homme entre 1,25:1 et 1,95:1 (Lim et al., 2018; Salk et al., 2017), tandis que le suicide est 1,5 à 3 fois plus important chez les hommes (Ilic & Ilic, 2022; World Health Organization, 2014).

Si le terme de « dépression » dans le contexte psychiatrique est apparu avec le développement de la psychologie au XIXème siècle, cette pathologie est décrite depuis plus de deux millénaires dans des ouvrages médicaux sous le nom de « mélancolie », remontant jusqu'au VIème siècle av. EC. dans les textes du médecin et philosophe grec Hippocrate (Radden, 2003), soulignant son impact notable sur la société à travers les âges. Aujourd'hui, elle est typiquement diagnostiquée selon les critères du manuel diagnostique et statistique des troubles mentaux (DSM), dans laquelle elle figure depuis sa première édition en 1952 (Fischer, 2012). Dans sa version la plus récente, le DSM-5-TR paru en 2022, le diagnostic du trouble dépressif caractérisé repose sur neuf symptômes (**Tableau 1**). Il est cependant important de noter qu'il n'existe pas de dépression « type ». La dépression est une pathologie particulièrement hétérogène et il existe 256 combinaisons différentes de symptômes permettant d'établir un diagnostic de trouble dépressif caractérisé (Buch & Liston, 2021).

Le DSM-5-TR précise également neuf spécifications du trouble : avec détresse anxieuse ; avec caractéristiques mixtes ou mélancoliques ou atypiques ou psychotiques congruentes à l'humeur, ou psychotiques non congruentes à l'humeur; avec catatonie ; avec début lors du péripartum ; avec caractère saisonnier.

Humeur dépressive présente pratiquement toute la journée, **presque tous les jours, signalée par le sujet ou observée par les autres**
Diminution marquée du plaisir pour toutes ou presque toutes les activités pratiquement toute la journée, presque tous les jours
Perte ou gain de poids significatif en absence de régime, ou diminution ou augmentation de l'appétit presque tous les jours
Insomnie ou hypersomnie presque tous les jours.
Ralentissement ou agitation psychomotrice presque tous les jours
Fatigue ou perte d'énergie presque tous les jours
Sentiment de dévalorisation ou de culpabilité excessive ou inappropriée
Diminution de l'aptitude à penser ou à se concentrer ou indécision presque tous les jours
Pensées de mort récurrentes, idées suicidaires récurrentes sans plan précis, tentative de suicide ou plan précis pour se suicider

Tableau 1 : Symptômes du trouble dépressif caractérisé tels que décrits dans le DSM-5-TR. Au moins 5 des symptômes listés doivent être présents pendant une même période d'une durée de 2 semaines, dont au moins un des deux symptômes en gras dans le tableau.

En plus de la présence d'un nombre plus ou moins grand des différents symptômes, la sévérité de la pathologie est reflétée par l'intensité de ceux-ci, qui peut être mesurée dans un cadre clinique grâce à l'échelle de Hamilton (HAM-D), un outil diagnostique mis au point en 1960 par le psychiatre Max Hamilton (Hamilton, 1960). Cet outil permet notamment de mesurer l'évolution de la pathologie, par exemple pour évaluer l'efficacité d'un traitement. C'est un outil largement utilisé avec une bonne fiabilité inter-évaluateurs et une bonne fiabilité test-retest (Trajković et al., 2011).

Comme c'est le cas pour de nombreuses maladies psychiatriques, la dépression présente d'importantes comorbidités, notamment les troubles anxieux, diagnostiqués chez environ 60 % des patients dépressifs, ou encore les troubles liés à l'usage de substances, présents dans 24 % des cas (Kessler et al., 2003).

En raison du caractère invalidant de ses symptômes (**Tableau 1**), et de sa prévalence grandissante, cette pathologie entraîne une charge socio-économique particulièrement importante et croissante. En Europe, son coût était estimé à 1 % du PIB en 2006 (Sobocki et al., 2006). Aux États-Unis, ce coût est passé d'environ 66 milliards à plus de 80 milliards de dollars entre 2005 et 2010 (Greenberg et al., 2015).

B) Perturbations émotionnelles, cognitives et végétatives

Les perturbations caractéristiques de la dépression peuvent être catégorisées en perturbations affectives, cognitivo-comportementales et neurovégétatives (Maj et al., 2020).

1) Perturbations affectives

Les perturbations affectives, incluant l'humeur déprimée et la diminution du plaisir, ou anhédonie, sont au cœur de la dépression, représentant des symptômes dont la présence est nécessaire pour un diagnostic selon le DSM-5 (**cf. Tableau 1**). L'humeur déprimée est caractérisée par la prédominance d'un affect fortement négatif incluant une détresse émotionnelle telle que la tristesse, la colère, ou la peur (Vittengl et al., 2022). L'anhédonie représente une réduction ou une perte de capacité à ressentir du plaisir de stimuli physiques ou sociaux, en particulier vis-à-vis des activités normalement plaisantes (Ho & Sommers, 2013). Elle est associée à un profil de dépression plus sévère et récurrent, à une réponse plus faible ou nulle au traitement et à un risque accru de suicide (Luca et al., 2024). L'expérience du plaisir peut être décomposée en trois phases : anticipation (appétitif), ressenti (consommatoire), souvenir (Strauss & Gold, 2012). L'anhédonie peut se manifester par une perte de plaisir appétitif, consommatoire ou les deux (Ho & Sommers, 2013). En plus de l'expérience du plaisir en tant que tel, ce symptôme implique des déficits motivationnels : les patients sont effectivement moins enclins, dans des tâches de motivation, à faire des efforts pour obtenir des récompenses et présentent une moindre capacité à utiliser des informations pour orienter leurs choix dans le but d'obtenir des récompenses (Treadway et al., 2012) ; ces déficits sont corrélés avec la sévérité de la pathologie (Yang et al., 2014).

2) Perturbations cognitivo-comportementales

Parmi les perturbations cognitivo-comportementales se trouvent les symptômes de la dévalorisation et/ou culpabilité, la difficulté à se concentrer ou prendre des décisions, et l'idéation suicidaire. Le premier est le plus représenté, décrit chez plus de 90 % des patients, et lié à des biais cognitifs négatifs dirigés vers soi ; selon les patients, ce symptôme génère davantage de détresse que les perturbations affectives (Zahn et al., 2015). Le symptôme de difficulté à se concentrer ou prendre des décisions provient de déficits des fonctions exécutives, affectant la vitesse de traitement de l'information et l'apprentissage (Kriesche et al., 2023), et de façon moins marquée, de déficits mnésiques ; l'intensité de ces déficits est corrélée avec la sévérité et la récurrence des épisodes dépressifs (Kriesche et al., 2023). Ces déficits peuvent parfois persister après la rémission, et sont considérés par certains auteurs comme des mécanismes à cibler à part entière dans les traitements

antidépresseurs (Rock et al., 2014). Le symptôme de pensées de mort, idéation suicidaire, plans ou tentatives de suicide, est observé chez environ 38 % des patients (Cai et al., 2021). Différents facteurs, comme une sévérité accrue de la dépression, une résistance aux traitements antidépresseurs, et des antécédents de comportements suicidaires prédisent la présence de ce symptôme (Kern et al., 2023; Riera-Serra et al., 2024). Environ 1 % des patients dépressifs se suicident chaque année, un chiffre atteignant 2,5 % chez ceux ayant des antécédents de comportements suicidaires (Lundberg et al., 2023).

Une perturbation cognitivo-comportementale d'intérêt dans la dépression, bien que peu souvent discutée, est l'inadaptation ou l'emploi de mauvaises stratégies de gestion face à des situations stressantes ou dans la résolution de problèmes (Almeida et al., 2021; Thompson et al., 2010). Les patients présentent effectivement des stratégies d'évitement, de passivité ou de désengagement comportemental qui sont associées à une sévérité accrue de la pathologie (Bucatoş et al., 2025; Ho et al., 2022) et une moins bonne issue thérapeutique (Bucatoş et al., 2025). Cette perturbation est considérée comme état-dépendante plutôt que trait-dépendante, reflétant davantage un état pathologique plutôt qu'un type de personnalité (Bucatoş et al., 2025).

3) Perturbations neuro-végétatives

Les troubles du poids dans la dépression sont définis par une variation, sur une durée d'un mois, du poids corporel de base excédant 5 %, en l'absence d'un régime particulier (DSM-5). Les données cliniques suggèrent une courbe en U dans la relation entre poids et dépression, la prévalence de cette dernière étant particulièrement accrue chez des patients en sous ou surpoids (Carey et al., 2014). Les gains de poids sont associés à une dépression caractérisée d'« atypique ». Au-delà de la modification soudaine du poids, des anomalies dans la masse corporelle sont souvent associées à la dépression, et davantage de patients sont en surpoids qu'en sous poids (Jieqiong et al., 2022). De plus, l'obésité est présente 1,5 fois plus chez des patients comparés à des sujets sains (Treviño-Alvarez et al., 2023) et elle affecte davantage les femmes que les hommes (Bannert et al., 2024; Li et al., 2017).

Un autre symptôme clé de la dépression concerne les troubles liés au sommeil, qui peuvent être catégorisés en insomnie ou hypersomnie (Murphy & Peterson, 2015). Les perturbations de l'architecture du sommeil représentent une des caractéristiques les plus rapportées dans la dépression (Geoffroy et al., 2018). Chez l'humain, le sommeil inclut deux états de vigilance principaux : le sommeil lent (non-rapid eye movement sleep, NREM sleep), et le sommeil paradoxal (rapid eye movement sleep, REM sleep) (Collop et al., 2008). Le sommeil humain est monophasique, avec une

architecture incluant environ cinq cycles sommeil NREM-sommeil REM d'une durée d'environ 70 à 90 minutes (Lam, 2006). En l'absence de traitement, qui peut moduler les perturbations de l'architecture du sommeil dans la pathologie (Lam, 2006), les patients présentent un endormissement qui tend à être plus tardif, une moindre durée de sommeil NREM, et un sommeil plus fragmenté en général avec des réveils plus fréquents et durables au cours de la nuit, comparativement à des sujets sains (Bovy et al., 2022; Lam, 2006). De plus, le sommeil REM survient plus tôt, les épisodes sont plus longs et les saccades oculaires plus fréquentes (Arıkan et al., 2024; Palagini et al., 2013). Les antidépresseurs dits « classiques » (**cf. p.18**18) réduisent la latence d'entrée en sommeil REM et la durée de ses épisodes (Bovy et al., 2022; Lam, 2006).

Les perturbations psychomotrices, qui constituent le dernier symptôme végétatif de la dépression, incluent le ralentissement et l'agitation. Le ralentissement psychomoteur est caractérisé par un ralentissement de la pensée et de la parole et par des mouvements plus lents, ainsi qu'une activité amoindrie. L'agitation est caractérisée par des mouvements accrus et plus erratiques (Bennabi et al., 2013; Wüthrich et al., 2024). Ces deux perturbations ne sont pas mutuellement exclusives, pouvant survenir chez un même patient de façon concomitante.

B) Neurobiologie de la dépression

La pathologie est caractérisée par un ensemble de perturbations du système nerveux central, à une échelle inter-structurelle, structurelle, cellulaire et moléculaire. Néanmoins, comme peuvent l'être les symptômes d'un patient à un autre, ces dysfonctionnements semblent particulièrement hétérogènes, et le lien entre cause et conséquence de la maladie demeure globalement peu clair (Buch & Liston, 2021). L'état de la recherche portant sur la physiopathologie est résumé ainsi par Grogans et collaborateurs : « Les travaux menés au cours de la dernière décennie ont permis de solides avancées dans notre compréhension de la dépression. Pourtant, les mécanismes neurobiologiques sous-jacents restent difficiles à cerner, les biomarqueurs exploitables demeurent hors de portée, les traitements existants sont loin d'être curatifs, et les rechutes et récidives sont fréquentes. » (Grogans et al., 2022). Afin de décrire au mieux la physiopathologie de la dépression, les données ci-après sont essentiellement issues de méta-analyses concernant des patients sans traitement médicamenteux.

C) Structures cérébrales : morphologie et fonctionnement

La morphologie de structures cérébrales peut être évaluée par des analyses tissulaires *post mortem*, ou *in vivo* par des techniques d'imagerie comme l'imagerie par résonance magnétique (magnetic resonance imaging, MRI). Des techniques comme l'imagerie par résonance magnétique fonctionnelle (functional MRI, fMRI) permettent d'étudier l'activité de structures cérébrales.

L'amygdale (AMY), au rôle central d'interface intégrative du traitement émotionnel, notamment par l'attribution d'une valence à des stimuli perçus (Gothard, 2020; Šimić et al., 2021), est une région clé dans la pathologie. S'il y a été décrit des pertes volumiques potentiellement causées par des pertes cellulaires (Hamilton et al., 2008; Nolan et al., 2020), les résultats concernant ces anomalies morphologiques ne sont pas consensuels et elles pourraient ne concerner que les patients les plus jeunes (18-39 ans) d'une cohorte adulte (Daftary et al., 2019). Sur le plan fonctionnel, l'amygdale des patients dépressifs présente une hyperréactivité aux stimuli de nature négative (Drevets, 2003; Hamilton et al., 2012), mais pas aux stimuli de nature positive (Hossein et al., 2023).

Au sein du circuit de la récompense, le striatum ventral et l'aire tegmentale ventrale, impliqués dans le traitement de la récompense, la motivation et la prise de décision (Floresco, 2015; Hou et al., 2024; Tremblay et al., 2009), présentent tous deux une hypoactivité, notamment lors de tâches d'anticipation de récompense (Levy et al., 2018).

L'habénula latérale (lateral habenula, LHb), parfois décrite comme un centre « anti-récompense », est mise en cause dans la physiopathologie de la dépression (Ables et al., 2023). Néanmoins, son étude chez l'humain présente des obstacles importants en raison de sa petite taille et de la difficulté à distinguer ses sous-parties, médiane et latérale (Cameron et al., 2024). Des pertes volumiques y sont constatées dans la dépression (Cameron et al., 2024), et ces perturbations morphologiques pourraient être sexe-dépendantes, certaines observations suggérant notamment une corrélation négative entre volume de l'Hb et sévérité de la pathologie chez les femmes uniquement (Kyuragi et al., 2024). En plus de son volume, l'activité de cette structure semble également perturbée dans la pathologie. Si dans les modèles animaux, une hyperactivité est typiquement décrite dans cette structure (Cameron et al., 2024; Gold & Kadriu, 2019), les observations cliniques concernant son activité au repos sont profondément contradictoires (Fortin et al., 2025). Face à une issue négative dans une tâche de récompense, représentée par une perte d'argent, les patients dépressifs présentent même une hypoactivation ou absence de réponse de l'Hb, comparativement à des sujets sains (Cameron et al., 2024).

L'hippocampe (HPC) est une structure particulièrement perturbée dans la dépression (Gray et al., 2020). Il est impliqué, *via* sa partie antérieure, dans la régulation émotionnelle, et, *via* sa partie postérieure, dans des processus mnésiques et la navigation spatiale (Nichols et al., 2023). Les perturbations hippocampiques sont caractérisées par des pertes volumiques (Santos et al., 2018) liées à des pertes de substance grise (Nolan et al., 2020) et à un amoindrissement de la densité synaptique (Holmes et al., 2019). L'étendue de ces pertes est associée à la durée et à la sévérité de la dépression (Tartt et al., 2022).

Les régions corticales présentent des altérations importantes chez les patients dépressifs. Le lobe frontal présente une réduction de son volume (Pandya et al., 2012; Pizzagalli & Roberts, 2022) associée à une réduction du nombre de cellules gliales (Trifu et al., 2020) et à un amoindrissement de la densité synaptique (Holmes et al., 2019). Le cortex préfrontal dorsal (dorsal prefrontal cortex, dPFC), impliqué dans certaines fonctions exécutives (Barbey et al., 2013), présente un métabolisme et une perfusion sanguine amoindris (Pandya et al., 2012) ainsi qu'une hyporéactivité aux stimuli de valence négative (Hamilton et al., 2012). D'autres régions corticales également perturbées dans la pathologie incluent le cortex préfrontal médian (medial prefrontal cortex, mPFC) et le cortex cingulaire antérieur (anterior cingulate cortex, ACC) ; leurs régions dorsales contribuant à l'expression et l'évaluation des émotions, tandis que leurs régions ventrales sont engagées dans leur régulation (Etkin et al., 2011). Le mPFC est atrophié (Belleau et al., 2019; Zheng et al., 2021) tout comme l'ACC, qui a également un métabolisme dans l'ensemble plus faible (Pandya et al., 2012), bien que sa partie subgenouillée (sgACC) ait au contraire un métabolisme accru (Belujon & Grace, 2017) et une hyperréactivité aux stimuli négatifs (Hamilton et al., 2012).

Le cortex insulaire, impliqué, entre autres, dans la régulation émotionnelle et la perception de la douleur physique et émotionnelle (Nieuwenhuys, 2012), a une activité accrue au repos, une hypoactivité lors de tâches cognitives, et une hyperactivité dans des paradigmes mettant en jeu les émotions (Sliz & Hayley, 2012) en particulier élicitées par des stimuli de valence négative (Hamilton et al., 2012).

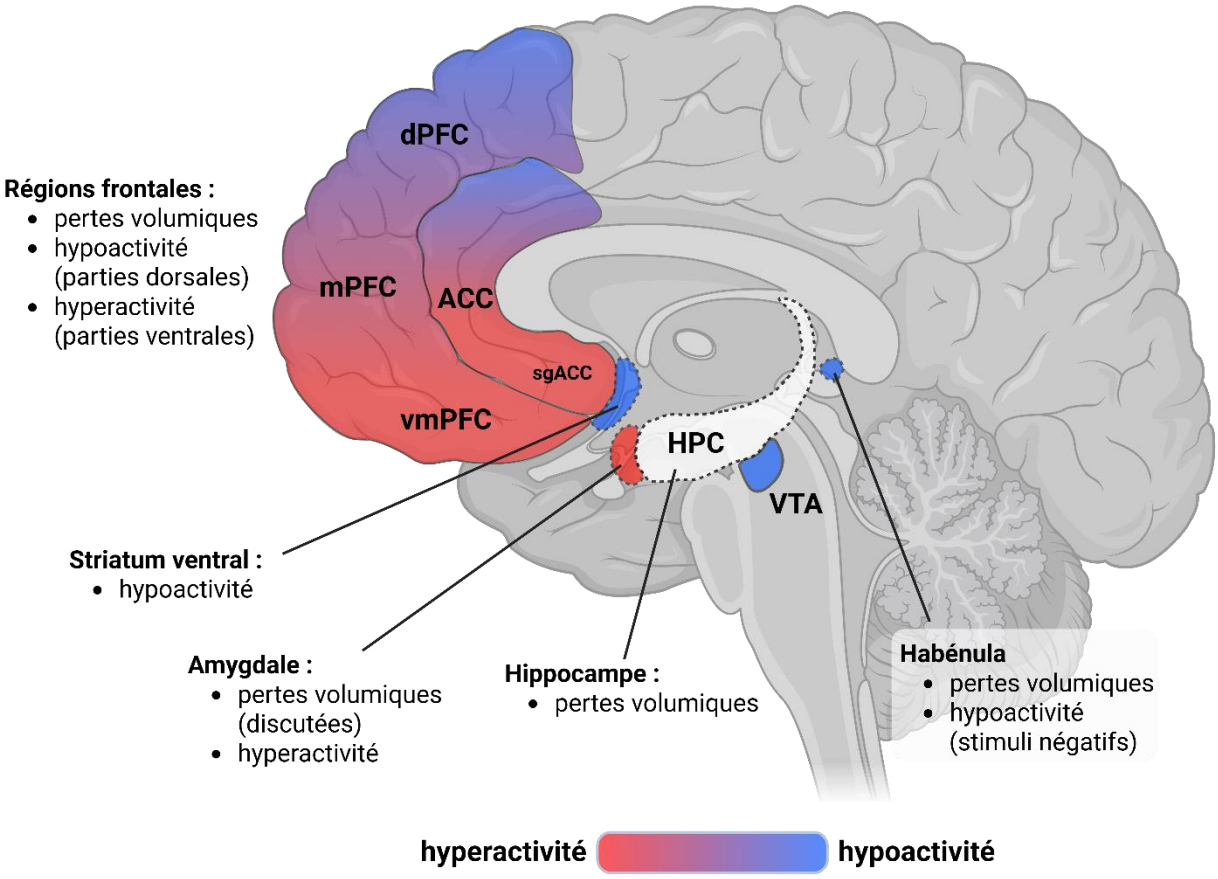

Figure 1 : Perturbations morpho-fonctionnelles dans la dépression (réalisé sur biorender). ACC : cortex cingulaire antérieur ; dPFC : cortex préfrontal dorsal ; HPC : hippocampe ; mPFC : cortex préfrontal médian ; vmPFC : cortex préfrontal ventromédian ; VTA : aire tegmentale ventrale.

Globalement, les patients dépressifs présentent donc une atrophie et une hypoactivité des régions impliquées dans la régulation émotionnelle, la motivation et le traitement de la récompense, tandis que les régions associées à la détection et au traitement des stimuli négatifs présentent au contraire une hyperactivité.

L'analyse de l'activité cérébrale de différentes structures permet également de refléter leur connectivité fonctionnelle (Gavrilescu et al., 2007). Cette dernière est définie comme la « corrélation temporelle de mesures électro/neurophysiologiques de différentes parties du cerveau » (Friston et al., 1993), par exemple l'activation synchrone de structures observée en fMRI (Nazari et al., 2019). L'analyse de données concernant l'activité cérébrale de patients peut ainsi mettre en évidence une hyperconnectivité, reflétée par une synchronisation accrue de l'activité de plusieurs régions, ou hypoconnectivité, reflétée par une moindre synchronisation, comparativement à des sujets sains. D'après des études en fMRI au repos, les patients présentent globalement une hyperconnectivité entre les régions du réseau par défaut, sous-tendant les pensées internes et dirigées vers soi (Kaiser et al.,

2015). En revanche, une hypoconnectivité est observée entre les structures du réseau frontopariétal, impliqué dans le contrôle cognitif et la régulation émotionnelle, et celles du réseau d'attention dorsale, dirigé vers l'environnement externe (Kaiser et al., 2015). Parmi les régions évoquées ci-dessus, une hypoconnectivité a été décrite entre le mPFC et l'AMY (Kaiser et al., 2015), entre le dPFC et l'AMY, plus précisément sa partie basolatérale (basolateral amygdala, BLA) (Hossein et al., 2023; Tahmasian et al., 2013), entre l'HPC et le dPFC (Tahmasian et al., 2013), et entre l'AMY et l'insula (Tang et al., 2018). À l'inverse, une hyperconnectivité a été décrite entre l'HPC et l'AMY (Tang et al., 2018). L'Hb présente également de nombreuses perturbations ambivalentes dans sa connectivité avec les autres structures cérébrales, incluant hypo et hyperconnectivité, qui sont corrélées avec la sévérité de la pathologie (Cameron et al., 2024).

Globalement, la dépression est caractérisée par des pertes volumiques, des anomalies dans l'activité et des dysfonctions dans la communication de structures cérébrales clés, en particulier concernant le traitement émotionnel (**Figure 1**).

1) Neurotransmission

En plus des altérations morphologiques et fonctionnelles de diverses régions cérébrales, des dysfonctions au sein de plusieurs systèmes de neurotransmissions sont également constatées dans la pathologie. Si un lien a longtemps été établi entre dépression et anomalies des systèmes monoaminergiques, incluant les systèmes sérotoninergique, dopaminergique et noradrénergique, les données empiriques les concernant s'avèrent peu concluantes et contradictoires (Moncrieff et al., 2023) (**cf. p. 14**). Néanmoins, plusieurs types de perturbations monoaminergiques ont été rapportés. Au sein du système sérotoninergique, une moindre fixation de ligands des récepteurs 5-HT_{1A} et 5-HT_{1B}, a été observée en tomographie par émission de positons (positron emission tomography scan, PET-scan) (Drevets et al., 2007; Nautiyal & Hen, 2017). Des dysfonctions dans la transmission dopaminergique, associées aux symptômes d'anhédonie, semblent également présentes dans la pathologie. En PET-scan, une moindre fixation de ligands des transporteurs de la dopamine (DA) (Meyer et al., 2001; Mizuno et al., 2023; Sarchiapone et al., 2006), des récepteurs excitateurs D1, ainsi qu'une fixation accrue de ceux des récepteurs inhibiteurs D2 (D'haenen & Bossuyt, 1994) ont été observées dans le striatum. Ces observations demeurent cependant largement discutées (Mizuno et al., 2023), les dysfonctions dopaminergiques apparaissant hautement hétérogènes et les études les concernant étant contradictoires (Belujon & Grace, 2017). Concernant le système noradrénergique, une moindre densité des récepteurs adrénergiques α2 dans le cortex frontal et l'hypothalamus (Klimek

et al., 1999) et une moindre fixation de ligands des transporteurs noradrénergiques dans le locus coeruleus (Klimek et al., 1997; Moret & Briley, 2011) ont été observées chez des patients. Néanmoins, le rôle de la noradrénaline (NA) dans la dépression semble, relativement à d'autres mécanismes biologiques, peu étudié et Chandley & Ordway notaient déjà en 2012 que « l'intérêt du rôle de la NA dans le suicide et la dépression a considérablement diminué au cours des dix dernières années » (Chandley & Ordway, 2012). Il est également notable que la monoamine oxydase A, enzyme de dégradation de la sérotonine (5-HT) et de la NA, présente une densité anormalement élevée dans le cerveau de patients dépressifs (Meyer et al., 2006) et corrélée à la sévérité des symptômes (Chiuccariello et al., 2014).

En dehors des systèmes monoaminergiques, la neurotransmission impliquant le glutamate (Glu), neurotransmetteur excitateur, semble globalement dysfonctionnelle dans la dépression. Des niveaux amoindris de Glu ou de son métabolite, la glutamine, ou de récepteurs glutamatergiques, sont constatés chez les patients dans le mPFC, l'ACC et l'HPC (Moriguchi et al., 2019; Sarawagi et al., 2021). Enfin, la neurotransmission impliquant l'acide γ-aminobutyrique (γ-aminobutyric acid, GABA), neurotransmetteur inhibiteur, est également anormale. Les niveaux périphériques et centraux de ce neurotransmetteur sont amoindris (Hasler, 2010; Luscher et al., 2011; Romeo et al., 2018; Sarawagi et al., 2021), en particulier dans les cas de dépression décrite comme « mélancolique », où l'humeur déprimée et l'anhédonie sont particulièrement marquées (Lorenzo-Luaces et al., 2021).

2) Perturbations de la neuroplasticité

La neuroplasticité est un phénomène physiologique permettant l'apprentissage et l'adaptation à son environnement (Albadawi, 2025). Celle-ci est notamment caractérisée par la capacité des neurones à créer, éliminer, renforcer ou affaiblir les connexions synaptiques qui les relient, incluant les mécanismes de potentialisation et dépression à long terme (long term potentiation/depression, LTP/LTD) (Bin Ibrahim et al., 2022). L'étude de la plasticité synaptique chez l'humain demeure très indirecte en raison de limites méthodologiques (Levy et al., 2018). Des indices obtenus par potentiels évoqués *via* des stimuli visuels suggèrent néanmoins une LTP affaiblie au niveau du cortex occipital chez les patients dépressifs (Normann et al., 2007). Comme évoqué précédemment, une densité synaptique amoindrie, corrélée avec la sévérité de la pathologie, a été constatée en PET-scan dans le dPFC, l'HPC et l'ACC (Holmes et al., 2019). De plus, un nombre amoindri de synapses a été observé *post mortem* en microscopie électronique dans le dPFC de patients dépressifs (Hj et al., 2012).

Au-delà des mécanismes synaptiques et des synapses elles-mêmes, les facteurs neurotrophiques régulant la plasticité présentent également des anomalies dans la pathologie. Celles-ci impliquent principalement le facteur neurotrophique dérivé du cerveau (brain-derived neurotrophic factor, BDNF) (Yang et al., 2020) au rôle central dans la formation, le maintien et le renforcement des synapses (Park & Poo, 2013). Les patients dépressifs ont typiquement des niveaux de BDNF amoindris au niveau périphérique (Tiwari et al., 2022), mais également central (Mousten et al., 2022). Les échantillons *post mortem* de victimes de suicide révèlent également des niveaux amoindris de BDNF dans l'HPC et le PFC (Eisen et al., 2015). Des niveaux affaiblis d'autres facteurs neurotrophiques sont également constatés chez les patients dépressifs, tel que le facteur de croissance des fibroblastes (fibroblast growth factor, FGF) dans le PFC et l'ACC (Evans et al., 2004) et le FGF-2 dans l'HPC (Gaughran et al., 2006). Plus récemment, un rôle potentiel d'autres facteurs neurotrophiques a été suggéré, incluant le facteur de croissance endothélial vasculaire (vascular endothelial growth factor, VEGF), le facteur neurotrophique dérivé des cellules gliales (glial cell line-derived neurotrophic factor, GDNF), le facteur de croissance analogue à l'insuline de type 1 (insulin-like growth factor 1, IGF-1), ou encore le facteur de croissance des nerfs (nerve growth factor, NGF). Néanmoins, ces derniers demeurent moins explorés, et les résultats les concernant dans la dépression sont contradictoires (Levy et al., 2018).

3) Perturbations neuroendocriniennes

L'axe hypothalamo-hypophyso-surrénalien (Hypothalamic–pituitary–adrenal axis, HPA), système neuroendocrine de réponse au stress (**Figure 2**) (Smith & Vale, 2006), présente des dérégulations importantes dans la dépression. Dans des conditions physiologiques, la détection de stimuli internes ou externes appelés stresseurs, susceptibles de menacer l'intégrité physique ou psychologique d'un individu, entraîne une activation de cet axe (McEwen, 2007). L'activation induit une sécrétion de corticolibérine par l'hypothalamus, qui stimule à son tour la sécrétion d'hormone adrénocorticotrope par l'hypophyse, qui entraine la sécrétion de cortisol par les glandes corticosurrénales. Le cortisol, hormone du stress, facilite une réaction appropriée de l'organisme face aux stresseurs. En agissant sur les récepteurs aux glucococorticoïdes (GR), le cortisol exerce également un rétrocontrôle inhibiteur sur sa propre libération en agissant directement sur l'axe HPA, au niveau de l'hypophyse et l'hypothalamus, mais également indirectement sur cet axe *via* le PFC et l'HPC. Ce rétrocontrôle inhibiteur limite ainsi l'activité de l'axe HPA et permet un retour à la situation physiologique basale (Ulrich-Lai & Herman, 2009).

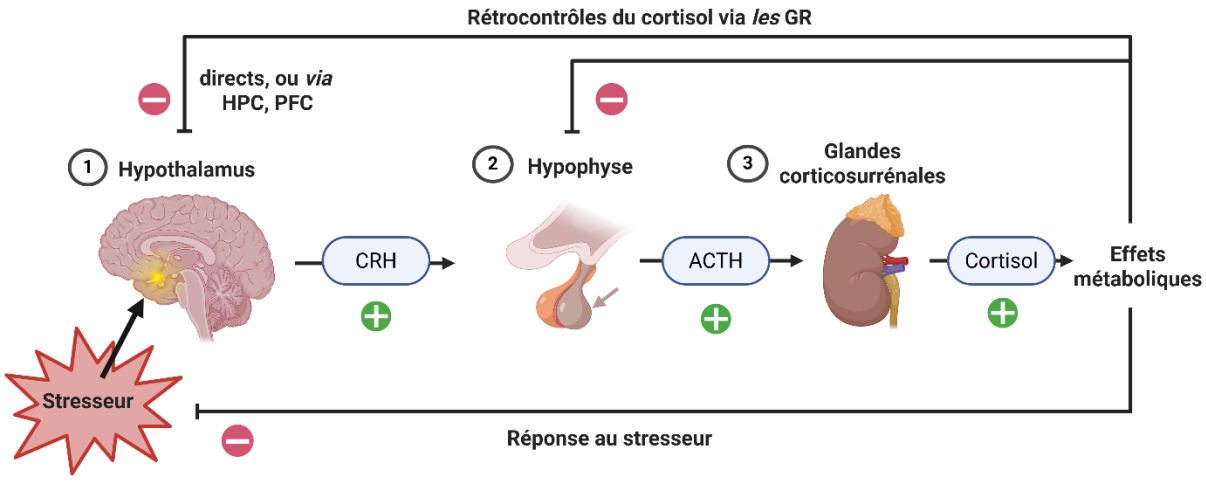

Figure 2 : Fonctionnement de l'axe hypothalamo-hypophyso-surrénalien (réalisé sur biorender).
ACTH : hormone adrénocorticotrope ; CRH : corticolibérine ; GR : récepteurs aux glucocorticoïdes.

Dans la dépression, les mécanismes de rétrocontrôle de l'axe HPA, évalués par le test de freinage à la dexaméthasone, sont souvent rapportés comme dysfonctionnels, en particulier chez des patients ayant subi un stress tôt dans leur vie (Jiang et al., 2025). Les taux circulants de cortisol sont typiquement anormaux chez les patients, qui présentent souvent une hypercortisolémie (Sahu et al., 2022) dont l'intensité est associée à la sévérité de la pathologie (Li et al., 2024).

4) Perturbations neuroinflammatoires

La dépression est parfois également associée à une inflammation et neuroinflammation. Les niveaux sériques de protéine C réactive, marqueur inflammatoire, sont élevés chez la moitié des patients, et atteignent un seuil considéré comme inflammatoire chez un quart des patients (Osimo et al., 2019). Les niveaux sanguins d'autres marqueurs inflammatoires dont TNF-α, IL-3, IL-6, IL-12, IL-18, sIL-2R sont également plus élevés (Osimo et al., 2020). Au niveau central, des niveaux accrus de protéine translocatrice (translocator protein, TSPO), marqueur neuroinflammatoire, sont typiquement observés dans le PFC, l'insula et l'HPC (Eggerstorfer et al., 2022).

5) Différences liées au sexe

Si des différences liées au sexe chez des patients concernent la prévalence et la présentation de certains symptômes, elles concernent également des perturbations des structures évoquées ci-dessus. D'après une récente méta-analyse d'études d'imagerie cérébrale (Mohammadi et al., 2023), ces perturbations impliquent notamment des différences volumiques de l'HPC, l'AMY, l'ACC et l'Hb, mais aussi des différences de fonction de structures comme le PFC ; cependant, les données concernant

ces différences sont contradictoires selon les études (Mohammadi et al., 2023). Des différences liées au sexe ont également été relevées dans l'hypercortisolémie : chez les femmes, les niveaux de cortisol sont particulièrement élevés au réveil et le soir, alors que chez les hommes l'élévation est globale sur l'ensemble du rythme circadien (Wang, et al., 2024). Le paramètre du sexe apparaît ainsi d'importance dans l'étude de la dépression.

Ainsi, la dépression est marquée non seulement par des perturbations structurelles, fonctionnelles et neurochimiques, mais également par des dysfonctions de la neuroplasticité, une dérégulation de l'axe de réponse au stress, et une neuroinflammation.

D) Étiopathologie

1) Hypothèses

Si les causes de la dépression ne sont pas encore clairement établies, deux hypothèses principales ont été élaborées concernant son étiologie. La première a été l'hypothèse monoaminergique, élaborée en 1967, selon laquelle des affaiblissements des niveaux de monoamines, en particulier de 5-HT, seraient à l'origine de la pathologie. Cette hypothèse est née du constat que des substances inhibant les enzymes de dégradation des monoamines avaient une efficacité thérapeutique chez les patients dépressifs, tandis que des substances déplétant les monoamines, comme la réserpine, pouvaient au contraire entraîner des symptômes dépressifs (Hirschfeld, 2000). Ce constat a conduit au développement d'antidépresseurs inhibant la dégradation ou la recapture des monoamines afin de favoriser leur action pharmacologique dans la synapse ; parmi ceux-ci, les inhibiteurs sélectifs de la recapture de la 5-HT sont devenus les antidépresseurs les plus prescrits (Pirraglia et al., 2003). Cependant, cette hypothèse est de plus en plus remise en question. D'abord, les observations concernant les niveaux de monoamines dans les cas de dépression sont contradictoires, et ne démontrent dans l'ensemble pas d'abaissement marqué. Une vaste revue « parapluie » récente a notamment montré l'absence de lien fiable entre perturbations sérotoninergiques et dépression (Moncrieff et al., 2023), bien que cette revue ait également été critiquée (Jauhar et al., 2023). Ensuite, l'effet thérapeutique des antidépresseurs ciblant les monoamines n'émerge qu'après plusieurs jours ou semaines de traitement, alors que l'inhibition de la recapture des monoamines est effective quelques heures seulement après leur administration (Hirschfeld, 2000). De plus, environ un tiers des patients ne répondent pas aux antidépresseurs monoaminergiques, mais peuvent répondre à d'autres types de thérapies (**cf. p. 20**). Enfin, l'hypothèse monoaminergique ne permet pas d'expliquer les perturbations observées dans les transmissions GABAergique et glutamatergique (Sanacora et al., 2012).

Face aux contradictions grandissantes entre l'hypothèse monoaminergique et les observations empiriques, une hypothèse alternative a été développée à la fin des années 90, centrée autour des facteurs neurotrophiques (Duman et al., 1997). Elle a émergé de l'observation de niveaux typiquement amoindris de BDNF chez les patients, cohérents avec les diverses perturbations volumiques et fonctionnelles observées ainsi que dans des modèles animaux de la pathologie (Neto et al., 2011). L'hypothèse neurotrophique est cohérente avec les observations cliniques, et permet de déterminer un facteur causal central de la dépression : le stress physiologique (**cf. p. 16**).

2) Facteurs de risque

Il existe de grandes différences interindividuelles en ce qui concerne la vulnérabilité à la dépression. En effet, le modèle de diathèse–stress stipule que la dépression se déclenche suite à l'expérience d'événements néfastes chez des individus ayant des prédispositions innées ou acquises (Harro, 2012). Ces différences interindividuelles sont liées à l'existence d'un nombre considérable de facteurs de risque de natures différentes. Parmi ceux-ci, les facteurs génétiques présentent une grande importance dans la dépression, dont l'héritabilité est estimée à 37 % (Sullivan et al., 2000). Deux cents quarante-trois *loci* « à risque » ont été identifiés dans le génome humain, et la pathologie est décrite comme « hautement polygénique » avec environ 11 000 variants expliquant 90 % de l'héritabilité liée aux polymorphismes mononucléotidiques (Als et al., 2023). Les polymorphismes les plus notables sont d'ailleurs liés au stress ou aux facteurs neurotrophiques (**Tableau 2 : Exemples de polymorphismes identifiés comme facteurs de risque dans la dépression.Tableau 2**).

Les facteurs de risque environnementaux concernent globalement l'expérience d'événements stressants et le mode de vie. L'expérience de stress précoce, incluant la maltraitance infantile (Humphreys et al., 2020; LeMoult et al., 2020; Zhao et al., 2025) ou l'exposition durant l'enfance à des blessures graves ou à la mort (Tang et al., 2014), est l'un des facteurs qui augmentent le plus fortement le risque de développer une dépression. Néanmoins, l'expérience d'événements stressants demeure un facteur important à l'âge adulte (Bjørndal et al., 2022; Cohen et al., 2019).

Gène	*Slc6a4*	*Bdnf*	*Nr3c1*	*IL6*	*Fkbp5*
Fonction	Transporteur de la 5-HT	Facteur neurotrophique	Récepteur aux glucocorticoïdes (GR)	Cytokine pro-inflammatoire	Protéine co-chaperone des GR
Poly-morphisme	5-HTTLPR (Caspi et al., 2003)	Val66Met (Hosang et al., 2014; Wang et al., 2023)	rs41423247 (Peng et al., 2018; Zhang et al., 2023)	rs1800795 (Kovacs et al., 2016)	rs3800373, rs4713916, rs1360780 (Fan et al., 2021; Hernández-Díaz et al., 2019; Zhang et al., 2024)

Tableau 2 : Exemples de polymorphismes identifiés comme facteurs de risque dans la dépression.

Le mode de vie peut également être source de risque de développer la pathologie. Certains régimes alimentaires sont mis en cause : la consommation d'ingrédients ultra-transformés, d'ingrédients pro-inflammatoires, mais également le fait de ne pas prendre de petit-déjeuner (Gorbachev et al., 2023; Kunugi, 2023; Selvaraj et al., 2022; Xu et al., 2021). Un manque d'activité physique et plus généralement un style de vie sédentaire, accompagné d'activités mentalement passives, sont également des facteurs de risques (Hu et al., 2025; Huang et al., 2020; Pearce et al., 2022). Enfin, l'isolement social et surtout le ressenti de solitude augmentent le risque de développer la pathologie (Erzen & Çikrikci, 2018; Matthews et al., 2016)

3) La place centrale du stress chronique dans la dépression

Le concept de « stress » a été développé par Hans Selye en 1925. Selye a défini trois étapes du « syndrome général d'adaptation » : la réaction d'alarme suite à la détection d'un stresseur, pendant laquelle les mécanismes de défense de l'organisme sont mobilisés ; le stade de résistance, pendant lequel l'organisme s'adapte au stresseur pour permettre un retour à son équilibre ; le stade d'épuisement, où une exposition prolongée au stresseur conduit à un épuisement des capacités d'adaptation de l'organisme, entraînant un état pathologique (Rochette et al., 2023; Selye, 1950). Ce concept a été complété par la notion d'allostasie. Les stresseurs génèrent une charge dite allostatique, nécessitant un certain degré d'adaptation de l'organisme afin d'assurer son équilibre face aux conditions (Sterling & Eyer, 1988). La perception d'un stresseur entraîne la « réaction d'alarme » en mobilisant, *via* l'activation de l'hypothalamus, le système sympatho-adrénergique, responsable d'une

réponse immédiate par la libération d'adrénaline facilitant la réponse « combat-fuite ». Une réponse plus lente, de l'ordre de dizaines de minutes, est générée par l'axe HPA, décrit plus haut (**cf. p. 12**) *via* la sécrétion de cortisol. Cette hormone stéroïdienne circule dans l'organisme, peut traverser la membrane plasmique et se fixer aux récepteurs des minéralocorticoïdes ainsi qu'aux GR dans le cytoplasme des cellules. Cette liaison entraîne la translocation du complexe dans le noyau, où il exerce des effets transcriptomiques (Kadmiel & Cidlowski, 2013). La signalisation GR facilite notamment la mobilisation d'énergie et permet l'inhibition de l'axe HPA dans une boucle de rétrocontrôle (**Figure 2**) (Ulrich-Lai & Herman, 2009). Ces mécanismes constituent les « défenses de l'organisme » permettant une adaptation de l'organisme, jusqu'à cessation du stresseur, puis retour à son équilibre. Cependant, la réponse aux stresseurs peut avoir des conséquences délétères pour l'organisme s'ils sont d'intensité et/ou de durée anormalement élevées. Un stresseur aigu d'intensité extrême peut par exemple générer des perturbations neurobiologiques durables et conduire à l'émergence de troubles post-traumatiques (Yehuda, 2002). L'exposition chronique à des stresseurs, même de faible intensité, génère une charge allostatique trop grande pour l'organisme, évoquant le « stade d'épuisement » (Logan & Barksdale, 2008), qui entraîne également des perturbations importantes comme l'affaiblissement ou l'effondrement des boucles de rétrocontrôle inhibiteur de l'axe HPA et donc l'hypercortisolémie. Conformément à l'hypothèse neurotrophique de la dépression, cette dérégulation cause un amoindrissement des niveaux de facteurs neurotrophiques, pouvant expliquer les dysfonctions neuroplastiques et fonctionnelles, entraînant l'atrophie de structures frontales et de l'HPC, voire de l'AMY, ainsi que les phénomène d'inflammation et de neuroinflammation (Krasner et al., 2025; Mariotti, 2015; Pittenger & Duman, 2008). La vie quotidienne entraine l'exposition à un grand nombre de stresseurs pouvant être légers, mais chroniques, provenant notamment des conditions de travail, de difficultés financières ou de problèmes relationnels (Hammen et al., 2009). Comme expliqué plus haut, l'expérience d'événements stressants, pendant l'enfance et à l'âge adulte, est fortement associée à la pathologie (Bjørndal et al., 2022; Cohen et al., 2019).

E) Traitements de la dépression

De nombreuses approches et stratégies thérapeutiques ont été élaborées. La plupart sont des pharmacothérapies (Cuijpers et al., 2020), qui ont connu de multiples évolutions. Les premières substances utilisées à des fins thérapeutiques ont été les amphétamines, dans les années 1930. Néanmoins, en raison d'une efficacité peu satisfaisante et de l'émergence d'effets secondaires indésirables importants, leur prescription pour cet usage a été graduellement réduite jusqu'à l'abandon total dans les années 1960 (Hirschfeld, 2012).

1) Les pharmacothérapies « classiques » et leurs limites

Les pharmacothérapies, incluant les tricycliques, les inhibiteurs des monoamines oxydases (monoamine oxydase inhibitors, MAOI), et les inhibiteurs sélectifs de la recapture des monoamines, sont souvent décrites collectivement comme « antidépresseurs classiques » (Ramaker & Dulawa, 2017).

Les tricycliques ont été mis sur le marché à la fin des années 1950, suivis de près par les inhibiteurs de la monoamine oxydase (Hillhouse & Porter, 2015; Hirschfeld, 2012). Ces deux familles de médicaments agissent en inhibant le système de la recapture des neurotransmetteurs comme la 5-HT et la NA, ou en empêchant leur dégradation, respectivement (Feighner, 1999). Néanmoins, les effets antidépresseurs des MAOI ont par la suite surtout été attribués à l'inhibition d'une des deux formes de monoamine oxydase, la MAO de type A (MAO_A) ; des MAO_AI, inhibiteurs sélectifs de cette forme d'enzyme, ont par la suite été développés et préférentiellement prescrits (Hillhouse & Porter, 2015).

Enfin, à partir des années 1980, les inhibiteurs sélectifs de la recapture des monoamines ont été développés, ciblant 5-HT et NA, DA et NA, ou NA uniquement (Hirschfeld, 2012). En 1987, la fluoxétine, premier inhibiteur sélectif de la recapture de la 5-HT (selective serotonin reuptake inhibitors, SSRIs) a été mis sur le marché. Les SSRIs, entraînant moins de contre-indications, sont graduellement devenus le traitement de première intention pour la dépression au cours des années 1990, éclipsant les autres traitements (Pirraglia et al., 2003; Hillhouse & Porter, 2015; Hirschfeld, 2012).

Cependant, l'efficacité de ces antidépresseurs classiques est relative. Pour un médicament donné, plusieurs semaines de traitement quotidien, généralement entre 4 et 6, sont typiquement nécessaires avant d'atteindre les effets thérapeutiques maximaux (Lee et al., 2012). Le taux de rechute après l'arrêt total du traitement, estimé à 40 %, est important (Kato et al., 2021). Ce traitement est souvent accompagné d'effets secondaires indésirables bénins mais pouvant également être sévères, voire fatals (**Tableau 3**).

Classe	Tricycliques	MAOAI	SSRI
Exemple	Imipramine (*Tofranil* ®)	Moclobémide (*Moclamine* ®)	Fluoxétine (*Prozac* ®)
Effets secondaires bénins et modérés	Bouche sèche, somnolence, constipation, étourdissements, sueurs, tremblements, vision floue, sédation, symptômes anticholinergiques, hypotension orthostatique, bouffées de chaleur. (Kamp et al., 2024)	Hypotension orthostatique avec étourdissements, prise de poids, œdème, dysfonction sexuelle, insomnie, sédation diurne, myoclonus. (Remick & Froese, 1990)	Nausées, vomissements, insomnie, somnolence, maux de tête, diminution de la libido, agitation, prise de poids, troubles du sommeil. (Edinoff et al., 2021)
Effets secondaires graves	Tachicardie, épilepsie, anomalies de la conduction cardiaque (Taylor et al., 2024)	Crises hypertensives, syndrome sérotoninergique (Remick & Froese, 1990)	Syndrome sérotoninergique, syndrome extrapyramidal, idéation suicidaire, prolongation de l'intervalle QT*. (Edinoff et al., 2021)

Tableau 3 : Effets secondaires des antidépresseurs classiques. ** L'intervalle QT est une mesure de l'activité cardiaque qui représente la durée entre le début de la contraction ventriculaire et la fin de la relaxation ventriculaire.*

Près de 40 % des patients ne répondent pas au premier traitement prescrit (Spigset & Mårtensson, 1999), et environ 30 % des patients présentent une dépression dite 'résistante' aux traitements (treatment-resistant depression, TRD). La TRD est définie par l'agence européenne des médicaments et l'agence américaine des produits alimentaires et médicamenteux (Food and Drug Administration, FDA) comme une dépression dans laquelle au moins deux stratégies thérapeutiques médicamenteuses différentes n'ont pas permis d'amélioration de la condition (McIntyre et al., 2023).

2) Approches non pharmacologiques

Des stratégies thérapeutiques non pharmacologiques, employées à la place ou en complément d'un traitement médicamenteux, ont également été élaborées. Un accompagnement psychiatrique/psychologique des patients peut par exemple être envisagé, comme la thérapie cognitivo-comportementale. Ce genre d'approche vise à identifier et remettre en question les biais cognitifs et les schémas de pensées négatives des patients, afin de favoriser un rétablissement et prévenir des rechutes (Driessen & Hollon, 2010; Roiser et al., 2012).

La stimulation magnétique transcrânienne (transcranial magnetic stimulation, TMS) répétée est une technique de stimulation cérébrale non invasive utilisée pour moduler l'activité neuronale, principalement par l'intermédiaire d'impulsions électromagnétiques appliquées à des régions perturbées dans la pathologie, la plus souvent le dPFC (Somani & Kar, 2019). Cependant, ses effets à long terme sont moins bien établis, avec un taux de rechute d'environ 50 % douze mois après cessation du traitement (Senova et al., 2019). Leur durabilité apparait négativement corrélée avec la sévérité de la maladie (Kedzior et al., 2015).

3) Approches pour la dépression résistante

Dans les cas de TRD, des approches thérapeutiques plus invasives peuvent être employées. Celles-ci reposent souvent sur l'électrophysiologie, comme l'électro-convulsivothérapie (ECT) (Trifu et al., 2021). L'ECT consiste à générer un état d'épilepsie sous anesthésie générale par l'application de courant électrique au cerveau (Kritzer et al., 2023). Malgré une efficacité notable, cette approche peut générer des troubles mnésiques (Hsieh, 2023; Subramanian et al., 2022) et elle présente un fort taux de rechute, à environ 80 % six mois après une session unique (Noda et al., 2013), et environ 30 % après des sessions répétées (Kellner et al., 2006).

Un autre traitement applicable dans les cas de TRD est la stimulation cérébrale profonde (deep brain stimulation, DBS). La DBS vise à appliquer une stimulation électrique contrôlée, via des électrodes implantées dans des régions liées aux dysfonctionnements observés dans la pathologie (Figee et al., 2022). Plusieurs régions peuvent être stimulées à des fins thérapeutiques, comme le striatum ventral (Bewernick et al., 2012; Hitti et al., 2021), ou d'autres régions liées au système de récompense, comme le faisceau médian du télencéphale (medial forebrain bundle) (Fenoy et al., 2022) ; la LHb, impliquée dans la régulation des monoamines, peut également être ciblée (Sartorius & Henn, 2007; Wang et al., 2024). Néanmoins, cette approche est particulièrement invasive et la cessation de la stimulation conduit à la rechute voire à l'aggravation des symptômes pré-traitement (Kilian et al., 2019).

Depuis les années 2000, un nouveau type de pharmacothérapie a vu le jour : les antidépresseurs à action rapide (rapid acting antidepressants, RAA). Comme leur nom le suggère, les RAA présentent des effets thérapeutiques rapides, de l'ordre de quelques heures, à opposer aux semaines pour les antidépresseurs classiques. Ils agissent sur des cibles différentes des systèmes monoaminergiques traditionnellement visés, incluant le système cholinergique, le système opioïdergique, l'axe HPA et le système glutamatergique (Machado-Vieira et al., 2017). Parmi ces substances, on trouve la

scopolamine, un anticholinergique, le rapastinel, un modulateur positif des récepteurs glutamatergiques ionotropiques N-méthyl-D-aspartate (NMDA-R), ou encore la (R/S)-kétamine (ci-après nommée Ket), un antagoniste non compétitif des NMDA-R (Witkin et al., 2018). Les RAA ont une efficacité notable chez les patients atteints de TRD (Yavi et al., 2021). Cependant, leurs mécanismes antidépresseurs sont encore mal connus. Ne sera développée ci-après que la Ket, employée dans les travaux de cette thèse.

4) La kétamine

a) Historique

Développée en 1962 à des fins d'anesthésie comme alternative à la phéncyclidine, dont l'effet sédatif était jugé trop long et les effets secondaires trop marqués, cette molécule de synthèse est toujours employée pour cet usage dans le domaine clinique et vétérinaire (Hirota & Lambert, 2022). Dès 1975, il a été constaté que la Ket, utilisée en tant qu'anesthésique, pouvait entraîner une amélioration de symptômes chez des patients dépressifs subissant une opération chirurgicale (Sofia & Harakal, 1975). Le premier essai clinique pour le traitement de la dépression, conduit en 2000 avec une dose sous-anesthésique de Ket, a démontré son efficacité thérapeutique (Berman et al., 2000). Depuis, un grand nombre d'essais cliniques l'ont confirmé, notamment dans la TRD (Corriger & Pickering, 2019; Schwartz et al., 2016; Zarate et al., 2006). En 2019, un de ses énantiomères, la (S)-Ket, a été approuvé sous forme de spray nasal pour le traitement de la TRD par la FDA et l'agence européenne des médicaments ; l'année suivante, la FDA a également autorisé son usage pour la dépression sans résistance aux traitements classiques (McIntyre et al., 2021).

b) Propriétés

La Ket est une molécule de synthèse appartenant à la famille des arylcyclohexylamine (Hancu et al., 2024). Elle existe en tant que mélange racémique de ses deux énantiomères : la (S)-Ket, également appelée eskétamine, et la (R)-Ket, ou arkétamine, qui présentent des propriétés différentes (Zanos et al., 2018). La biodisponibilité de la Ket varie selon la voie d'administration : elle est virtuellement de 100 % par voie intraveineuse, de 45 à 50 % par voie intranasale, et de 16 à 29 % par voie orale (Zanos et al., 2018). A dose égale, la (S)-Ket permet d'atteindre une concentration plasmatique similaire ou supérieure à celles de la (R)-Ket ou du mélange racémique (Zanos et al., 2018). L'élimination de la substance est relativement rapide, avec une demi-vie chez l'humain adulte de l'ordre de deux à quatre heures (Domino et al., 1982). Elle est métabolisée par les enzymes hépatiques de la famille des cytochromes P450 en plusieurs métabolites, comme la

déhydronorkétamine ou la norkétamine, elle-même métabolisée en hydroxynorkétamine (HNK), dont il existe plusieurs formes (Nguyen et al., 2024; Zanos et al., 2018).

L'effet pharmacologique de la Ket correspond à un antagonisme des NMDA-R : la molécule se lie au site de fixation de la phéncyclidine au sein du canal formé par le récepteur, agissant comme bloqueur non compétitif et prévenant le passage de cations normalement déclenché par l'activation du récepteur par son ligand, le Glu (Iadarola et al., 2015; Zanos et al., 2018). À l'échelle d'une synapse, l'excitation du neurone post-synaptique est ainsi prévenue. L'affinité de la (S)-Ket pour son site de fixation est environ quatre fois supérieure à celle de la (R)-Ket et deux fois supérieure à celle du mélange racémique (Zanos et al., 2018).

Si la Ket se fixe et agit principalement sur les NMDA-R, elle peut également interagir avec d'autres récepteurs, notamment cholinergiques, GABAergiques, dopaminergiques, sérotoninergiques, opioïdergiques, sigma, et également avec des canaux sodiques et calciques (Zanos et al., 2018).

c) Effets indésirables

Bien que les effets secondaires à long terme soient encore mal connus, les traitements antidépresseurs de Ket, qu'ils soient administrés par voie intraveineuse ou, de façon moins marquée, par spray nasal, entraînent des effets secondaires transitoires (Short et al., 2018), incluant des effets psychomimétiques, tels que la dissociation ou des hallucinations, des effets sympathomimétiques, incluant de l'hypertension ou de la tachycardie et des symptômes vestibulaires, incluant des étourdissements, nausées et vomissements (Yavi et al., 2022). Les effets les plus fréquents sont les maux de têtes, suivi des étourdissements et de la dissociation (Short et al., 2018). Dans le cadre d'un usage récréatif et de traitements réguliers pour la douleur chronique, des effets de toxicité hépatique et urinaire, liés à une utilisation fréquente et à forte de dose, ont été caractérisés (Juneja et al., 2024). Si de tels effets n'ont jamais été décrits dans le cadre d'un traitement antidépresseur, ils mettent en garde en cas de mauvaise application thérapeutique.

d) Mécanismes antidépresseurs

En raison de son action rapide, permettant une amélioration des symptômes dans les deux heures suivant l'administration (Papadopoulos, 2025), y compris dans les cas de TRD, il peut être supposé que le mécanisme d'action de la Ket cible plus précisément et efficacement les dysfonctions inhérentes à la pathologie. Si son mécanisme thérapeutique exact reste mal connu, le fait que les effets antidépresseurs persistent bien après l'élimination de la substance de l'organisme suggère l'implication de cascades de réactions cellulaires (Pham & Gardier, 2019). La Ket semble promouvoir et/ou restaurer

la neuroplasticité par stimulation de la voie mTORC1 promotrice de synthèse protéique (Li et al., 2010). Cette stimulation dépend de l'activation des récepteurs α-amino-3-hydroxy-5-méthylisoxazol-4-propionate (AMPA) et de la production de BDNF (Suzuki et al., 2023) en particulier dans le mPFC (Pham & Gardier, 2019). Ces mécanismes permettraient un rétablissement de la communication neuronale dans les réseaux dysfonctionnels (Shin & Kim, 2020).

Plusieurs hypothèses, qui ne sont pas mutuellement exclusives, ont été proposées pour expliquer les effets antidépresseurs de la Ket. Dans une revue, Zanos et Gould ont fait la synthèse de ces hypothèses (**Figure 3** et ci-dessous) (Zanos & Gould, 2018).

Désinhibition des neurones pyramidaux (Figure 3-1). Les neurones pyramidaux, dans des structures clés comme le mPFC, seraient maintenus toniquement dans un état d'inhibition par des interneurones GABAergiques. La Ket ciblerait sélectivement les NMDA-R situés sur ces interneurones (Zorumski et al., 2016), prévenant l'inhibition GABAergique et désinhibant ainsi les neurones pyramidaux. En conséquence, la communication glutamatergique serait facilitée (Moghaddam et al., 1997), déclenchant les mécanismes expliqués ci-dessus (Li et al., 2025; Yavi et al., 2022; Zanos & Gould, 2018).

Blocage de la transmission glutamatergique spontanée (Figure 3-2). Il existe une libération de Glu présynaptique spontanée qui entraîne une activation des récepteurs NMDA post-synaptiques, générant des courants post synaptiques excitateurs dits miniatures (miniature excitatory postsynaptic currents, mEPSC) (Espinosa & Kavalali, 2009) malgré la présence d'un ion Mg^{2+} (Reese & Kavalali, 2015). Les mEPSC inhiberaient indirectement la production de BDNF *via* la voie de signalisation de la kinase facteur d'élongation eucaryote 2 (eukaryotic elongation factor 2 kinase, eEF2K). La Ket permettrait d'empêcher la genèse des mEPSC (Autry et al., 2011), ce qui désinhiberait donc la production de BDNF (Li et al., 2025; Yavi et al., 2022; Zanos & Gould, 2018).

Blocage des récepteurs NMDA extra-synaptiques (Figure 3-3). Des récepteurs NMDA contenant la sous-unité GRIN2B (ou GluN2B) situés en dehors de la synapse sur des dendrites adjacentes à des cellules gliales, sont activés par des faibles niveaux de Glu (Pál, 2018). L'activation de ces récepteurs inhibe de façon tonique la voie mTORC1, prévenant la synthèse protéique ce qui maintient l'homéostasie (Miller et al., 2014). La Ket, en bloquant ces récepteurs, préviendrait temporairement cette inhibition de la voie mTORC1, permettant de désinhiber la synthèse protéique (Li et al., 2025; Zanos & Gould, 2018).

Inhibition de la LHb (Figure 3-4). Cette structure cérébrale est dysfonctionnelle dans la dépression (**cf. p. 7**). Dans les modèles animaux, une activité NMDA-dépendante en « burst », c'est-à-dire en rafale, est observée et mise en cause dans la genèse des perturbations comportementales (Yang et al., 2018). La Ket préviendrait ce phénomène, permettant d'atténuer ces perturbations (Li et al., 2025; Yavi et al., 2022; Zanos & Gould, 2018).

Métabolites de la Ket (Figure 3-5). Les métabolites HNK ont longtemps été considérés comme des métabolites inactifs du fait de leur incapacité pharmacologique à bloquer les récepteurs NMDA mais aussi de leur incapacité à induire un effet anesthésique (Leung & Baillie, 1986). Ce point de vue est remis en question puisque qu'un nombre grandissant d'études précliniques leur attribuent un potentiel antidépresseur, en particulier sous la forme (2R,6R)-HNK et dans une moindre mesure (2S,6S)-HNK, bien que ces résultats ne soient pas consensuels (Nguyen et al., 2024). L'effet antidépresseur des métabolites HNK dépendrait d'une activation des récepteurs AMPA, bien que le mécanisme sous-tendant cet effet reste mal compris (Li et al., 2025; Zanos & Gould, 2018).

Implication d'autres systèmes. L'effet antidépresseur de la Ket pourrait impliquer le système opioïdergique (Hess et al., 2022; Papadopoulos, 2025). Une étude clinique a montré que l'antagonisme des récepteurs opioïdergiques atténue la réponse thérapeutique de la Ket (Williams et al., 2018). Les systèmes monoaminergiques pourraient également être impliqués : la Ket conduit à une augmentation des niveaux de 5-HT, DA et NA dans le PFC (Hess et al., 2022; Papadopoulos, 2025). Une étude préclinique a notamment démontré qu'une administration directe de Ket dans le mPFC réduit l'immobilité dans le FST de façon corrélée avec une augmentation des niveaux de 5-HT, mécanisme nécessitant l'activité de récepteurs AMPA dans le raphé dorsal (Pham et al., 2017).

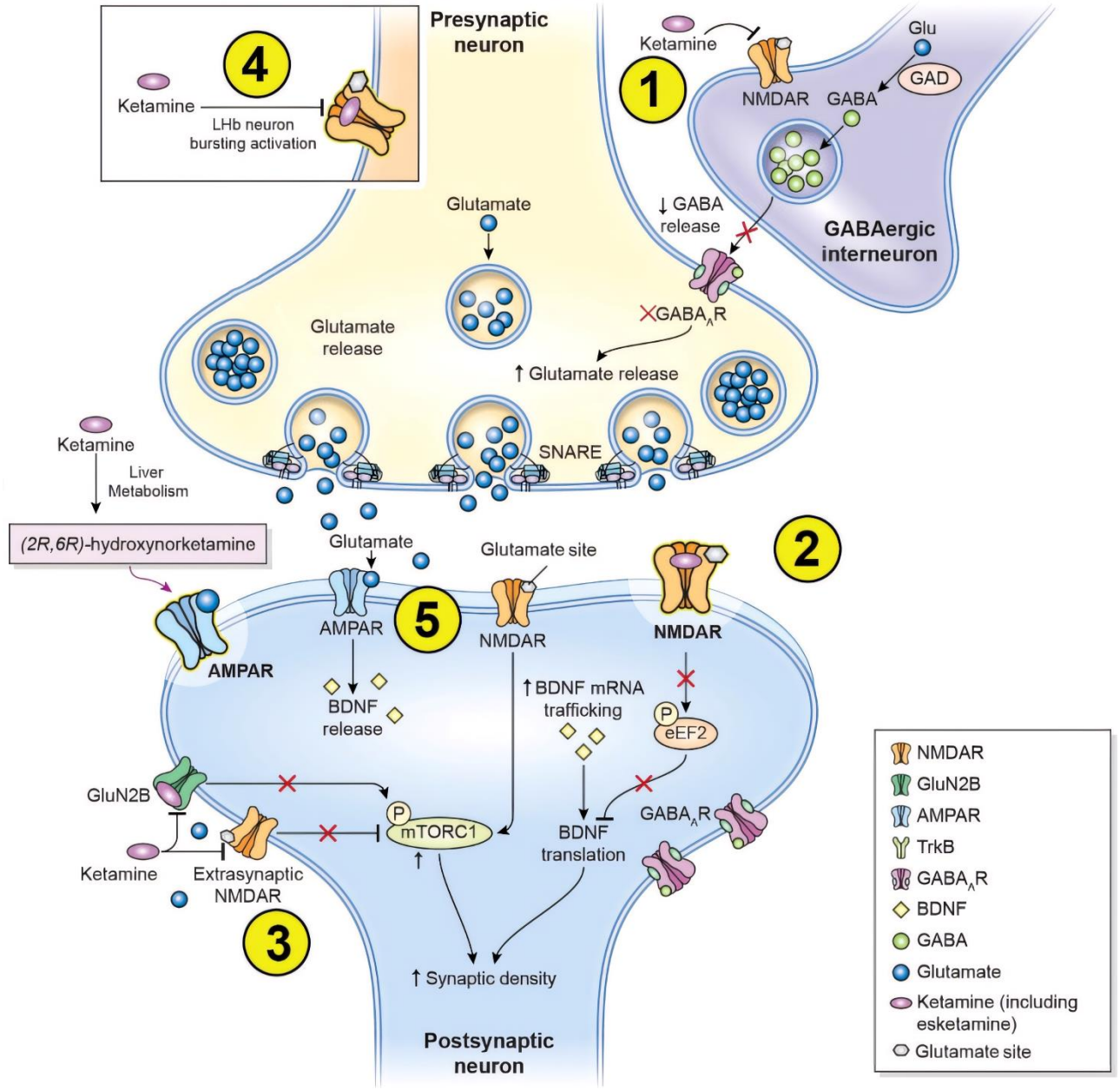

Figure 3 : Hypothèse des principaux mécanismes antidépresseurs de la Ket. Adaptée de « Ketamine treatment for depression: a review » (Yavi et al., 2022).

Certains auteurs ont émis des doutes sur la nécessité d'un antagonisme des NMDA-R, citant le paradoxe d'un potentiel antidépresseur plus marqué de l'énantiomère (R)-Ket malgré son affinité plus faible pour le récepteur, d'un effet thérapeutique des métabolites n'ayant pas d'action antagoniste sur les NMDA-R, ainsi que de l'absence d'effet antidépresseur d'un grand nombre d'autres substances antagonistes des NMDA-R (Wei et al., 2022). Outre son mécanisme, la modalité de traitement optimale de la Ket demeure mal maîtrisée. L'effet thérapeutique d'un traitement aigu semble s'estomper dans les jours ou semaines qui suivent (Shin & Kim, 2020). Des traitements répétés peuvent

être employés, mais il n'y a pas de consensus sur la fréquence idéale pour une telle approche, et le risque d'abus ou de dépendance à la substance n'est pas encore clairement connu (Fountoulakis et al., 2025; Reddy et al., 2024).

II) Modélisation animale de la dépression

A) Généralités

Afin de mieux comprendre les mécanismes physiopathologiques de la dépression, ainsi que les mécanismes thérapeutiques de traitements comme la Ket, l'utilisation d'animaux modèles de la pathologie est nécessaire et repose presque intégralement sur des modèles murins, rats ou souris (Baker et al., 2020). Néanmoins, comme pour celle des pathologies psychiatriques en général, la modélisation de la dépression chez le rongeur présente deux obstacles majeurs : l'absence d'une conscience de soi et l'impossibilité de verbaliser directement le ressenti. Ces deux critères rendent difficile, voire impossible, la modélisation de certains symptômes, par exemple concernant l'humeur, la culpabilité ou l'idéation suicidaire (Krishnan & Nestler, 2011; Markov & Novosadova, 2022).

S'il existe des modèles issus de croisements sélectifs, des modèles induits par traitement pharmacologique ou par opérations chirurgicales (Barr et al., 2002; Becker et al., 2021; Franklin et al., 2012), la plupart des modèles reposent sur l'exposition à des stresseurs (Gururajan et al., 2019), et présentent ainsi une bonne validité théorique étant donné la place prépondérante du stress dans la pathologie. Est développé ci-après le paradigme de stress léger chronique employé lors des différents travaux de cette thèse.

B) Le paradigme de stress léger chronique

1) Principe et caractéristiques générales

Le paradigme de stress léger chronique (chronic mild stress, CMS ; également chronic unpredictable stress, ou chronic unpredictable mild stress), est l'un des paradigmes les plus employés pour modéliser la dépression chez le rongeur. En 2021, plus de 1700 études employant ce modèle chez le rat ont été dénombrées (Strekalova et al., 2022). Développé dans les années 1980 par Katz et collaborateurs, le paradigme de CMS consiste à exposer des rongeurs à une série de stresseurs légers, non-traumatiques, incontrôlables, et parfois imprévisibles, appliqués pendant plusieurs semaines afin de mimer le stress chronique de faible intensité mis en cause dans l'étiologie de la dépression chez l'humain (**cf. p. 16**) (Katz et al., 1981; Willner, 2017b). Ces stresseurs sont de nature variée (**Figure 4**): physique (comme l'exposition à du bruit ou de la lumière constante), physiologique (comme des

privations intermittentes de nourriture et/ou d'eau) et sociale (comme l'intrusion d'un congénère dans la cage). Ils sont appliqués de façon à limiter une habituation par changement régulier du stresseur utilisé. La durée d'application du protocole de CMS est variable, pouvant s'étendre de 3 semaines à 3 mois (Strekalova et al., 2022). Le protocole de CMS est depuis longtemps présenté comme reproductible (Willner, 1997a), affirmation appuyée par un questionnaire envoyé à différents laboratoires employant ce paradigme (Willner, 2017a), et par une méta-analyse indépendante (Antoniuk et al., 2019). Néanmoins, certains auteurs ont alerté sur la possibilité d'une surestimation de cette fiabilité et reproductibilité, liée à de potentiels résultats négatifs non publiés et de problèmes non rapportés (Markov & Novosadova, 2022). Notamment, plus de la moitié des utilisateurs sondés ont refusé de répondre à la question de la reproductibilité (Willner, 2017a). Il n'en reste pas moins que le protocole de CMS est considéré comme l'un des modèles de dépression avec les meilleures validités théorique, d'aspect et prédictive (voir ci-dessous) (Nollet, 2021; Petković & Chaudhury, 2022; Ratajczak et al., 2024; Willner & Belzung, 2015).

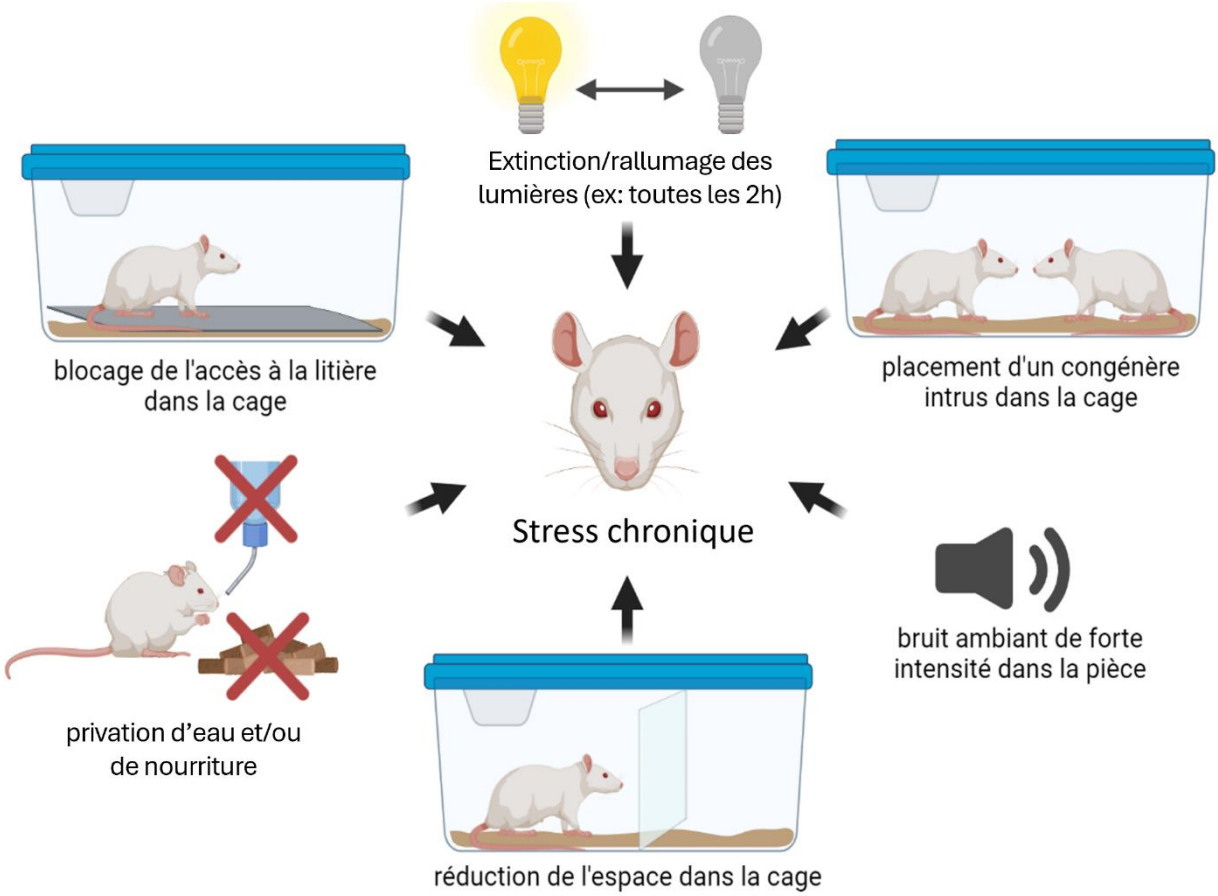

Figure 4 : Principe du protocole de CMS. L'exposition à des stresseurs variés régulièrement changés génère un stress léger, mais chronique.

2) Différences interindividuelles

De même que la vulnérabilité à la dépression varie selon les personnes, une variabilité interindividuelle existe chez des animaux soumis à un protocole de CMS (Harro, 2012). Si pendant longtemps, et encore majoritairement, cet aspect était négligé dans les études reposant sur l'exposition au CMS, l'intérêt d'identifier des sous-groupes parmi les animaux exposés au CMS a commencé à être considéré dans les années 2000 (Strekalova et al., 2022). Non seulement cette approche permet d'étudier des paramètres de résilience aux conséquences du stress chronique, mais elle est également plus écologique, les animaux vulnérables représentant une population modèle plus pertinente qu'un ensemble d'animaux stressés, vulnérables ou non, pris sans distinctions (Scharf & Schmidt, 2012). Typiquement, cette distinction repose sur le comportement d'anhédonie (Strekalova et al., 2022; Willner, 2017b), mais certaines études utilisent d'autres paramètres comme le poids ou l'anxiété (José Jaime et al., 2016). Une étude a également mis en évidence, *via* une analyse factorielle exploratoire, que les variables de poids et la locomotion prédisaient plus fidèlement l'appartenance au groupe CMS que l'anhédonie, représentant donc des indicateurs plus fiables de l'impact du protocole (Sequeira-Cordero et al., 2019).

3) Différences liées au sexe

La question de différences liées au sexe suite à l'application d'un protocole de stress chez le rongeur est d'importance dans le cadre de la dépression, pour laquelle une prédominance féminine est établie. Malgré cela, l'inclusion de femelles dans les études liées au stress chronique reste peu commune (Campbell et al., 2024), à l'image de la recherche préclinique en général (Beery & Zucker, 2011). Au-delà des conséquences comportementales et physiologiques engendrées par le stress chronique, il est notable que la réponse de l'organisme à des stresseurs présente elle-même un dimorphisme sexuel, avec une activation accrue de l'axe HPA chez les femelles (Markov & Novosadova, 2022; Novais et al., 2017). Ce dimorphisme sexuel est également influencé par des différences espèce- et surtout souche-dépendantes (Franceschelli et al., 2014; Novais et al., 2017). Les différences liées au sexe dans la réponse au CMS sont rapportées ci-après.

4) Conséquences du stress léger chronique

Les données suivantes sont focalisées sur les paramètres évalués lors de cette thèse.

a) Perturbations comportementales

Anhédonie

Le test de consommation et le test de préférence au saccharose (Sucrose consumption/ preference test, SCT/SPT) sont basés sur l'appétence des rongeurs pour les aliments sucrés. La consommation d'une solution de saccharose mise à disposition du rongeur, ou la préférence pour celle-ci par rapport à de l'eau, peuvent être évaluées. Une consommation ou préférence amoindrie peut refléter un comportement d'anhédonie (Gencturk & Unal, 2024). Il existe cependant une grande variabilité dans les protocoles de SPT (Primo et al., 2023). Globalement, le CMS entraîne une baisse graduelle de la préférence au saccharose (Pothion et al., 2004; Strekalova et al., 2022; Willner, 2005). Cet effet n'est cependant pas uniforme ; les études analysant la réaction individuelle au CMS révèlent qu'une partie seulement des animaux, entre 35 et 70 %, présente une baisse marquée de la préférence (Markov, 2022). Les perturbations observées suite au CMS sont rétablies par un traitement chronique d'antidépresseurs classiques ou un traitement aigu de Ket (Mishra et al., 2018; Pothion et al., 2004; Willner, 2005). Néanmoins, une augmentation, plutôt qu'une diminution, de la préférence au saccharose suite au CMS a également été rapportée (Markov, 2022). L'anhédonie induite par l'exposition au CMS semble plus marquée chez les mâles que chez les femelles (Dalla et al., 2011; Franceschelli et al., 2014), mais des différences basales dans la préférence au saccharose, influencées par les hormones sexuelles, pourraient biaiser les résultats (Franceschelli et al., 2014). Il existe également des différences marquées entre les souches ou lignées utilisées (Pothion et al., 2004) et entre rat et souris (Strekalova & Steinbusch, 2010) dans des conditions identiques de test.

Si le SPT reste le paradigme principal pour l'évaluation de l'anhédonie chez le rongeur, sa reproductibilité est mise en question (Markov, 2022), pour des raisons similaires à celles de la reproductibilité et fiabilité du CMS en général (Willner, 2017a). De plus, de nombreux protocoles incluent une période de privation d'eau et/ou de nourriture avant le test, jugée non nécessaire et source de biais dans une méta-analyse récente (Berrio et al., 2024).

Activité locomotrice

L'activité locomotrice spontanée des rongeurs peut être quantifiée dans un environnement familier, le plus souvent leur cage d'hébergement. Le peu d'études, qui ont évalué l'impact du CMS sur ce type d'activité, ont donné des résultats contradictoires, i.e. une absence d'effet (Castelhano-

Carlos et al., 2014), ou une diminution de l'activité sur une évaluation courte de 10 minutes (George et al., 2022) ou sur 24 heures (Rudyk et al., 2019). Une étude employant un modèle d'exposition répétée à un stresseur unique de contention suggère néanmoins qu'une diminution de l'activité pourrait dépendre de l'âge (Furukawa et al., 2025). Une évaluation réalisée sur une période d'au moins 24 heures permet de déterminer le rythme d'activité nycthéméral et ainsi de mettre à jour des perturbations de ce rythme d'activité telles qu'elles peuvent exister chez les patients dépressifs (Germain & Kupfer, 2008; Robillard et al., 2013). Le peu de résultats disponibles semblent contradictoires, i.e. une augmentation (Strekalova et al., 2011) ou une réduction (Gorka et al., 1996) de l'activité locomotrice en période nocturne.

L'activité locomotrice des rongeurs peut également être évaluée en réponse à la nouveauté. Dans ce cas, elle est le plus souvent quantifiée dans un champ ouvert (open field test, OFT), une enceinte typiquement carrée ou circulaire, ouverte sur le dessus. La distance parcourue, ainsi que des comportements comme les redressements, peuvent être évalués (Gould et al., 2009). Comme pour l'activité spontanée, les résultats concernant l'impact du CMS sur l'activité locomotrice observée dans l'OFT sont contradictoires : certaines études constatent une réduction (Chen et al., 2022; Joodaki et al., 2024; Nowacka-Chmielewska et al., 2017; Qiao et al., 2020; Yang, 2015), d'autres une augmentation (Fitzgerald et al., 2019; Strekalova & Steinbusch, 2010) ou une absence de changements (Alqurashi et al., 2022; Mutlu et al., 2012). Certains travaux rapportent que l'administration chronique d'antidépresseurs classiques peut rétablir à un niveau normal une activité amoindrie (Joodaki et al., 2024). En revanche, une étude rapporte qu'une administration de Ket n'a pas eu d'effet sur l'activité locomotrice en condition contrôle ou sur l'hyperactivité locomotrice induite par le CMS (Fitzgerald et al., 2019), bien que l'effet ait été évalué 8 jours après l'injection. Lorsque des différences liées au sexe sont observées, elles concernent une moindre activité locomotrice chez les femelles suite au CMS (Dalla et al., 2011; Franceschelli et al., 2014).

Anxiété

L'anxiété est l'un des paramètres fréquemment évalués dans la modélisation de la dépression (Becker et al., 2021). Si elle ne figure pas directement dans les symptômes de la pathologie selon le DSM-V, elle est incluse parmi les ses caractéristiques. De plus, près de deux tiers des patients dépressifs présentent une anxiété comorbidités chez deux-tiers des patients (Kessler et al., 2003). Incluse dans certaines échelles cliniques évaluant la dépression comme la HAM-D, elle est corrélée avec la sévérité de la pathologie, et a été décrite comme une caractéristique centrale de celle-ci (Vaccarino et al., 2008). Chez le rongeur, le test d'anxiété le plus utilisé est le test du labyrinthe en

croix surélevé (elevated plus maze test, EPM) (Danduga & Kola, 2024). L'EPM s'appuie sur leur aversion naturelle pour les espaces exposés, ouverts sur le vide, et lumineux. Dans ce test, l'animal est placé au centre d'un labyrinthe en forme de croix surélevé par rapport au sol, avec deux bras « ouverts » (BO) exposés, et deux bras « fermés » par des parois. Une exploration amoindrie des bras ouverts suggère une anxiété accrue. L'EPM présente une forte validité prédictive (Danduga & Kola, 2024; Walf & Frye, 2007). L'exposition au CMS conduit de manière consistante à une réduction de l'exploration des bras ouverts (Strekalova et al., 2022; Willner, 2017b), qui est rétablie par traitement aigu ou chronique aux antidépresseurs classiques (Kurt et al., 2000; Willner, 2017b). Si une anxiété accrue suite au CMS a été rapportée chez les mâles dans une étude (Xia et al., 2023), la majorité d'entre elles ne rapporte pas de différences liées au sexe (Aranđelović et al., 2024; Crockett et al., 2025; Medina-Saldivar et al., 2024).

Réaction face à une situation stressante sans échappatoire

Le test de la nage forcée (forced swim test, FST), évalue le comportement de rongeurs face à une situation stressante sans échappatoire. Dans le FST, le rongeur est placé dans un cylindre rempli d'eau et la durée de différents comportements est mesurée, comprenant l'activité, incluant la nage, la plongée et les tentatives d'escalade hors du cylindre, ou l'absence d'activité ou immobilité i.e. un comportement immobile de flottaison à la surface (Slattery & Cryan, 2012). L'immobilité a souvent été, et est toujours, décrite par certains auteurs comme reflétant un « désespoir comportemental », ou un état « dépressif » en soi ; cependant, cette interprétation est de plus en plus remise en question et l'immobilité est, depuis quelques années, davantage vue comme stratégie de « coping », ou d'adaptation, engagée face à l'inévitabilité de la situation (Commons et al., 2017; de Kloet & Molendijk, 2016). En effet, l'engagement de stratégies inadaptées représente une perturbation cognitivo-comportementale d'importance notable dans la dépression (**cf. p. 4**). De plus, ce test peut présenter un intérêt dans l'étude des traitements antidépresseurs (Castagné et al., 2011), ayant été initialement développé comme test de criblage de ces composés (Porsolt et al., 1977). Cette validité ne concerne que le potentiel thérapeutique des antidépresseurs plutôt que la dynamique temporelle de leur efficacité, car un traitement antidépresseur aigu suffit à réduire l'immobilité dans le FST, tandis que l'effet thérapeutique chez l'humain nécessite un traitement chronique (de Kloet & Molendijk, 2016). Les effets induits par le CMS dans le FST sont consensuels, avec un accroissement systématique de l'immobilité (Strekalova et al., 2022; Weston et al., 2021; Willner, 2005, 2017b). Les antidépresseurs classiques (Willner, 2005) mais également la Ket (Weston et al., 2021) réduisent l'immobilité. Des différences contradictoires liées au sexe ont été relevées suite au CMS, avec les

femelles plus immobiles que les mâles (Hodes, 2018), ou les mâles plus immobiles que les femelles (Franceschelli et al., 2014).

b) Perturbations physiologiques et neurophysiologiques

Poids

Les perturbations liées au poids, le plus souvent liées à une moindre prise de poids, semblent être un des effets du CMS les plus robustes car rapportés dans plus de quatre études sur cinq (Markov & Novosadova, 2022; Moreira et al., 2016; Sequeira-Cordero et al., 2019; Willner, 1997b). Elles peuvent être rétablies par un traitement antidépresseur classique chronique (Xing et al., 2013). Ces perturbation apparaissent indépendantes de la vulnérabilité comportementale, puisqu'elles sont observées à la fois dans des populations classées vulnérables ou non (Lamanna et al., 2022; Wiborg, 2013). Les perturbations liées au poids apparaissent également sexe-spécifiques, n'affectant que les mâles ou de façon plus marquée les mâles que les femelles (Hodes, 2018; Liang et al., 2008; Murison & Hansen, 2001; Sterrenburg et al., 2011; Xing et al., 2013).

Rythme veille-sommeil

Si l'emploi de modèles animaux pour l'étude du rythme veille-sommeil est courant, l'impact du CMS sur ce dernier demeure paradoxalement très peu exploré (Olini et al., 2017). Néanmoins, plusieurs perturbations du rythme veille-sommeil sont constatées suite à l'exposition à un protocole de CMS. Elles concernent une fragmentation accrue du sommeil, c'est-à-dire un plus grand nombre d'épisodes dans les trois états de vigilance et des épisodes plus courts (Grønli et al., 2012), une plus grande latence d'endormissement (Zhang et al., 2023) et une durée réduite de sommeil NREM (Zhang et al., 2023), parfois seulement en phase nocturne (Dalanon et al., 2021). De plus, le CMS réduit la latence d'entrée en sommeil REM (Cheeta et al., 1997) et entraîne une durée de sommeil REM accrue (Cheeta et al., 1997; Grønli et al., 2004; Zhang et al., 2023), bien qu'une réduction ait également été observée (Grønli et al., 2012). Ces études n'ont pas examiné les effets d'antidépresseurs, mais une autre a montré qu'un traitement antidépresseur chronique permet de normaliser les conséquences délétères d'un stress social (Matsuda et al., 2021).

Potentialisation et dépression à long terme des synapses

Les perturbations dans la dépression incluent des anomalies dans la plasticité synaptique, notamment des mécanismes de LTP et de LTD (**cf. p.11**). S'il est possible de les étudier dans diverses régions cérébrales, leur exploration est surtout effectuée dans l'hippocampe. Il s'agit non seulement de la structure dans laquelle ce mécanisme biologique a été mis en évidence (Bliss & Lomo, 1973),

mais comparée à d'autres régions, sa circuiterie en une boucle trisynaptique (**Figure 5**) est « idéale pour stimuler les axones présynaptiques (voie des collatérales de Schaffer depuis CA3) et enregistrer l'activité des neurones postsynaptiques innervés (région CA1) » (Brown et al., 2022), facilitant donc l'étude de ces mécanismes.

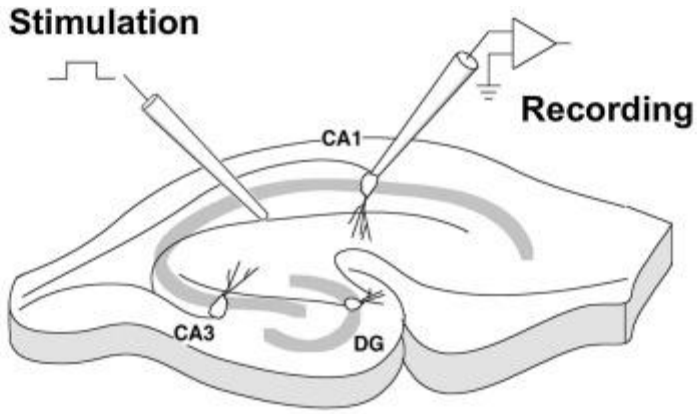

Figure 5 : Disposition d'électrodes de stimulation et d'enregistrement pour étude de la plasticité hippocampique dans la région CA3-CA1 au sein de la boucle trisynaptique. CA : *cornu ammonis*, DG : gyrus denté (dentate gyrus).

D'importantes perturbations de la plasticité hippocampique ont été décrites suite au CMS : un affaiblissement de la LTP (Alfarez et al., 2003; Shang et al., 2017; Yang et al., 2019) et un renforcement de la LTD (Holderbach et al., 2007), même si le blocage de cette dernière a également été constaté (Shang et al., 2017). Ces perturbations peuvent être contrecarrées par traitement chronique d'antidépresseurs classiques (Holderbach et al., 2007), par traitement aigu de Ket (Yang, et al., 2018) ou par d'autres approches comme la TMS (Yang et al., 2019). Une LTP amoindrie a également été constatée dans le mPFC suite au CMS (Zhang et al., 2011).

Communication inter-structurelle

La dépression est caractérisée par une communication anormale entre des structures corticales et sous corticales (**cf. p. 9**). Cette communication peut être étudiée par analyses électrophysiologiques *in vivo*, permettant de mesurer les oscillations générées au sein de structures d'intérêt. Ces oscillations, générées par l'activité synchrone d'une population neuronale, peuvent être catégorisées selon différentes bandes de fréquence associées à différents processus comportementaux (Adamantidis et al., 2019). Elles incluent les bandes delta (δ ; 1-4 Hz) associée au sommeil NREM, (Hernan et al., 2017), thêta (θ ; 4-12 Hz), associée au sommeil REM et à l'exploration d'un environnement (Hernan et al., 2017), alpha (α ; 8-14 Hz) et bêta (β ; 12-32 Hz) associée aux processus attentionnels (Phillips & Uhlhaas, 2015), et gamma (γ ; 25–125 Hz) associée aux fonctions exécutives et au traitement de

l'information et impliquée dans la consolidation mnésique durant le sommeil NREM (Hernan et al., 2017; Weber et al., 2021). Les oscillations à haute fréquence (high frequency oscillations, HFO ; 130–180 Hz) constituent une autre bande d'intérêt puisqu'elles peuvent être induites par la ket (Hunt et al., 2011; Wróbel et al., 2024).

La théorie de la communication à travers la cohérence stipule que des structures présentant une synchronie dans une certaine bande de fréquence sont fonctionnellement connectées et communiquent entre elles (Fries, 2015). Les résultats d'études explorant l'effet du CMS sur la communication intracérébrale indiquent des altérations du niveau de cohérence entre différentes structures : une augmentation dans les bandes δ et θ entre le mPFC [région prélimbique (PRL)] et le noyau accumbens (Akhoondian et al., 2023) ; une baisse dans les bandes δ, θ, β, et γ entre le mPFC et le vHPC (Oliveira et al., 2013); une baisse dans la bande θ entre le mPFC et le noyau dorsal latéral du thalamus (Zheng et al., 2015) ; une baisse dans les bandes δ, θ, et α entre le PFC et le dHPC (Mirbolouk et al., 2023) ; une baisse dans la bande θ lors de l'exploration d'un nouvel environnement entre le mPFC et la BLA (Cao et al., 2023; Qi et al., 2020).

Expression transcriptomique

L'exposition au CMS entraîne d'importantes perturbations transcriptomiques d'une multitude de gènes, dont l'expression est accrue ou amoindrie, au sein de différentes régions du cerveau, en particulier dans le mPFC, la BLA et l'HPC (Gururajan, 2022; Khan et al., 2020; Li et al., 2022). Si ces gènes interviennent dans un grand nombre de mécanismes, ils apparaissent particulièrement liés aux phénomènes de plasticité synaptique (Gururajan, 2022; Li et al., 2022), à la réponse au stress et au fonctionnement de l'axe HPA (Terenina et al., 2019), à la transmission glutamatergique (Gururajan, 2022; Newton et al., 2020), GABAergique ou monoaminergique (Ma et al., 2016) ou aux mécanismes inflammatoires (Zhang et al., 2022). L'impact de l'exposition au stress chronique sur l'expression de ces gènes, comme par exemple *Fkbp5*, impliqué dans la réponse au stress, est particulièrement région-spécifique, présentant notamment des différences entre l'HPC, le PFC et l'AMY (Flati et al., 2020); cela souligne l'intérêt d'étudier l'impact du stress sur différentes régions en parallèle (Chen et al., 2022; McEwen et al., 2015; Valdez et al., 2023).

C) Modélisation de la dépression résistante au traitement : le rat Wistar-Kyoto exposé à un protocole de stress léger chronique

Le rat Wistar-Kyoto (WKY) est issu de croisements sélectifs du rat Wistar dans un projet de génération d'un modèle d'hypertension spontanée (Spontaneously hypertensive rat, SHR) (Okamoto & Aoki, 1963), constituant l'équivalent non hypertendu du rat SHR et servant de témoin pour ce dernier. Par la suite, une sensibilité et une vulnérabilité accrue au stress ont été constatées au sein de cette lignée, ainsi que des caractéristiques comportementales évoquant un phénotype dépressif (Redei et al., 2023). La distribution de la lignée WKY à différents fournisseurs a débuté dans les années 70, avant qu'une forte consanguinité ne soit atteinte par croisements sélectifs. Ainsi, des différences phénotypiques et génotypiques marquées existent entre les WKY de différents fournisseurs (Paré & Kluczynski, 1997; Zhang-James et al., 2013). Constatant ces différences et une variabilité entre animaux issus d'une même colonie, il a même pu être démontré que des « sous-lignées » de WKY avec des caractéristiques comportementales différentes pouvaient être obtenues par croisements sélectifs d'individus d'un même fournisseur (Will et al., 2003). Sur le plan physiologique, diverses perturbations ont été décrites. Elles concernent les systèmes glutamatergique et monoaminergique (Millard et al., 2020), la plasticité synaptique intra-hippocampique et entre le PFC et l'AMY (Aleksandrova et al., 2019), et l'axe HPA (Millard et al., 2020; Redei et al., 2023), bien que pour ce dernier point, les observations sur la nature de ce dysfonctionnement soient contradictoires (Armario et al., 2023). Enfin, le rat WKY présente une moins grande réponse aux antidépresseurs classiques (Willner & Belzung, 2015).

Des différences entre rats WKY mâles et femelles ont également été rapportées. Les perturbations comportementales spontanées et en réponse au stress chronique semblent plus marquées chez les femelles (Paré et al., 1999; Paré & Redei, 1993) exceptées pour l'anhédonie qui n'a été observée que chez les mâles (Burke et al., 2016). L'anxiété se manifesterait de façon différente entre mâles et femelles selon les tests employés (Burke et al., 2016). Une étude employant des « sous-lignées » de WKY suggère que les perturbations comportementales pourraient n'émerger qu'à l'âge adulte chez les femelles (Mehta et al., 2013), et a révélé une expression différentielle de gènes dans l'HPC (Mehta et al., 2013). Des auteurs alertent néanmoins sur le manque important de considération du sexe dans cette lignée de rats (Millard et al., 2020).

Dans l'ensemble, le rat WKY constitue une lignée de fort intérêt préclinique de plus en plus utilisée dans le cadre de la dépression ces 50 dernières années (**Figure 6**), avec au total plus de 600 articles de recherche répertoriés selon une estimation en 2023 (Redei et al., 2023),

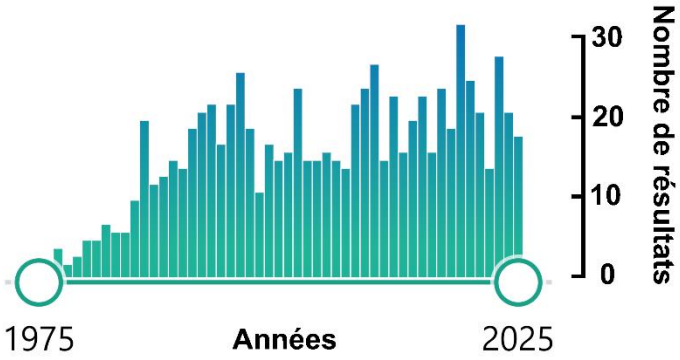

Figure 6 : Graphique des résultats obtenus avec une recherche Pubmed « Wistar-Kyoto Depression » par année.

L'intérêt pour le WKY exposé à un protocole de CMS dans la dépression remonte à 1994, avec une première exploration isolée du modèle (Tejani-Butt et al., 1994). Plus récemment, une étude princeps de Willner et collaborateurs a démontré que les troubles comportementaux induits par le CMS chez le rat WKY ne pouvaient pas être améliorés par des antidépresseurs classiques, tandis que c'était le cas de rats Wistar également soumis au CMS. En revanche, la DBS ou un traitement sous-anesthésique de Ket, approches efficaces dans la TRD, ont permis de le faire (Willner et al., 2019).

Sur le plan comportemental, le modèle du rat WKY-CMS présente une baisse de la préférence au sucrose (Papp et al., 2019, 2020, 2023; Willner et al., 2019), une immobilité accrue dans le FST (Tejani-Butt et al., 1994), une moindre exploration dans l'OFT (Papp et al., 2023; Tejani-Butt et al., 1994; Virijevic et al., 2024), et une anxiété accrue dans l'EPM (Papp et al., 2019; Virijevic et al., 2024; Willner et al., 2019). Sur le plan physiologique, le CMS accroit la corticostérone circulante (Valdez et al., 2023) et ralentit la prise de poids (Willner et al., 2019) des rats WKY. L'expression de divers gènes ou protéines est anormale dans le mPFC ou l'HPC, avec une moindre expression protéique et génique du facteur neurotrophique BDNF, de kinases pro-plasticité synaptique, et une expression accrue de cytokines inflammatoires et de kinases pro-inflammatoires (Papp et al., 2019; Valdez et al., 2023; Virijevic et al., 2024). Des perturbations de la neurotransmission noradrénergique ont également été mises en évidence dans le cortex somatosensoriel, l'HPC, l'AMY et l'hypothalamus de rats WKY soumis au CMS (Tejani-Butt et al., 1994).

III) Objectifs de la thèse

L'objectif général de cette thèse est double. Le premier est de mieux caractériser l'impact du CMS chez le rat WKY. Le second est d'évaluer les effets de la Ket, à dose sous-anesthésique, sur certaines des perturbations induites par le CMS. La modalité de traitement, aiguë ou chronique, demeurant une question centrale dans l'utilisation de la Ket comme antidépresseur, une partie des travaux a impliqué la comparaison des effets de ces deux régimes.

La première partie vise à approfondir la caractérisation comportementale du modèle utilisé, notamment en étudiant des paramètres peu souvent explorés dans les modèles précliniques comme le rythme d'activité nycthéméral. Elle concerne également l'étude de potentielles différences liées au sexe, étant donné l'importance de ce facteur sous-représenté dans les modèles animaux de dépression, et le manque de données chez le rat WKY (Millard et al., 2020). Enfin, un dernier objectif est d'étudier la variabilité interindividuelle dans la vulnérabilité à un protocole de CMS chez le rat WKY (**cf. p. 28**), chez lequel une variabilité génétique subsiste. Cette vulnérabilité est évaluée selon deux paramètres : la stratégie d'adaptation employée face à l'inévitabilité de la situation dans le FST, et l'anxiété, évaluée dans l'EPM. Ces deux paramètres sont effectivement d'un grand intérêt dans l'étude de la dépression, le premier étant décrit comme reflétant un état pathologique (**cf. p. 5**), et le second étant décrit comme une caractéristique centrale de la pathologie.

Une deuxième partie, réalisée chez des rats CMS-vulnérables mâles, vise à évaluer les conséquences du CMS sur la plasticité synaptique (LTP et LTD) dans l'HPC. Cette étude est réalisée par technique d'électrophysiologie *ex vivo* au niveau de la synapse CA3-CA1. Les capacités de la Ket à restaurer d'éventuels déficits de ces mécanismes de plasticité, selon une modalité de traitement aiguë ou chronique, sont également évaluées.

Une troisième partie de la thèse, réalisée chez des rats CMS-vulnérables mâles, concerne : **i**) l'étude de l'impact du CMS sur l'organisation du rythme veille/sommeil et sur l'activité du réseau cérébral constitué par trois régions fondamentales dans la physiopathologie de la dépression, i.e. le mPFC (région PRL), l'HPC (partie dorsale, région CA1) et la BLA, et **ii**) l'évaluation des conséquences des traitements aigu et chronique sur les potentielles perturbations observées. Pour mener à bien cette troisième partie, des enregistrements électrophysiologiques de 24 heures sont réalisés *in vivo*, chez des animaux caractérisés comme vulnérables au CMS, à trois reprises (avant traitement puis suite au traitement aigu et suite au traitement chronique).

La quatrième et dernière partie de la thèse vise à évaluer, au sein des structures cérébrales d'intérêt (PFC, HPCd, BLA), l'impact du CMS sur l'expression de sept gènes codant pour des protéines impliquées dans des mécanismes dysfonctionnels dans la dépression et/ou les effet de la Ket.

Nom du *gène* (protéine)	Expression génique	Expression protéique	Sources
Bdnf **(BDNF)**	↓	↓	(Abbasi Mehmandost Sofla et al., 2025; Luoni et al., 2015; Osacka et al., 2021; Willner, 2017b)
Fkbp5 **(FKPB5)**	↑	↑	(Guidotti et al., 2013; Wei et al., 2016; Xing et al., 2015)
Gr/Nr3c1 **(GR)**	↑↓	↓	(Guidotti et al., 2013; Wei et al., 2016; Xing et al., 2015)
Gria1/Glua1 **(GRIA1/GLUA1)**	↓	↓	(Duric et al., 2013; He et al., 2023)
Grin2b/Glun2b **(GRIN2B/GLUN2B)**	↓	**?**	(Elhussiny et al., 2021)
Mao$_a$ **(MAO$_A$)**	↑	↑	(Geng et al., 2021; Harris et al., 2015; Lin et al., 2019; Stefanovic et al., 2016)
Psd95/Dlg4 **(PSD95)**	↓	↓	(Lu et al., 2019; Willner, 2017b; Zhuang et al., 2016)

Tableau 4 : Expression de gènes (et protéines associées) étudiés dans la quatrième partie de la thèse suite au CMS.

L'expression de ces gènes est perturbée par le CMS (**Tableau 4**). Ils sont impliqués dans : **i**) la plasticité synaptique : *Bdnf* au rôle étiologique central dans la dépression et dans les effets antidépresseurs de la Ket, et *Psd95/Dlg4* codant pour la protéine PSD-95, impliquée dans le maintien des synapses ; **ii**) la neurotransmission glutamatergique : *Gria1/Glua1*, codant pour une sous-unité des AMPA-R fondamentale pour la plasticité synaptique, car permettant leur acheminement à la membrane (Diering & Huganir, 2018), et *Grin2b/Glun2b* codant pour une sous-unité des NMDA-R impliquée dans un mécanisme antidépresseur supposé de la Ket (Miller et al., 2014) ; **iii**) la réponse au stress : *Nr3c1* et *Fkbp5*, codant pour les GR et la protéine co-chaperone des GR, respectivement, impliqués dans les rétrocontrôles de l'axe HPA et dont des polymorphismes sont mis en cause dans la vulnérabilité à la dépression (**cf. p. 15**) ; **iv**) la régulation des monoamines : *Mao$_a$* codant pour la

MAO_A. Cette étude est la plus complète de ce projet car elle en aborde tous les aspects clés, c'est-à-dire le sexe (utilisation d'animaux femelles et mâles), la vulnérabilité face au CMS (utilisation d'animaux vulnérables et non vulnérables), et les effets de la Ket (uniquement en administration aiguë).

MÉTHODOLOGIE GÉNÉRALE

Bien que le matériel et les méthodes utilisés dans chaque étude soient décrits au sein des articles correspondants, cette section du manuscrit vise à en offrir une présentation plus détaillée et didactique. J'y propose notamment des illustrations photographiques et schématiques des dispositifs expérimentaux et des procédures mises en œuvre, qui me semblent essentielles pour faciliter la compréhension et la reproductibilité des présents travaux. Par ailleurs, j'ai jugé nécessaire d'inclure une partie théorique consacrée à l'origine biologique des signaux électrophysiologiques enregistrés lors des études in vivo et ex vivo. Ces développements permettront au lecteur de mieux appréhender les fondements physiologiques des mesures réalisées et l'interprétation des résultats obtenus.

I) Généralités

A) Sujets et conditions d'hébergement

Ce travail a été mené sur des rats WKY mâles et femelles provenant du centre d'élevage Charles River (Sulzfeld, Allemagne). Cinq cohortes de rats ont été utilisées, pour un total de 294 individus. La première cohorte, comportant 30 mâles et 40 femelles, a été consacrée à l'étude comportementale, avec des animaux sélectionnés pour les études d'électrophysiologie *ex vivo* et *in vivo*. La seconde cohorte, comportant 60 mâles, a permis d'agrandir les effectifs pour les études d'électrophysiologie *ex vivo* et *in vivo*. La troisième cohorte, comprenant 8 mâles, a servi d'animaux témoins pour les études d'électrophysiologie *in vivo*. Enfin, la quatrième (40 mâles et 38 femelles) et la cinquième (40 femelles et 38 mâles) cohortes ont été consacrées à l'étude transcriptomique, bien que des animaux de la cinquième aient également été sélectionnés pour compléter l'effectif de l'étude *ex vivo*.

Les animaux sont arrivés au laboratoire à l'âge de 4 à 5 semaines et ont été placés par groupes de cinq animaux du même sexe dans des cages Makrolon type IV (545 × 330 × 200 mm) contenant une litière composée de sciure de bois. Après une semaine d'acclimatation aux conditions du laboratoire, chaque animal a été réparti dans l'un des deux groupes expérimentaux : le groupe témoin non stressé (NS) et le groupe CMS, à l'exception de la cohorte 3 (témoins uniquement, reçus à un âge correspondant à celui des animaux CMS à la fin du protocole). Cette répartition a été réalisée de façon aléatoire puis ajustée si nécessaire pour garantir une équivalence de la masse corporelle entre les groupes.

Pour chaque cohorte, les animaux assignés au groupe témoin ont été hébergés par paires du même sexe dans des cages Makrolon type IV dans une salle avec une température ambiante maintenue à 23 ± 1 °C, et un éclairage selon un cycle lumière/obscurité de 12h/12h (allumage à 07h00) (**Figure 7A**). Les animaux assignés au groupe CMS ont été hébergés individuellement dans des cages Makrolon type III (365 x 220 x 180 mm) dans une salle d'hébergement différente, non adjacente à celle des animaux du groupe témoin (**Figure 7B**), avec des conditions similaires, sauf pour le cycle lumière/obscurité parfois modifié lors du protocole de CMS (**cf. p. 43**). Lorsque la cohorte incluait les deux sexes, les animaux mâles et femelles d'une même salle étaient séparés d'au moins 2 mètres.

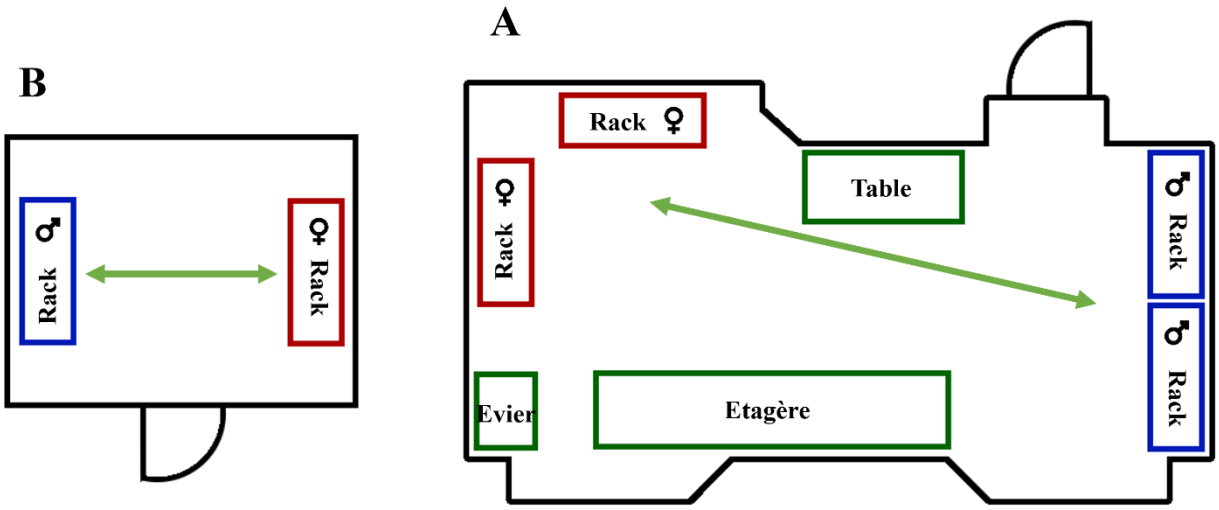

Figure 7 : Plan des salles d'hébergement. A : salle d'hébergement des rats témoin. Distance entre les racks mâles et femelles (flèche verte) : 2 m ; B : salle d'hébergement des rats soumis au protocole de CMS. Distance entre les racks mâles et femelles (flèche verte) : 4,5 m.

Les rats ont eu un accès *ad libitum* à la nourriture (référence n°1324, Altromin International, Allemagne) et à l'eau, mise à disposition *via* un biberon en plastique pour chaque cage, sauf lors de phases de privation incluses dans le cadre du protocole de CMS pour les rats y étant exposés.

Toutes les procédures expérimentales ont été réalisées en conformité avec la réglementation en vigueur relative à la protection des animaux utilisés à des fins scientifiques (directive 2010/63/UE transposée en droit national), et ont été approuvées par le comité d'éthique local (CREMEAS), ainsi que par le Ministère français de l'Éducation nationale, de l'Enseignement supérieur et de la Recherche avec les numéros d'autorisation APAFIS #25270 et APAFIS #43839.

B) Déroulé expérimental global

Les animaux des différentes cohortes ont suivi un déroulé expérimental similaire sur 11 semaines (**Figure 8**). Après réception et acclimatation aux conditions du laboratoire (semaine 1), les animaux ont été répartis dans les groupes expérimentaux (semaine 2). Les semaines 3 à 5 ont été consacrées aux tests comportementaux pré-CMS, incluant l'exposition au saccharose pour le SPT, la mesure de l'activité locomotrice spontanée et une première session de SPT pour obtenir une première valeur basale. Le protocole de CMS a ensuite été appliqué de la semaine 6 à la semaine 9 durant lesquelles la préférence au saccharose et la masse des animaux ont été évaluées une fois par semaine (le lundi et le mercredi, respectivement). Les évaluations comportementales post-CMS, incluant le FST et l'EPM, permettant le phénotypage comportemental des animaux en fonction de la vulnérabilité

ou non au CMS, se sont déroulées pendant les semaines 10 et 11. Hormis pour l'étude comportementale, les animaux ont également été exposés à deux semaines additionnelles de CMS avant l'évaluation des divers paramètres étudiés. Il est à noter que le CMS a débuté à la semaine 5 pour la cohorte 1, la mise en place d'une deuxième séance de SPT afin d'avoir des mesures basales plus stables n'ayant pas encore été introduite. La cohorte 3, constituée uniquement de témoins non stressés destinés à subir les enregistrement électrophysiologiques, a été reçue à un âge équivalent aux animaux post-CMS, et n'a pas été exposée aux tests comportementaux, certains d'entre eux comme le FST, pouvant générer un stress important (Bernal-Morales et al., 2018; de Kloet & Molendijk, 2016) et donc biaiser les données électrophysiologiques.

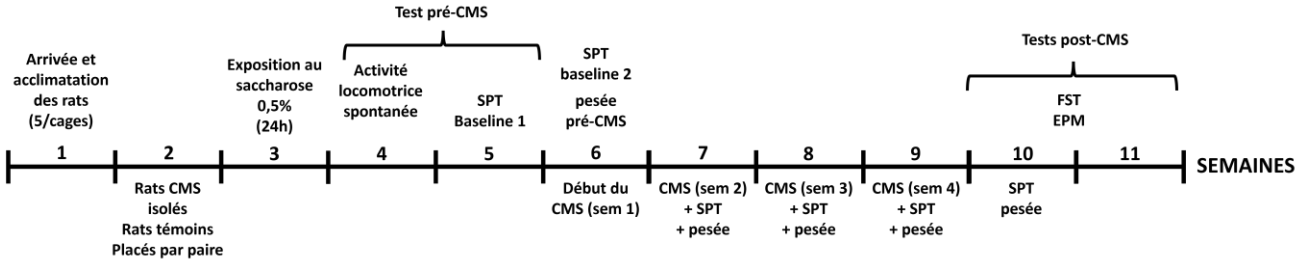

Figure 8 : Déroulé d'une cohorte « type ».

C) Protocole de stress léger chronique

Le protocole de CMS a consisté en l'exposition des rats à dix différents stresseurs de nature physique, physiologique ou sociale, organisée en schéma hebdomadaire.

Le blocage d'accès à la litière a été réalisé le mardi de 12:00 à 17:00, par le dépôt sur la litière d'une plaque en acier (dimensions : 33 × 20 cm).

L'exposition à la lumière stroboscopique a été réalisée lors de la phase d'obscurité du mardi au mercredi, de 20:00 à 01:00, puis de 02:00 à 06:00, *via* des stroboscopes (référence 83473285, Leroy Merlin, France), à une fréquence de 2 Hz (soit 120 flashs par minute).

La privation hydrique et/ou alimentaire (**Figure 9A**) a été réalisée mercredi, jeudi et dimanche de 09:00 à 17:00, pendant laquelle le biberon d'eau et/ou la nourriture ont été temporairement retirés des cages.

La restriction de l'espace disponible dans la cage (**Figure 9B**) a été réalisée du mercredi au jeudi et du samedi au dimanche, de 18:00 à 9:00, par l'ajout d'un couvercle de cage pour souris dans

la cage d'hébergement, réduisant l'espace disponible à la zone située sous la mangeoire, tout en maintenant l'accès à l'eau et à la nourriture.

Les séances d'intrusion dans la cage d'hébergement (**Figure 9C**) ont été réalisées du jeudi au vendredi de 18:00 à 09:00. Chaque rat CMS a été alternativement assigné au rôle d'intrus ou de résident d'une semaine à l'autre. Les paires formées ont été différentes à chaque séance, de façon à éviter la familiarisation des animaux, qui réduit le stress généré dans cette situation (Parmigiani & Brain, 1983). À la fin de la séance, le résident a été transféré dans la cage de l'intrus, tandis que l'intrus est resté dans la cage du résident.

La modification du cycle lumière/obscurité a été réalisée durant la phase d'obscurité habituelle (19:00–07:00) du jeudi au vendredi. Les lumières de la salle d'hébergement des animaux CMS ont été allumées et éteintes selon la séquence suivante : de 19:00 à 21:00, extinction ; de 21:00 à 23:00, allumage ; de 23:00 à 01:00, extinction; de 01:00 à 03:00, allumage; de 03:00 à 05:00, extinction; de 05:00 à 06:00 allumage ; de 06:00 à 07:00 extinction, puis rallumage des lumières à 07:00 et retour au cycle standard 12 heures/12 heures.

La diffusion de bruit blanc de forte intensité a été réalisé le samedi de 09h00 à 17h00, à l'aide d'un lecteur MP3 connecté à une enceinte audio. L'intensité sonore mesurée à la sortie de l'enceinte a été de 91 dB et d'environ 74 ± 1 dB au niveau des différents racks, tandis que le niveau sonore basal de la pièce a été d'environ 47 ± 1 dB.

Le détrempage de la litière (**Figure 9D**) a été réalisée du dimanche au lundi de 18h00 à 09h00, par le versement d'environ 900 mL d'eau dans la cage afin d'imbiber la sciure.

Ce protocole a été appliqué pendant quatre semaines consécutives, avec un changement des stresseurs le matin et le soir (**Tableau 5**).

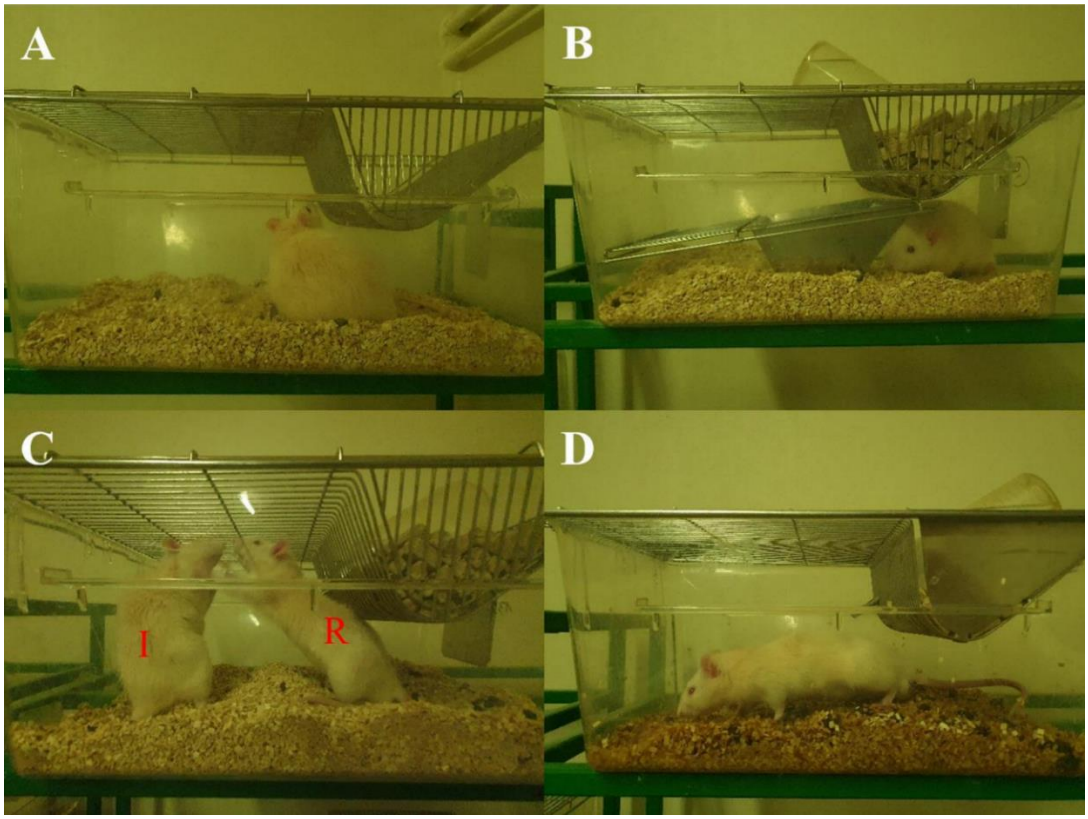

Figure 9 : Photographies d'illustrations de certains stresseurs. A : privation d'eau et de nourriture. B : restriction d'espace dans la cage. C : séance d'intrusion ; exemple d'un rat résident (R) confrontant un rat intrus (I). D : détrempage de la litière.

Jour de la semaine	Matin	Après-midi/soir
Lundi	08:00 Changement des cages	
Mardi	12:00 blocage d'accès à la litière	20:00 Lumière stroboscopique
Mercredi	09:00 Privation de nourriture	18:00 Restriction d'espace dans la cage
Jeudi	09:00 Privation hydrique	18:00 Séance d'intrusion
Vendredi	09:00 Retour en hébergement individuel	21:00 – 07:00 Modification du cycle lumière/obscurité
Samedi	09:00 Bruit blanc de forte intensité	18:00 Restriction d'espace dans la cage
Dimanche	09:00 Privation hydrique et de nourriture	18:00 Détrempage de la litière

Tableau 5 : Organisation du protocole de CMS.

D) Tests comportementaux communs aux études

Les tests comportementaux ont été réalisés entre 9h00 et 13h00, période du rythme circadien où la concentration basale de corticostérone est stable et minimale (Atkinson & Waddell, 1997), excepté pour le SPT (voir ci-dessous) et la mesure de l'activité locomotrice spontanée, réalisés sur une période 24 heures. Étant donné le nombre élevé d'animaux, il n'a pas été possible d'évaluer tous les sujets les mêmes jours dans les différents tests, à l'exception du SPT. Les animaux ont donc été répartis aléatoirement en sous-groupes, de 14 à 26 individus, comprenant chacun une proportion équivalente d'animaux des groupes CMS et témoin. Les sous-groupes ont ensuite été équilibrés de manière à assurer une équivalence de la masse corporelle entre les groupes.

1) Test de préférence au saccharose

La plupart des études utilisant le SPT emploient des solutions avec une concentration de saccharose de 1 ou 2 % (Primo et al., 2023). La concentration optimale est considérée comme la concentration minimale permettant d'observer une préférence ; n'existant pas de valeur consensuelle pour celle-ci, il est recommandé de la déterminer en amont de l'étude (Markov, 2022). Les résultats d'études antécédentes réalisées au laboratoire ont permis de sélectionner la concentration de 0,5 %. La solution a été préparée à partir de saccharose en poudre (Erstein, France) et d'eau, puis versée dans chaque biberon à raison de 200 mL. Un biberon identique ne contenant que de l'eau a également été préparé (**Figure 10**). Durant la troisième semaine du protocole (**Figure 8**), une première mise à disposition a été réalisée afin de familiariser les animaux avec la présence des deux biberons, ainsi qu'à la solution sucrée pendant 24 heures. Des séances hebdomadaires de SPT ont été réalisées chaque lundi à partir de la cinquième semaine, les deux premières permettant d'obtenir des mesures basales de la préférence avant l'application du protocole de CMS (excepté pour la cohorte 1, n'ayant compris qu'une seule séance de mesure basale). La position des deux biberons a été alternée d'une semaine sur l'autre afin d'éviter des biais de consommation dus à leur emplacement. La pesée de chaque biberon, avec une balance de précision, avant et après chacune des séances a permis de déterminer la masse de solution de saccharose consommée, ainsi que la masse d'eau consommée. Pour estimer la consommation individuelle des animaux témoins, hébergés par paires, la valeur de chaque biberon de la cage a été divisée par deux. Enfin, un index de préférence a été calculé par la formule :

$$index\ de\ préférence = \frac{masse\ consommée\ (saccharose)}{masse\ consommée\ (saccharose) + masse\ consommée\ (eau)} * 100$$

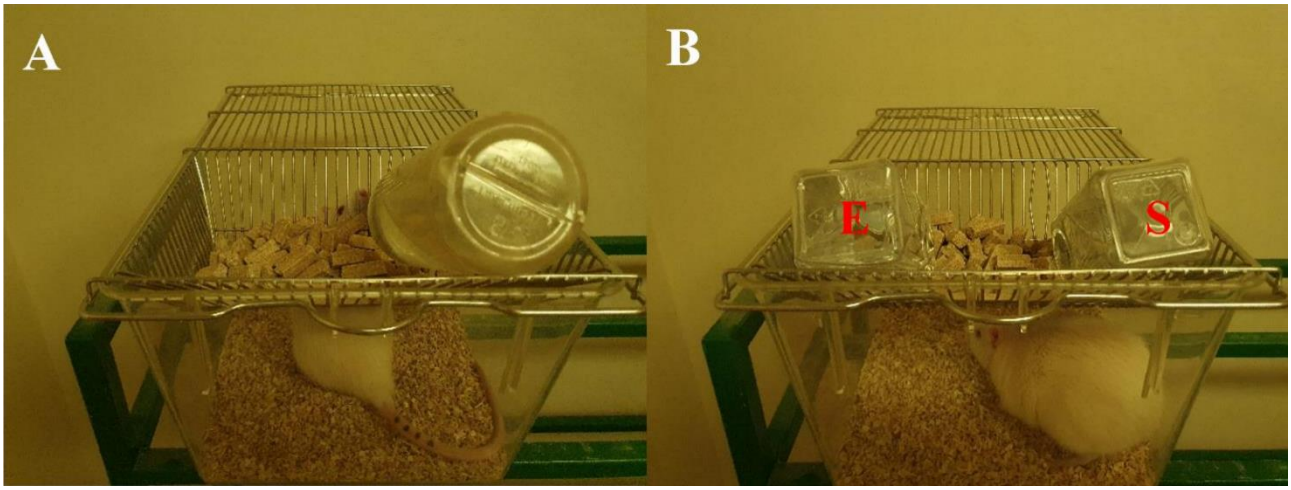

Figure 10 : Photographies du test de préférence au saccharose. A : Disposition du biberon d'eau habituel ; B : Exemple de disposition des biberons d'eau (E) et de solution de saccharose (S) lors du test.

2) Mesure de l'activité locomotrice spontanée

L'activité locomotrice spontanée a été évaluée dans une cage identique à la cage d'hébergement contenant peu de sciure. Chaque cage a été disposée entre 2 paires de cellules photoélectriques placées à 2 cm du sol et à 5 cm de chaque extrémité de la cage. L'interruption consécutive des 2 faisceaux de lumière infrarouge, représentant une traversée de la cage, a été enregistrée par un ordinateur par intervalle d'une heure (**Figure 11**).

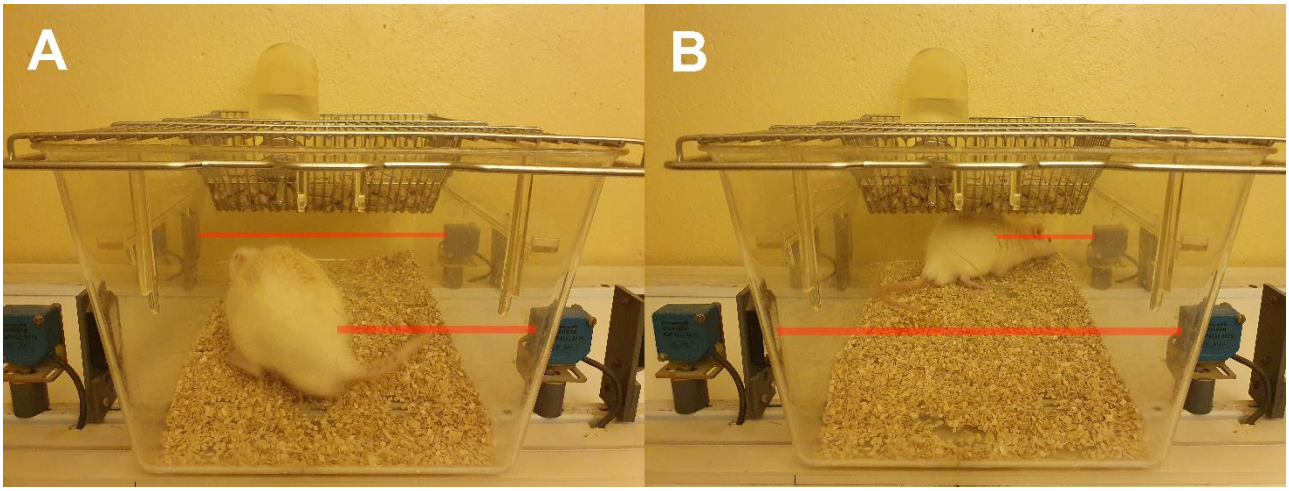

Figure 11 : Principe de la mesure de l'activité locomotrice spontanée. A : interruption d'un rayon infrarouge ; B : Interruption de l'autre rayon infrarouge de la cage, entraînant la comptabilisation d'une traversée de la cage.

Les rats ont été amenés dans la salle de test dans leur cage d'hébergement, puis placés à 11h00 dans les cages de test pour une durée de 24 heures couvrant un nycthémère entier, c'est-à-dire un cycle

lumière/obscurité de 12h/12h (allumage à 07h00) (**Figure 12**). Le nombre de traversées de la cage durant la première heure d'enregistrement, reflétant la réaction locomotrice face au changement d'environnement, a été analysé séparément.

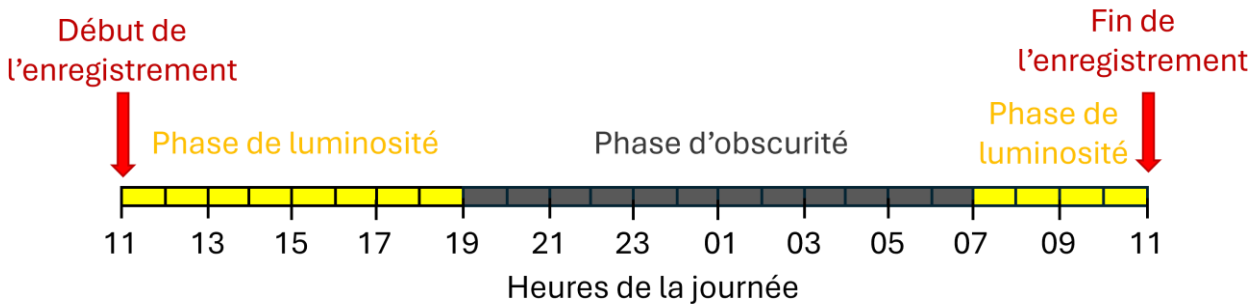

Figure 12 : Déroulé de l'enregistrement d'activité locomotrice spontanée.

3) Test de la nage forcée

Deux cylindres en plexiglas transparent (63 cm de hauteur et 20 ou 30 cm de diamètre) ont été utilisés, ce qui a permis de tester les animaux deux par deux. Une analyse des résultats en fonction du cylindre utilisé a permis de déterminer que la différence de diamètre n'a pas eu d'influence sur les résultats dans le FST. Ces cylindres ont été remplis d'eau à une température contrôlée (24 ± 1 °C) jusqu'à une hauteur de 40 cm afin que les rats, mesurant environ 30 cm du museau au bout de la queue à l'âge adulte, ne puissent pas toucher le fond lorsqu'ils se trouvaient à la surface. Une caméra USB placée face à chaque cylindre, reliée à un ordinateur, a permis, *via* le logiciel OBS Studio, d'enregistrer les séances pour permettre une analyse *a posteriori* du comportement des animaux. Un miroir a été disposé derrière chaque cylindre afin de permettre une observation du comportement des animaux quand ils se trouvaient de dos par rapport à la caméra (**Figure 13**).

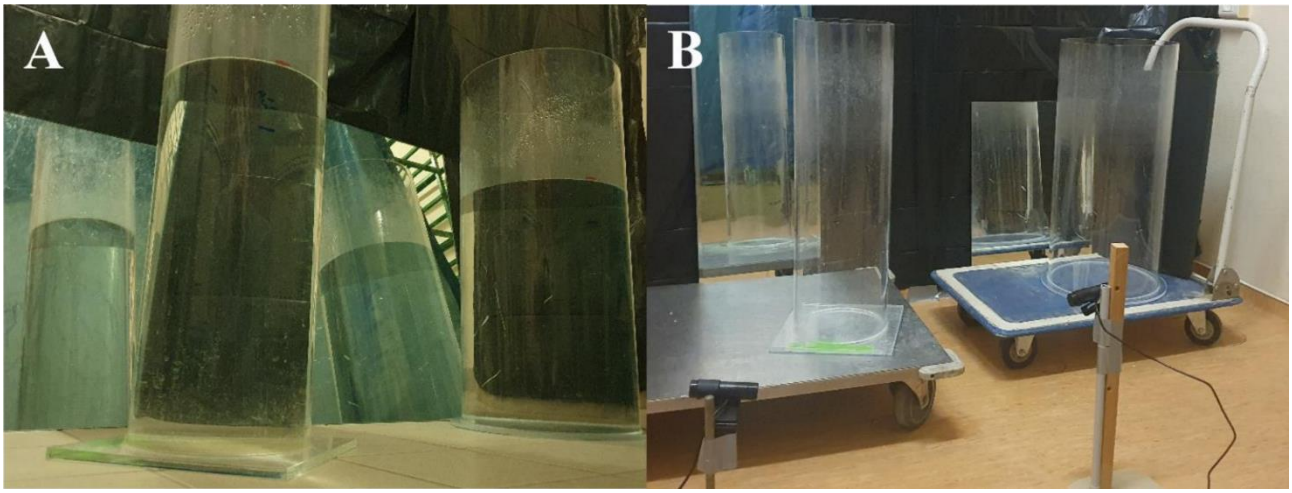

Figure 13 : Photographies d'illustration du FST. A : Cylindres utilisés lors du test et leur réflexion dans un miroir. B : configuration avec caméras.

Le FST, basé sur le protocole de Slattery & Cryan (2012), a été constitué de deux séances (pré-test et test) séparées de 24 heures. Durant le pré-test, les rats ont été placés dans un cylindre pendant 15 minutes. Durant le test, ils ont été replacés dans le même cylindre pendant 6 minutes. Pour chacune de ces séances, les deux rats de la paire testée ont été amenés dans leur cage d'hébergement (soit deux cages pour les rats du groupe CMS, hébergés individuellement, et une pour les rats du groupe témoin, hébergés par paire testée en même temps). Les rats ont été sortis de leur cage et déposés à la surface de l'eau, puis retirés des cylindres à la fin de la séance, et séchés avec une serviette avant d'être replacés dans leur cage. Après chaque passage, l'eau des cylindres a été remplacée suite au rinçage de ces derniers. La durée d'immobilité de l'animal pendant le test, caractérisée par une absence d'activité des quatre membres de l'animal, a été chronométrée par un expérimentateur. Ce temps a été rapporté en pourcentage de la durée totale du test.

4) Test du labyrinthe en croix surélevé

L'EPM a été réalisé dans un dispositif en plexiglas noir surélevé de 73 cm par rapport au sol, constitué de quatre bras perpendiculaires deux à deux, mesurant chacun 50 cm de longueur et 10 cm de largeur, séparés par une zone centrale de 10 cm de côté. Les bras fermés étaient entourés de parois opaques de 40 cm de hauteur. Les bras ouverts avaient des bordures de 1,5 cm de hauteur. Le dispositif a été placé au centre d'une salle avec une lampe halogène dans chacun des coins, générant une intensité lumineuse de 2 lux au niveau des bras fermés, 7,5 lux au niveau des bras ouverts, et 5 lux au centre du labyrinthe (**Figure 14**). Une caméra a été fixée à environ 1 m au-dessus du centre du dispositif.

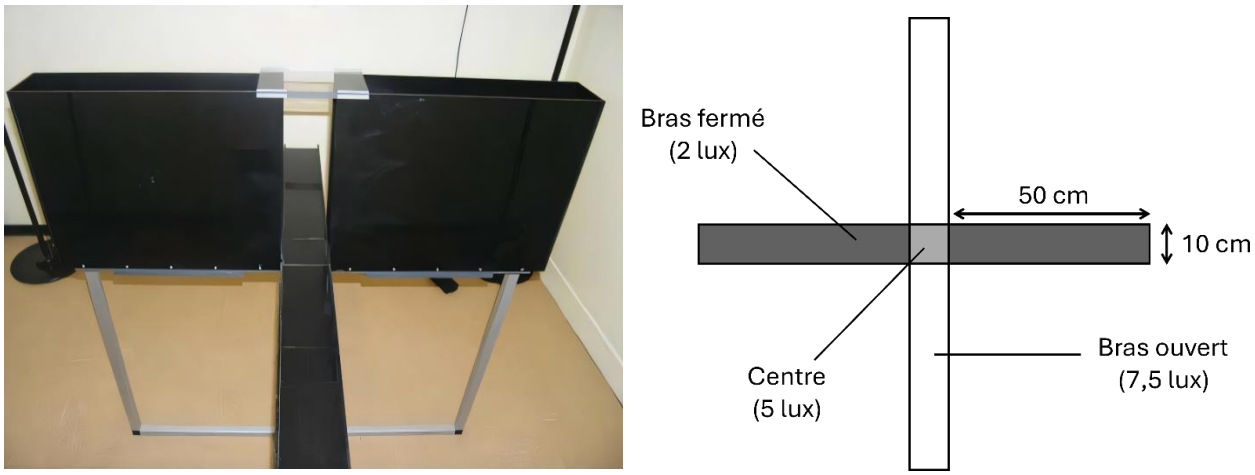

Figure 14 : Labyrinthe en croix surélevé. Photo (gauche) et schéma (droite) du dispositif.

Une heure avant le début du test, les rats ont été amenés dans une salle adjacente à la salle de test. Chaque animal a ensuite été apporté dans la salle d'expérimentation dans une cage de transport vide et propre, et laissé sur une table pendant 5 minutes. L'animal a ensuite été déposé au centre du labyrinthe, museau orienté vers un bras fermé. Les déplacements de l'animal dans chaque section du dispositif, i.e. le centre et les quatre bras, ont été observés en temps réel pendant 5 minutes par l'expérimentateur *via* un moniteur relié à la caméra. Les entrées de l'animal dans chaque section, comptabilisées lorsque les quatre pattes de l'animal avaient franchi la délimitation, et le temps passé par l'animal dans chaque section ont été enregistrés par l'expérimentateur *via* le logiciel Chronos (développé au laboratoire par E. Cosquer). Le dispositif a ensuite été nettoyé à l'eau distillée puis avec une solution d'éthanol à 70 %. Le pourcentage de temps passé dans les bras ouverts, par rapport au temps total passé dans les bras ouverts et fermés a ensuite été déterminé.

E) Identification des animaux vulnérables ou non-vulnérables au CMS

1) Approche

L'identification d'animaux vulnérables ou non au protocole de CMS a été basée sur leur comportement dans l'EPM et le FST (**cf. p. 37**), c'est-à-dire le pourcentage de temps passé dans les bras ouverts de l'EPM et le pourcentage de temps passé en immobilité dans le FST. Pour classifier les données comportementales en groupes vulnérables ou non-vulnérables, la méthode des k-moyenne (k-means) a été employée.

2) Principe des k-moyennes

La méthode des k-moyennes repose sur un algorithme d'apprentissage-machine (machine learning) non supervisé. Elle permet de classifier des données sans catégorisation prédéfinie pour entraîner l'algorithme et ne nécessite pas non plus de définition d'un seuil comme dans la classification par hiérarchisation (Yuan & Yang, 2019). Néanmoins, elle dépend d'un nombre prédéfini de groupes, ou « clusters », qui peut être déterminé arbitrairement, ou qui peut être estimé selon plusieurs méthodes, notamment la méthode du coude (Yuan & Yang, 2019). L'algorithme associe des points, représentant chacun une observation dans un espace multidimensionnel, dispersés selon plusieurs axes, dans le cas présent selon la valeur des pourcentages d'immobilité dans le FST et de temps passé dans les bras ouverts de l'EPM, à un nombre k de centroïdes. L'emplacement des centroïdes est généré aléatoirement, et chaque point est associé au centroïde dont il est le plus proche, formant les clusters. La somme, sur tous les points, des distances euclidiennes au carré au centroïde, appelée la somme des erreurs au carré (sum of squared errors, SSE) est calculée. L'algorithme est répété plusieurs fois, avec une mise à jour des coordonnées des centroïdes en y assignant la valeur moyenne des coordonnées de chaque point du groupe, ainsi qu'un nouveau calcul de la SSE à chaque itération. Ainsi, le centroïde se stabilise graduellement jusqu'à atteindre un point de convergence, où la SSE est minimale et les clusters les plus compacts possible.

4) Méthode du coude

La méthode du coude (elbow method) permet de déterminer la valeur k optimale, c'est-à-dire le nombre de clusters le plus approprié selon la dispersion des données. Elle consiste à réaliser une classification par k-moyennes avec un nombre croissant de clusters sur le même jeu de données. Pour chaque valeur de k, la compacité des clusters, évaluée par la SSE résiduelle, est déterminée. Plus la valeur de k, c'est-à-dire le nombre de centroïdes, est grande, plus la SSE diminue, car la distance d'un point donné au centroïde le plus proche devient plus courte. Enfin, une représentation graphique de la SSE en fonction de la valeur k est réalisée. Un point d'inflexion, appelé le « coude », correspondant à la valeur k après laquelle la diminution de la SSE devient moins prononcée, est considéré comme le nombre de clusters optimal à employer (**Figure 15**). Cette détermination reste cependant visuelle (Shi et al., 2021).

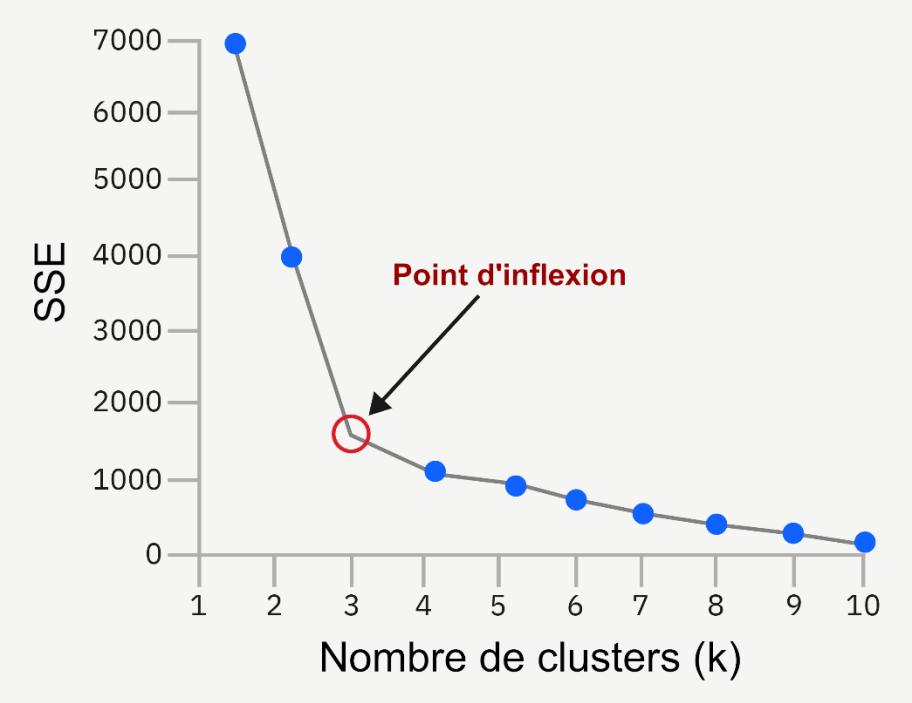

Figure 15 : Principe de la méthode du coude. La valeur SSE diminue en fonction du nombre de clusters. Le point d'inflexion (k=3 sur la figure) représente le nombre optimal de clusters.

La méthode du coude a été appliquée *via* des scripts rédigés en langage MATLAB (version 2022b ; MathWorks, Natick, États-Unis).

F) Traitements pharmacologiques

Les administrations de Ket (Imalgen 1000®, Boehringer Ingelheim, Allemagne) ou de solution de chlorure de sodium à 0,9 % (Aguettant, France), réalisées dans toutes les études sauf l'étude comportementale, ont été effectuées par voie intra-péritonéale (ip) ou sous-cutanée (sc). La Ket a été administrée à raison de 10 mg/kg. Toutes les injections ont été réalisées avec un volume correspondant à 1 mL/kg. Les traitements ont été administrés selon deux modalités : aiguë (administration unique) et chronique (administrations répétées). Pour cette dernière, les animaux ont reçu une injection quotidienne de Ket pendant sept jours consécutifs.

G) Statistiques

Les analyses statistiques ont été réalisées sur le logiciel Statistica (TIBCO Software, États-Unis) et *via* des scripts rédigés en langage MATLAB. Les divers tests statistiques, paramètres et facteurs utilisés sont décrits au sein des différents articles.

II) Etude comportementale

A) Déroulé

. L'étude comportementale a été conduite sur les animaux de la première cohorte, soit 30 mâles et 40 femelles. Elle a consisté en l'évaluation de divers paramètres avant et/ou après l'application du protocole de stress. Une première mesure de l'activité locomotrice spontanée a été réalisée à la semaine 4. Le nombre de traversées, regroupé par intervalles de deux heures, a permis, *via* une analyse de régression non linéaire COSINOR, de déterminer le rythme nycthéméral d'activité locomotrice. Le SPT a été réalisé au long du protocole de CMS et indexé sur la valeur basale obtenue juste avant l'application du protocole. Après l'application du protocole, une seconde mesure de l'activité locomotrice spontanée a été réalisée post-CMS (semaine 10), afin de comparer son évolution. La réaction locomotrice des animaux à un changement d'environnement a été mesurée dans le test du champ ouvert (open field test, OFT ; voir ci-dessous). Enfin, les performances des animaux dans le FST et dans l'EPM ont été évaluées.

B) Description du test du champ ouvert

1) Dispositif

L'OFT a été réalisé dans une enceinte carrée ouverte (70 x 70 x 50 cm, **Figure 16**). L'enceinte a été posée sur une plaque transparente en Plexiglas, sous laquelle a été placée une feuille quadrillée (carreaux de 14 cm de côté) permettant de quantifier les déplacements de l'animal. Cette quantification a été réalisée en temps réel par l'expérimentateur, situé dans une pièce adjacente, grâce à une caméra USB, reliée à un ordinateur, placée au-dessus de l'enceinte.



Figure 16 : Photographie du dispositif pour le test du champ ouvert.

2) Déroulé du test

Les animaux ont été amenés dans une pièce adjacente à la salle de test une heure avant le début de ce dernier. Chaque rat testé a été ensuite transporté dans la salle de test dans sa cage d'hébergement, ou *via* une cage de transport dans le cas des animaux témoins, ces derniers étant hébergés par paire. Il a ensuite été déposé museau face à la paroi dans un des quatre coins du dispositif. La locomotion a été évaluée par le déplacement de l'animal d'une case à l'autre, comptabilisé lorsque les quatre pattes de l'animal avaient passé la ligne séparant deux cases. Les redressements de l'animal sur ses pattes arrière ont également été comptabilisés. Le comportement de l'animal a été évalué pendant 10 minutes. À la fin du test, l'animal a été replacé dans sa cage, et le dispositif nettoyé avec de l'eau distillée puis une solution d'éthanol 70 %.

III) Étude *ex vivo* de la plasticité hippocampique

A) Prélèvement des tranches cérébrales

L'étude de la plasticité hippocampique a été réalisée au sein de quatre groupes d'animaux mâles issus des cohortes 1, 2 et 5. Après deux semaines additionnelles de CMS post-phénotypage, les animaux ont été traités ; deux groupes ont reçu une injection de NaCl (témoin-NaCl, n = 8 ; CMS-vulnérable-NaCl, n = 10) ; les deux autres groupes ont reçu un traitement aigu de Ket (CMS-vulnérable-Ket-1, n = 10), ou chronique (CMS-vulnérable-Ket-7, n = 8). Deux heures après l'injection

aiguë (NaCl ou Ket) ou après la dernière injection du traitement chronique de Ket, les animaux ont été anesthésiés par injection intrapéritonéale d'uréthane [CH3CH2OC(O)NH2 ; 1,9 g/kg]. Suite à la perte des réflexes palpébraux et de retrait de la queue et de la patte après un pincement léger, les rats ont été placés sur de la glace, ont subi une laparotomie, puis une perfusion intracardiaque a été réalisée avec du liquide céphalo-rachidien artificiel (artificial cerebro-spinal fluid, aCSF) enrichi en saccharose, maintenu à basse température (0–4 °C) et continuellement alimenté en carbogène gazeux (95 % O_2, 5 % CO_2).

Type d'aCSF	Composition
Enrichi en saccharose	248 mM saccharose, 11 mM glucose, 26 mM $NaHCO_3$, 2 mM KCl, 1,25 mM KH_2PO_4, 2 mM $CaCl_2$, 1,3 mM $MgSO_4$ et 2,5 mM acide kynurénique
Standard	126 mM NaCl, 26 mM $NaHCO_3$, 2,5 mM KCl, 1,25 mM NaH_2PO_4, 2 mM $CaCl_2$, 2 mM $MgCl_2$ et 10 mM glucose

Tableau 6 : Composition des deux types de liquide céphalo-rachidien artificiel utilisés

Après 10 minutes de perfusion, les rats ont été décapités et leur cerveau rapidement extrait puis fixé sur une platine par le côté postérieur suite au retrait du cervelet. Des coupes transverses de 450 µm d'épaisseur, incluant la région de l'hippocampe dorsal (dHPC), ont été réalisées au vibratome (VT1200S ; Leica, Nussloch, Allemagne) et placées dans de l'aCSF standard à température ambiante, continuellement alimenté en carbogène gazeux (**Figure 17**). Dans la mesure du possible, au moins deux coupes cérébrales ont été prélevées pour chaque animal. Chaque coupe a ensuite été utilisée pour l'étude d'un mécanisme de plasticité synaptique, la LTP ou la LTD. Ceci a ainsi permis d'étudier les deux mécanismes pour chaque animal.

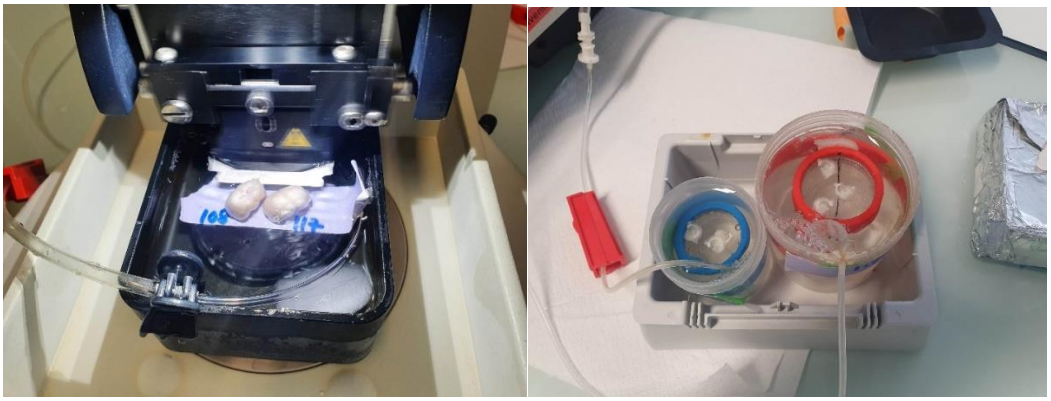

Figure 17 : Photographie des étapes de prélèvement des coupes contenant le dHPC. A : prélèvements au vibratome de cerveaux fixés à la platine ; B : coupes placées dans l'aCSF alimenté en carbogène gazeux.

B) Mesure des potentiels de champ

Pour l'enregistrement des potentiels de champs, les coupes individuelles ont été placées dans une chambre d'enregistrement et perfusées avec de l'aCSF standard, alimenté au carbogène à un débit de 2,5–3 mL/min (**Figure 18**). Les électrodes de stimulation et d'enregistrement, fabriquées à partir de capillaires en verre borosilicaté (diamètre interne 1,20 mm, diamètre externe 1,69 mm ; Warner Instruments, Harvard Apparatus, États-Unis), ont été placées dans la *stratum radiatum* de la tranche, dans la région CA1 (**Figure 18**). La résistance des deux électrodes a été d'environ 3 MΩ une fois installées et remplies d'aCSF.

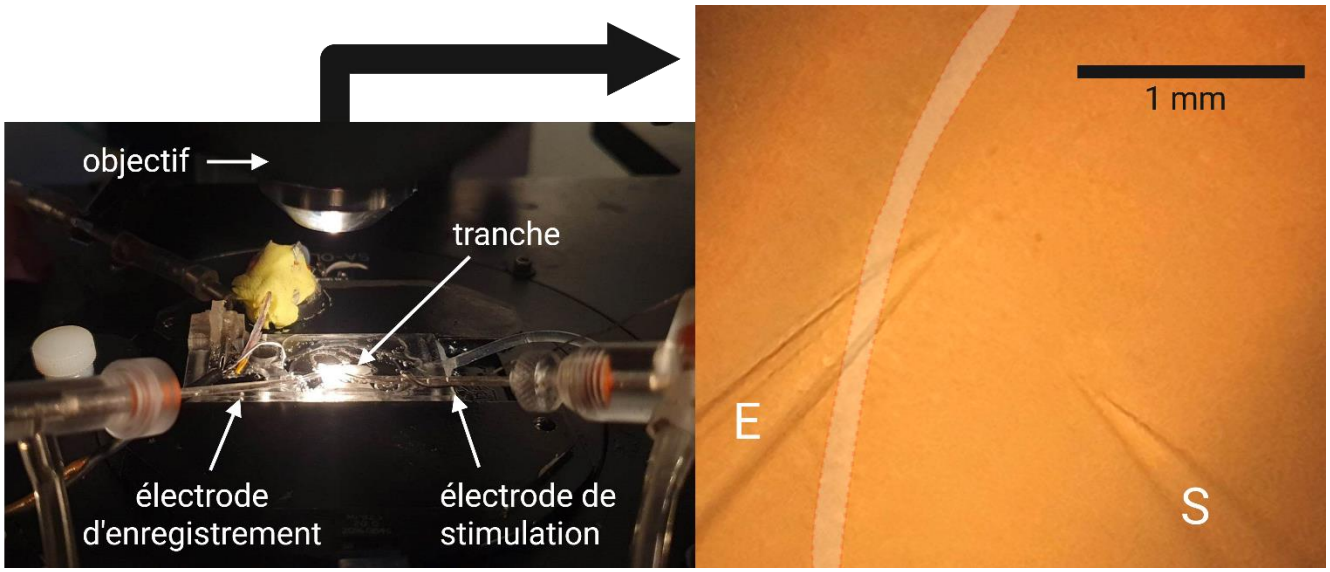

Figure 18 : Dispositif d'enregistrement des tranches. Tranche en chambre d'enregistrement sous microscope (gauche) ; Aperçu au microscope (droite) des électrodes placées dans la *stratum radiatum* de la région CA1. E : électrode d'enregistrement, S : électrode de stimulation. La zone délimitée par les pointillés correspond à la zone pyramidale contenant les corps cellulaires des neurones dont la réponse est enregistrée.

Les mécanismes de LTP et de LTD sont reflétés par l'amplitude de la réponse post-synaptique à une stimulation excitatrice, ci-après nommé « efficacité synaptique ». Lorsque la synapse est potentialisée, l'efficacité synaptique est accrue, tandis que lorsque la synapse est déprimée, l'efficacité synaptique est diminuée. Pour mesurer l'efficacité synaptique, le potentiel de champ post-synaptique excitateur (field excitatory postsynaptic potentials, fEPSP) évoqué par une stimulation présynaptique peut être enregistré. En situation de potentialisation, les courants post-synaptiques générés suite à la stimulation excitatrice entraîneront une déflection plus grande du potentiel mesuré qu'en situation basale. A l'inverse, en situation de dépression de la synapse, cette déflection sera moins ample. Des

protocoles de stimulation à haute fréquence (high frequency stimulation, HFS) ou basse fréquence (low frequency stimulation, LFS) sont employés pour induire une LTP ou LTD.

L'efficacité synaptique basale a été mesurée en enregistrant les fEPSP dans la région CA1 à une fréquence de stimulation de 0,033 Hz (une impulsion toutes les 30 secondes), n'induisant pas de potentialisation ou de dépression de la synapse, donc de modifications de l'efficacité synaptique dans des conditions physiologiques. L'intensité de stimulation, de l'ordre de 0,1 mA, a été progressivement augmentée afin de déterminer l'intensité à laquelle le fEPSP atteignait une amplitude maximum. Pour éviter la saturation des réponses, les enregistrements suivants ont été réalisés avec une intensité de stimulation évoquant une amplitude de fEPSP correspondant aux deux tiers du maximum. Les enregistrements ont ainsi été réalisés en condition basale pendant 20 minutes. Par la suite, un protocole de HFS ou LFS a été appliqué dans le but d'induire une LTP ou LTD, respectivement. La HFS a consisté en l'application de trains de cinq impulsions à 100 Hz, répétés six fois avec des intervalles de 10 secondes. La LFS a consisté en l'application d'impulsions à 1 Hz pendant 15 minutes. Des enregistrements des fEPSP en condition post-induction ont été réalisés pendant 50 minutes. Le coefficient de la pente (« slope ») des fEPSPs générés, plutôt que la valeur du potentiel lui-même, a été utilisé pour évaluer l'efficacité synaptique (**Figure 19**). En effet, l'amplitude du fEPSP représente une estimation moins fiable car il est sujet à des artéfacts et du bruit électrique. Les enregistrements ont été réalisés *via* un amplificateur MultiClamp 700A (Molecular Devices). Les signaux ont été échantillonnés à 20 kHz, numérisés *via* une interface d'acquisition BNC-2110 (National Instruments), et acquis à l'aide du logiciel WinWCP (Strathclyde Electrophysiology Software, John Dempster, University of Strathclyde, Royaume-Uni).

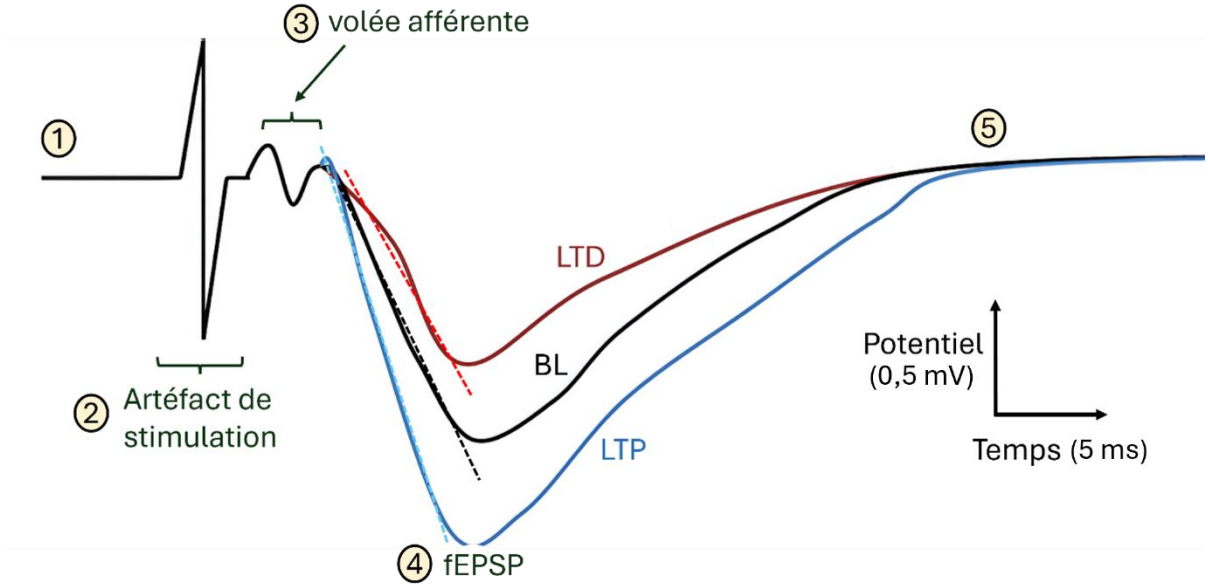

Figure 19 : Représentation de la réponse générée par à une stimulation. Le fEPSP (potentiel de champ excitateur post-synaptique) généré par une stimulation, représenté par une baisse du potentiel mesuré dans l'espace extracellulaire (**cf. p. 59**), est d'amplitude plus ou moins grande selon l'efficacité synaptique ① Potentiel au repos pré-stimulation ② Artéfact lié à la stimulation ③ Volée afférente générée par des fibres pré-synaptiques stimulées directement ④ fEPSP ⑤ Retour au potentiel au repos. BL : fEPSP en condition basale ; LTD : fEPSP en situation de dépression à long terme ; LTP : fEPSP en situation de potentialisation à long terme. Pointillés rouges, noirs et bleus : pente liée à l'amplitude du fEPSP généré en LTD, BL ou LTP, respectivement. L'échelle est arbitraire pour donner un ordre de grandeur.

C) Analyse des données

Pour évaluer la stabilité de la réponse synaptique en condition basale, puis l'effet de l'induction du protocole de LFS ou HFS, les données de chaque coupe ont été normalisées sur les 5 dernières minutes d'enregistrement en condition basale. Les coefficients de pente des fEPSPs ont ensuite été moyennés par blocs de 10 minutes, constituant donc 2 blocs en condition basale, et 5 blocs en condition post-induction.

IV) Enregistrements électrophysiologiques *in vivo*

A) Origine du signal enregistré

Les populations neuronales et gliales sont le lieu de flux ioniques transmembranaires, qui génèrent des courants électriques. L'ensemble de ces courants électriques au sein d'un volume de tissu cérébral contribue à générer un potentiel à l'amplitude variable, représenté par la superposition des contributions de ces courants à un point de l'espace donné, calculé à partir d'un point de référence. Ce potentiel, mesuré en volts, peut être enregistré dans différentes zones. Il peut par exemple être mesuré sur le scalp (électroencéphalogramme, EEG), à la surface du cortex (électrocorticogramme, ECoG) ou au sein du parenchyme cérébral (potentiel de champ local ou local field potential, LFP) (Buzsáki et al., 2012).

L'ECoG offre une mesure plus directe de l'activité électrique que l'EEG mesuré sur le scalp, qui est déformé par la traversée du crâne et des tissus intermédiaires comme la peau. Il résulte de la sommation des courants électriques présents dans la zone la plus superficielle du cortex. L'analyse de ce type de signal permet notamment de distinguer différents états de vigilance.

Le LFP représente le potentiel mesuré dans un champ électrique local au sein du parenchyme cérébral. Il est généré par les contributions électriques émanant des courants transmembranaires de la population cellulaire environnante, bien que des contributions non locales puissent provenir de sources plus distantes par le phénomène de conduction volumique. Si l'ensemble des courants transmembranaires contribue à générer le potentiel mesuré, incluant les potentiels d'actions rapides ou des fluctuations des potentiels de membranes des cellules gliales, sa contribution la plus importante est composée par les courants générés par l'activité synaptique des neurones (Buzsáki et al., 2012).

À l'échelle d'un neurone, l'activation de récepteurs AMPA ou NMDA par une stimulation excitatrice entraîne un influx de cations (Na+, Ca2+) générant un courant excitateur entrant dans le neurone. Le potentiel extracellulaire à cet endroit devient donc négatif car des charges positives quittent le milieu extracellulaire. L'influx de ces charges positives dans le neurone entraîne ailleurs un efflux de charges positives par principe d'électroneutralité, qui génère un courant dit passif ou de retour en quittant le neurone. Le potentiel extracellulaire à cet autre endroit devient donc positif car des charges positives le rejoignent. Un dipôle électrostatique est donc formé, générant un champ électrique.

À l'échelle d'une population cellulaire cérébrale intégrée dans un réseau, l'amplitude d'un LFP dépend du champ électrique généré dans l'environnement de la zone d'enregistrement (d'où le nom potentiel de champ local), qui est principalement dominée par les fluctuations ioniques dues à l'activité synaptique des neurones (Buzsáki et al., 2012). La mesure du LFP permet donc d'étudier l'activité électrique d'une structure d'intérêt. Des boucles locales excitatrices et inhibitrices *via* des neurones pyramidaux et interneurones GABAergiques génèrent des oscillations de l'activité électrique à des fréquences différentes. Dans l'hippocampe, par exemple, les oscillations rapides dans la bande gamma (30–120 Hz) dépendent des interneurones GABAergiques (Antonoudiou et al., 2020; Kullmann, 2011). Les oscillations permettent une activité coordonnée des populations neuronales, favorisant leur synchronie, et donc leur communication (Fell & Axmacher, 2011). En effet, les pics oscillatoires correspondent à un pic d'excitabilité d'une population neuronale au sein d'une structure cérébrale, car le potentiel de membrane est élevé et les neurones sont donc dépolarisés. Ces pics représentent des fenêtres temporelles pour la communication avec des populations dans d'autres structures. Si des populations de deux structures différentes présentent une synchronisation de phase (**Figure 20A**), des stimulations parvenant de l'une à l'autre, en phase de forte excitabilité, pourront provoquer une réponse importante (**Figure 20C**). À l'inverse, en l'absence de synchronisation (**Figure 20B**) des stimulations parvenant à une population en dehors des pics d'excitabilité ont peu de chance de générer une réponse. Deux populations synchrones de deux structures différentes projetant conjointement sur une troisième population pourront générer une plus grande excitation dans cette dernière (**Figure 20D**).

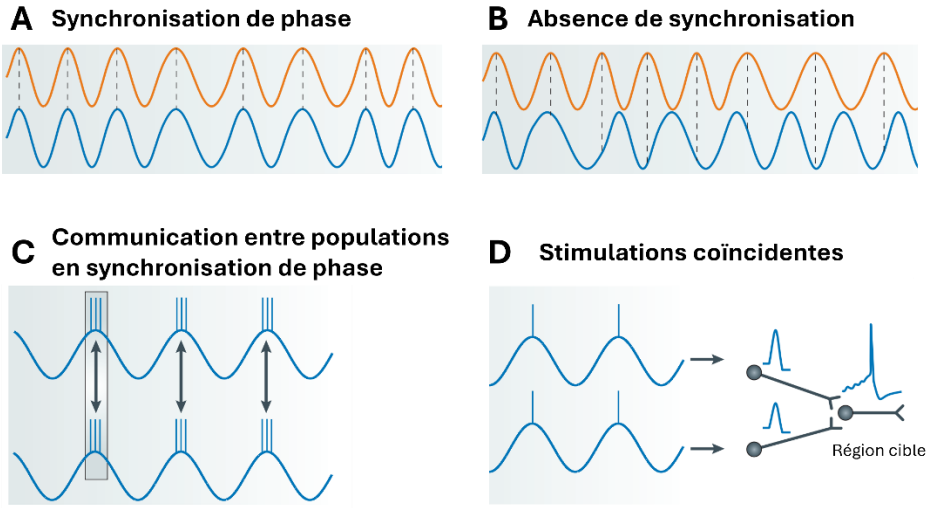

Figure 20 : Mécanismes liés à la synchronisation de phase. A : Oscillations en synchronisation de phase ; B : Oscillations sans synchronisation de phase ; C : Synchronisation de phase entre deux populations permettant des transferts d'information (représentés par les flèches) efficaces lors des pics d'excitabilité ; D : Synchronisation de phase de populations neuronales permettant des stimulations coïncidentes sur une région cible permettant de générer une plus grande excitation. Adapté de « The role of phase synchronization in memory processes » (Fell & Axmacher, 2011).

De plus, deux structures ne communiquant pas directement entre elles peuvent également présenter une activité en phase si elles sont synchronisées par une source commune. La synchronie favorise donc le transfert d'information unilatéral ou bilatéral entre différentes structures, et reflète un fonctionnement des structures en réseau. Ces mécanismes sont appelés « communication par la cohérence » (Fries, 2015). La synchronisation de l'activité de ces structures reflète également le phénomène de connectivité fonctionnelle (**cf. p. 9**) Il est donc possible d'étudier la cohérence des signaux LFP de structures différentes afin d'estimer la connectivité fonctionnelle de ces dernières.

B) Chirurgie

Les enregistrements *in vivo* pour les études des états de vigilance et des LFP ont été réalisés au sein de deux groupes d'animaux mâles : témoin (n = 8) constituant la cohorte 3, et CMS-vulnérable (n = 9) sélectionnés parmi la cohorte 1 et 2. La procédure chirurgicale d'implantation des électrodes d'enregistrement a été réalisée sous anesthésie gazeuse *via* de l'isoflurane (IsoFlo® 100 %, Zoetis, USA), à 4 % pour l'induction dans une chambre isolée en plexiglas, puis à 1,5 % pour le maintien pendant l'opération. Les animaux ont ensuite été placés dans un cadre stéréotaxique avec un tapis

chauffant afin de prévenir l'hypothermie durant l'opération. Des anesthésiques locaux, incluant un mélange de lidocaïne, 2 mg/kg (Lidor, Axience, France), et de bupivacaïne, 2 mg/kg (Mylan, USA), ainsi qu'un anti-inflammatoire non stéroïdien, le méloxicam, 1 mg/kg (Metacam®, Boehringer Ingelheim, Allemagne) ont été administrés par injection au niveau du scalp afin de limiter la douleur et permettre une bonne récupération de la chirurgie. Les barres d'oreilles du cadre stéréotaxique ont été enduites d'une crème lidocaïne/prilocaïne (2,5 g de chaque substance dans 100g de crème, Biogaran, France). Du gel oculaire (Lubrithal, Dechra, Royaume-Uni) a été appliqué pour prévenir le dessèchement des yeux pendant l'opération. Une fois les rats placés dans le cadre stéréotaxique, le sommet de la tête a été rasé, désinfecté à la bétadine, et incisé sur la ligne médiane pour exposer le crâne. Les coordonnées stéréotaxiques pour l'implantation d'électrodes afin d'enregistrer le LFP au sein de chaque structure cible dans l'hémisphère droit ont été marquées (en mm, par rapport à Bregma, AP = antéropostérieur, ML = médiolatéral, DV = dorsoventral) : cortex préfrontal médian, partie prélimbique (PRL, +2,6 ; -0,7 ; -3), amygdale basolatérale (BLA, -2,5 ; -4,9 ; -6,9) et hippocampe dorsal, région CA1 (dHPC, -3,8 ; -2,7 ; -2,2).

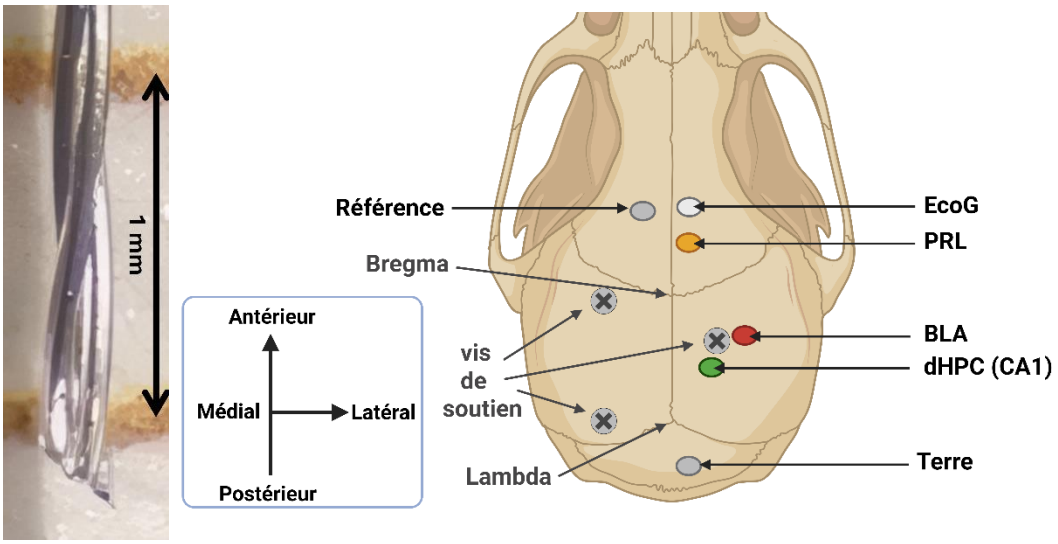

Figure 21 : Photographie d'électrodes (gauche) et schéma des zones d'implantation par rapport au crâne du rat (droite). EcoG : électrocorticogramme ; PRL : partie prélimbique du cortex préfrontal médian ; BLA : amygdale baso-latérale ; dHPC (CA1) : hippocampe dorsal (région *Cornu Ammonis* 1)

Des trous ont été percés à l'aide d'une fraiseuse aux emplacements d'intérêt, et des microélectrodes, dont l'extrémité était enduite de marqueur fluorescent lipophile DiIC18(3) (DiI) à 2 % (Sigma-Aldrich, France), ont été implantées dans les structures d'intérêt pour l'enregistrement des

LFP. Ces microélectrodes étaient composées de 3 brins de tungstène, permettant 3 canaux d'enregistrement par structure (0,004 pouces, PFA-coated, A-M Systems, USA). Une vis corticale a été posée à la surface du cortex en position frontale pour l'enregistrement de l'ECoG. Des électrodes de référence et de terre ont été fixées au niveau du cortex frontal gauche et du cervelet, respectivement. Trois vis de soutien ont été fixées, deux sur la partie gauche du crâne, et une autre sur la partie droite (**Figure 21**), auxquelles ont été fixées les électrodes grâce à de la colle UV (Metafil FLO, SUN Medical Co., Moriyama-shi, Japan**)**. Les fils des électrodes ont été soudés à une carte d'interface électronique (electronic interface board, EIB-16, Neuralynx, États-Unis) (**Figure 22**), et l'ensemble a été fixé avec de la colle (Super-Bond C&B®, Sun Medical Co., Moriyama-shi, Japan), puis recouvert de ciment dentaire (Paladur®, Kulzer, Allemagne), laissant accessible le connecteur de l'EIB pour les enregistrements.

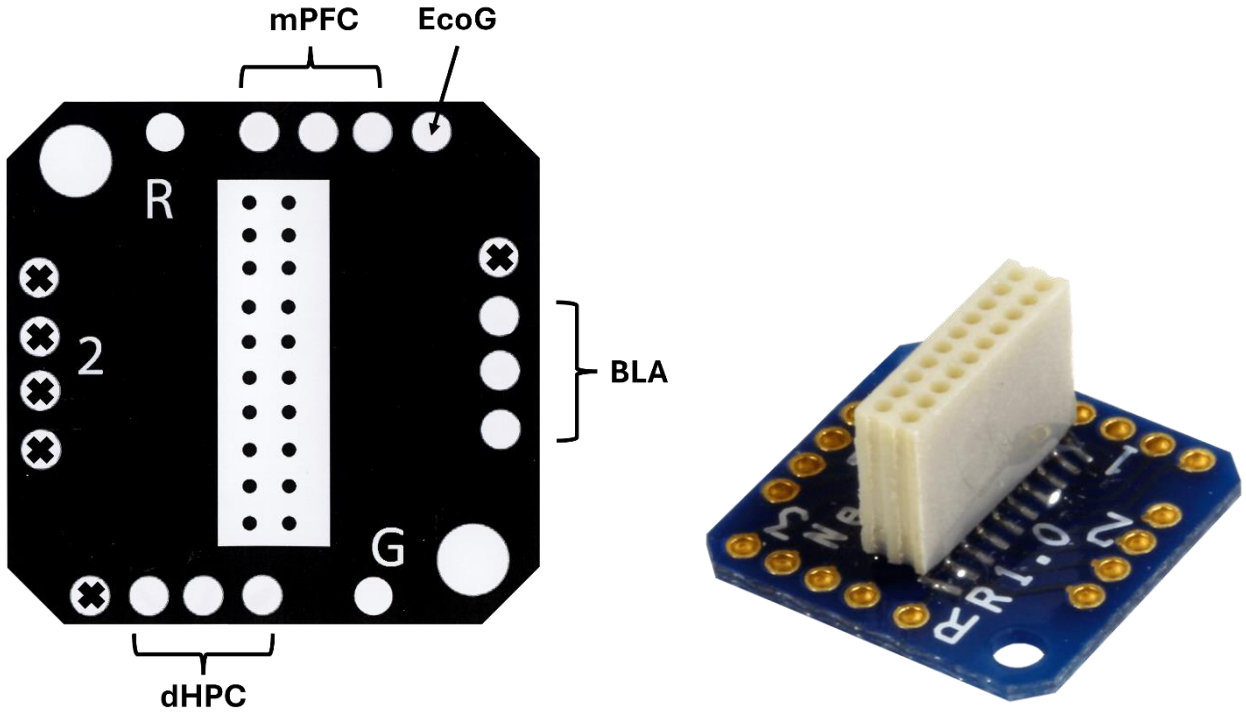

Figure 22 : Interface électronique (electronic interface board, EIB). A : Plan de l'EIB ; G : Terre (Ground) ; R : Référence ; dHPC : hippocampe dorsal ; BLA : Amygdale basolatérale ; PRL : partie prélimbique du cortex préfrontal médian ; EcoG : électrocorticogramme. B : Photographie du modèle d'EIB employé (EIB-16, Neuralynx).

Suite à la chirurgie, les animaux ont été placés dans une cage propre sous une lampe chauffante jusqu'à leur réveil complet. Ils ont ensuite été hébergés individuellement pendant deux semaines pour récupération, avec suivi quotidien du poids corporel et du comportement. Les animaux témoins ont par la suite à nouveau été hébergés par paire.

C) Déroulé des enregistrements

Après la période de récupération de la chirurgie, les rats CMS ont été exposés au protocole de CMS pendant deux semaines (**Figure 24**). Les animaux ont été soumis à une phase d'habituation au système d'enregistrement pendant 3 jours consécutifs (3 h/jour) avant le début de l'acquisition des données. Pendant les enregistrements, la cage d'hébergement des animaux a été installée à l'intérieur d'une cage de Faraday afin de minimiser les interférences électromagnétiques. Un préamplificateur (modèle C3334, Intan Technologies, États-Unis) a été connecté à l'EIB permettant le transfert du signal, *via* un câble léger, à un amplificateur placé au-dessus de la cage de Faraday. Un connecteur tournant a permis une libre mobilité de l'animal dans la cage sans torsion du câble (**Figure 23**). Les signaux électrophysiologiques ont été acquis à l'aide d'un système d'enregistrement Intan (modèle C3334, Intan Technologies, États-Unis). Ils ont été amplifiés et numérisés à une fréquence d'échantillonnage de 1 000 Hz (1000 valeurs successives de l'amplitude du LFP pour chaque seconde), avec un filtre analogique passe-haut à 1,26 Hz et un filtre analogique passe-bas à 500 Hz, puis sauvegardés par fichiers consécutifs de 15 minutes au format .rhd.

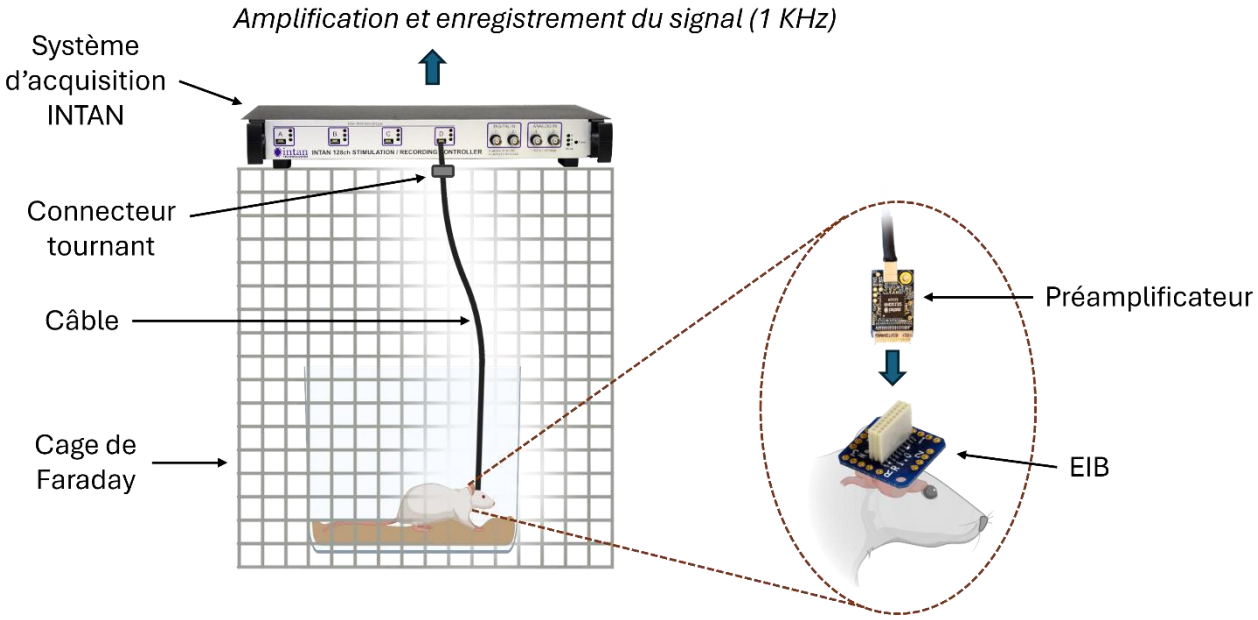

Figure 23 : Dispositif d'acquisition du signal.

Trois enregistrements ont été réalisés dans une approche intra-sujet sur un cycle lumière/obscurité complet (12 h/12 h, extinction des lumières de 19:00 à 07:00). Vingt-quatre heures après la fin du CMS, les animaux CMS-vulnérables, ou témoins non stressés (NS) d'un âge équivalent, ont reçu une injection sc. de NaCl 0,9 %, et le premier enregistrement (Session 1) a été immédiatement démarré, à 09:00, pour une durée de 24 heures. Vingt-quatre heures après la fin du premier

enregistrement, les animaux CMS ont reçu une injection sc. de Ket (10 mg/kg), tandis que les animaux témoins ont reçu une injection sc. de NaCl 0,9 %, et le second enregistrement (Session 2) a été immédiatement démarré, à 09:00, pour une durée de 24 heures. Par la suite, les animaux ont reçu quotidiennement (toujours à 09:00) une injection sc. (Ket pour les CMS-vulnérables, NaCl pour les NS), jusqu'à atteindre 7 injections, et le dernier enregistrement (Session 3) a été lancé à 09:00 immédiatement suite à la dernière injection, pour une durée de 24 heures (**Figure 24**).

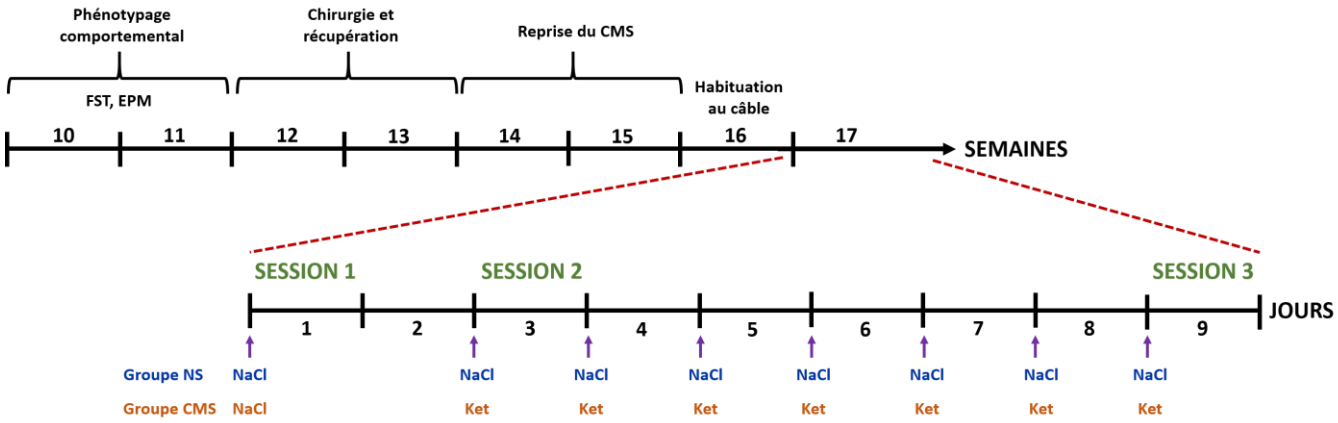

Figure 24 : Déroulé des enregistrements (en vert). Les flèches en violet représentent les administrations de NaCl ou Ket. NS : témoins non stressés ; CMS : animaux CMS-vulnérables.

D) Vérifications histologiques

Après la fin du dernier enregistrement, les rats ont été anesthésiés par injection ip d'un mélange de Ket (100 mg/kg) et de xylazine (10 mg/kg, Rompun®, Bayer, Allemagne), puis, après perte des réflexes palpébraux et de retrait de la queue ou de la patte après un pincement léger, ont subi une perfusion intracardiaque avec une solution de tampon phosphate salin (phosphate-buffered saline, PBS) 0,1 M à 4 °C (environ 200 mL), suivie de paraformaldéhyde (PFA) 4 % (Carl Roth, Allemagne ; pH 7,4 ; 4 °C) (environ 300 mL). Les cerveaux ont été extraits et post-fixés dans la même solution de PFA (4 h à 4 °C), puis placés dans une solution de saccharose 20 % (dans PBS 0,1 M, à 4 °C) jusqu'à saturation (48 h) pour assurer une cryoprotection des tissus. Enfin, les cerveaux ont été congelés par immersion dans de l'isopentane (Carl Roth, Allemagne) à -35 °C pendant 1 minute. Des sections coronales de 40 µm ont été effectuées avec un cryostat (Cryostat NX70, Epredia *via* Microm Mictotech, France) et collectées sur des lames gélatinées. Elles ont ensuite été montées entre lame et lamelle dans un milieu (Fluoromount-G, Thermo Fisher Scientific, États-Unis) contenant un fluorochome, le DAPI (4',6-diamidino-2-phénylindole), permettant de visualiser les noyaux cellulaires. Les traces des électrodes d'enregistrement des LFP, dont l'extrémité avait été enrobée de

DiI, révélées par la fluorescence, ont été visualisées à l'aide d'un microscope Apotome (Zeiss Axio Imager M2, Zeiss®, Allemagne) avec le logiciel Zen (Blue Edition). La position des électrodes a été confirmée dans les régions cérébrales (PRL, dHPC, BLA) par comparaison avec un atlas de cerveau de rat (Paxinos, 7e édition, 2014 ; **Figure 25**).

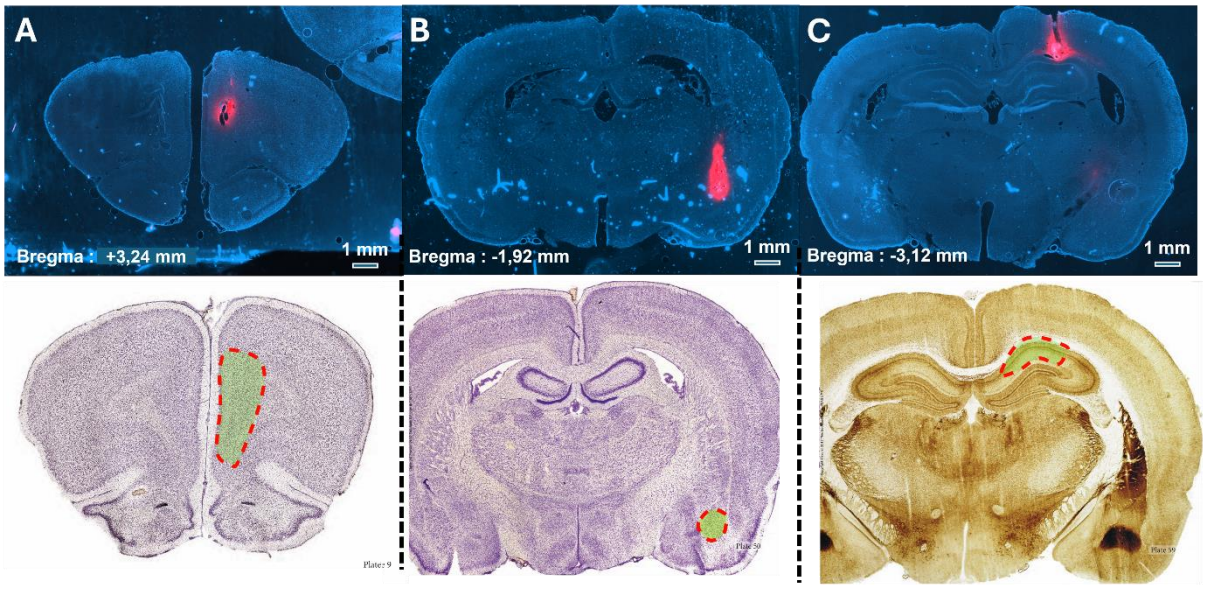

Figure 25 : Vérifications histologiques sur coupes frontales. Bleu : marquage DAPI ; Rouge : marquage DiI. A : Coupe du PRL (cortex préfrontal médian , partie prélimbique). B : Coupe de la BLA (amygdale baso-latérale). C : Coupe du dHPC, région CA1 (hippocampe dorsal, région *Cornu Ammonis* 1). Zone en pointillés : zone ciblée sur des photographies de l'atlas Paxinos, 7e édition, 2014.

V) Étude du rythme veille-sommeil

A) Identification des états de vigilance et élaboration des hypnogrammes

Les signaux de l'ECoG, enregistrés en format .rhd, ont été convertis au format .edf à l'aide de scripts personnalisés développés sur MATLAB, puis importés dans le logiciel Profusion Sleep (version 4.0, Compumedics, USA). Pour chacun des rats, chacun des trois enregistrements de 24 heures a été segmenté en époques de 4 secondes, une approche permettant une analyse fine de la microarchitecture du sommeil (McShane et al., 2010). Pour chaque époque, l'état de vigilance a été déterminé visuellement sur la base des motifs d'oscillations. L'état de veille (Wake) est caractérisé par des oscillations rapides et irrégulières dominées par les ondes thêta θ et gamma γ, le sommeil NREM est dominé par des ondes delta δ, lentes et amples, tandis que le sommeil REM est marqué par des oscillations rapides et très régulières, dominées par la bande thêta θ (**Figure 26**) (Hoshino et al., 2016).

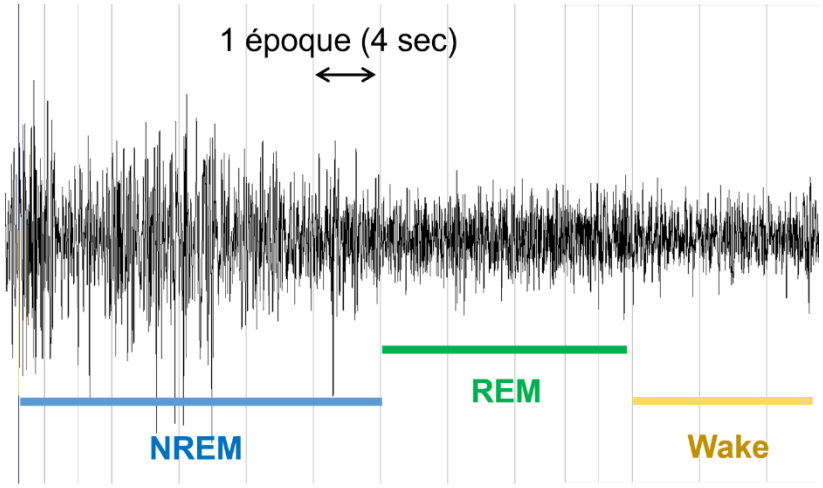

Figure 26 : Exemple de tracé de l'électrocorticogramme, subdivisé en époques de quatre secondes (lignes horizontales grises). NREM : sommeil marqué par des oscillations amples et lentes ; REM : sommeil marqué par des oscillations rapides et régulières ; Wake : état de veille, marqué par des oscillations rapides mais irrégulières.

Afin de garantir une précision du scorage des états de vigilance, celui-ci a été supervisé par un autre expérimentateur. Le scorage de toutes les époques d'un enregistrement a permis de générer un hypnogramme, correspondant à la distribution des différents états de vigilance au cours du temps. Les hypnogrammes ont été exportés depuis Profusion Sleep au format Excel .xlsx.

B) Analyses des données

Les hypnogrammes ont été importés dans MATLAB, permettant leur analyse *via* des scripts personnalisés. Ces derniers ont permis d'extraire, pour chaque enregistrement, la durée totale, le nombre et la durée moyenne des épisodes de chaque état de vigilance (Wake, sommeil NREM, sommeil REM). Ils ont également permis de calculer la latence de première entrée en sommeil NREM (temps séparant le début de l'enregistrement et le début du premier épisode de sommeil NREM), et la latence de première entrée en sommeil REM (temps séparant le début du premier épisode de sommeil NREM et le début du premier épisode de sommeil REM) (**Figure 27**). Hormis les latences, les différents paramètres ont été déterminés pour chacune des 24 heures d'enregistrement. Celles-ci ont enfin été analysées par intervalles de 2 heures, (en déterminant la somme pour le nombre d'épisodes et pour la durée totale, et la moyenne pour la durée moyenne des épisodes) organisés selon la phase lumineuse : 5 blocs de 2 heures de phase lumineuse (09h00 à 19h00, début de l'enregistrement à l'extinction des lumières), et 6 blocs de 2 heures de phase d'obscurité (19h00 à 07h00).

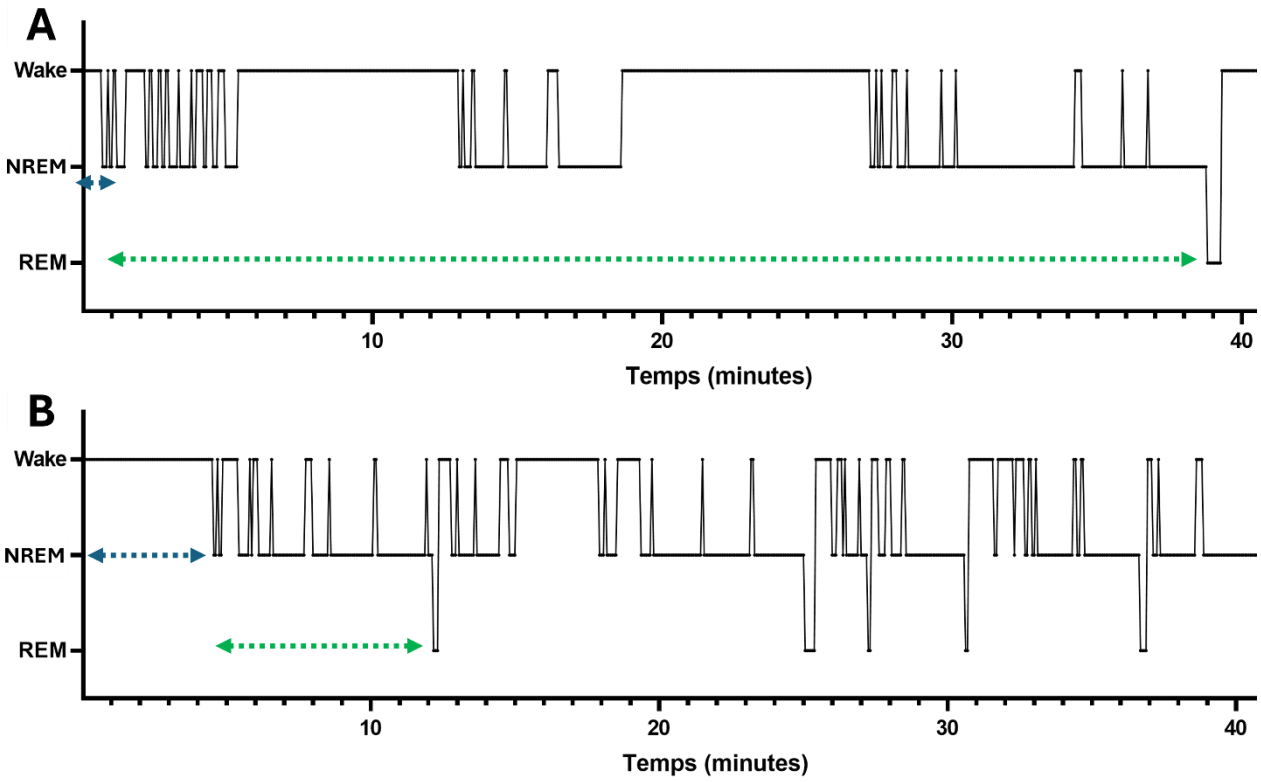

Figure 27. Exemples d'hypnogrammes de rat témoin (A) et CMS-vulnérable (B) sur les 40 premières minutes d'enregistrement. La flèche en pointillés bleue représente la latence d'entrée en sommeil NREM. La flèche en pointillés verte représente la latence d'entrée en sommeil REM.

VI) Étude des potentiels de champ local

A) Étapes de prétraitement

1) Nettoyage du signal

Les LFP étudiés étaient constitués de trois signaux pour le PRL, la BLA et le dHPC, car enregistrés *via* trois électrodes pour chaque site implanté. Ces signaux ont été extraits des fichiers .rhd *via* MATLAB, et analysés à l'aide d'une série de scripts. Pour chaque structure, deux des trois signaux présentant la meilleure qualité ont été identifiés visuellement sur la première heure d'enregistrement. Cette identification a été effectuée dans le domaine temporel, c'est-à-dire les variations de la valeur du potentiel au cours du temps, et basée sur une moindre présence d'artéfacts observés (**Figure 28A**). Elle a également été effectuée dans le domaine fréquentiel, c'est-à-dire le spectre fréquentiel calculé à partir du signal (**cf. p. 71**), basée sur une moindre déformation du spectre causée par le bruit et les artéfacts

(**Figure 28B**). Le troisième a été exclu. Les deux signaux conservés ont été soustraits l'un à l'autre afin de générer des dérivations bipolaires et d'établir une référence locale et donc de minimiser les contributions électriques de sources plus distantes. Effectivement, la référence locale permet de virtuellement neutraliser le phénomène de conduction volumique, par lequel des signaux générés en dehors de la zone étudiées sont conduits par les tissus à la zone d'enregistrement et contaminent le signal (Trongnetrpunya et al., 2016).

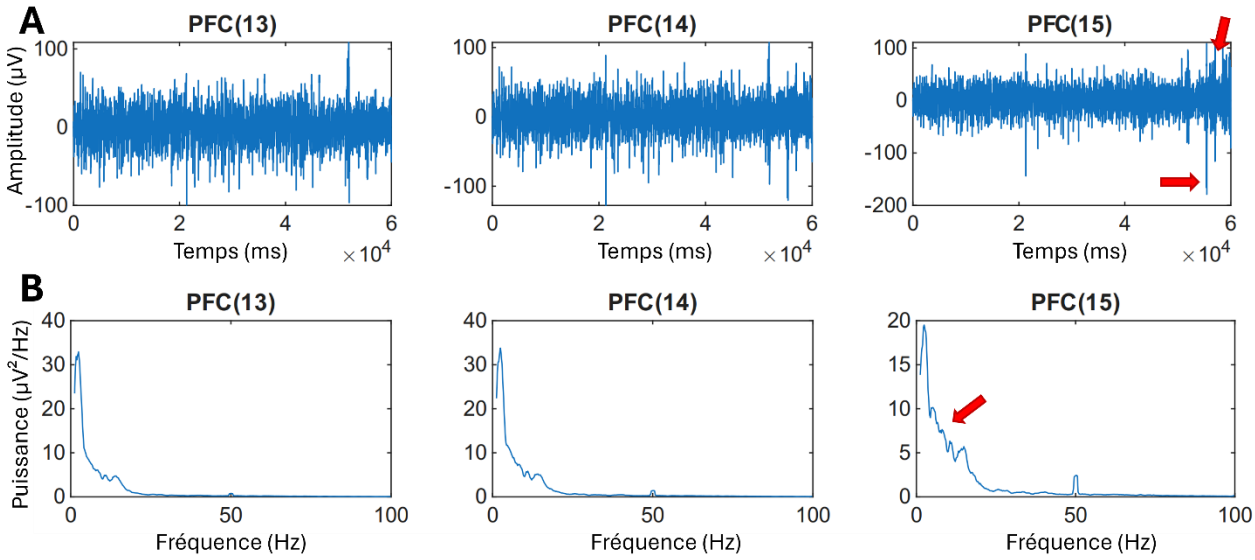

Figure 28 : Exemple du signal enregistré par les trois électrodes dans le PRL. Les numéros de canal (13, 14 et 15) sont attribués lors de l'importation du fichier MATLAB. A : signal dans le domaine temporel. B : signal dans le domaine fréquentiel. Flèches rouges en A : exemples d'artéfacts d'enregistrements dans le canal 15 non présents dans les canaux 13 et 14. Flèche du haut : plus grand nombre d'artéfacts, flèche du bas : artéfact de plus grande amplitude (remarquez l'échelle) ; Flèche rouge en B : déformations du spectre correspondant dues au bruit et artéfacts non présentes dans les deux autres canaux. Dans cet exemple, le canal 15 a donc été exclu de la triade d'électrodes.

Le signal issu de la dérivation bipolaire a ensuite été filtré avec un filtre Butterworth dans la bande passante 1,26–200 Hz, et les artéfacts ont été supprimés à l'aide d'un algorithme basé sur la transformée par ondelettes (wavelet transform). Après ces étapes, certains signaux présentaient une contamination résiduelle due à la fréquence du secteur électrique utilisé en Europe apparaissant comme un pic à 50 Hz. Dans ces cas-là, un filtre « notch » Butterworth à cette fréquence adaptée à la contamination a été appliqué (**Figure 29**).

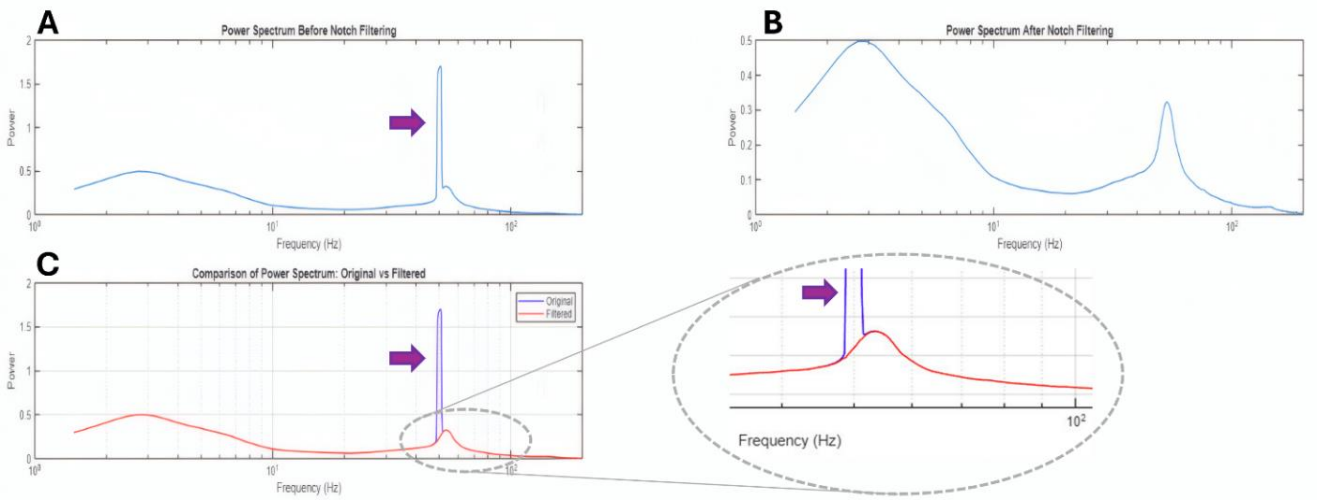

Figure 29 : Application du filtre « notch » pour retirer la contamination électrique à 50 Hz. A : Spectre avant filtrage. B : Spectre après filtrage. C : superposition des spectres avant (bleu) et après (rouge) filtrage, sans distorsion du signal générant un « trou » dans la bande gamma (45-65 Hz). Flèche violette : contamination à 50 Hz.

Après filtrage, le signal a été segmenté par intervalle d'une heure selon les trois différents états de vigilance déterminés durant l'étude du rythme veille-sommeil, c'est-à-dire la veille (Wake), le sommeil NREM et le sommeil REM. Ainsi, pour chacune des 24 heures, les segments du signal correspondant à un même état de vigilance ont été regroupés. Pour éviter les phases de transitions d'un état à l'autre, deux époques (8 secondes de signal) au début et à la fin de chaque segment ont été retirés.

2) Détermination des bandes de fréquences

Étant donné la grande variabilité dans la définition des différentes bandes de fréquence dans les études électrophysiologiques chez le rongeur, celles utilisées dans cette étude ont été sélectionnées sur la base d'une recherche bibliographique portant sur les effets du stress et de la Ket dans des modèles rongeurs de dépression (**Tableau 7**). Certaines bandes voisines pouvant parfois se chevaucher, telles que le thêta (4–12 Hz) et l'alpha (8–14 Hz), les intervalles sélectionnés pour l'étude ont été définis afin de séparer chaque bande d'au moins 1 Hz.

Bande	Intervalle maximum	Intervalle sélectionné	Références
delta δ	1-4 Hz	**1–4 Hz**	(Ahnaou et al., 2017; Amat-Foraster et al., 2019; Cordon et al., 2015; Hunt et al., 2011; Pal et al., 2015; Pothorszki et al., 2024)
thêta θ	4-12 Hz	**5–8 Hz**	(Ahnaou et al., 2017; Amat-Foraster et al., 2019; Cordon et al., 2015; Hinman et al., 2013; Kittelberger et al., 2012; Pal et al., 2015; Pothorszki et al., 2024; Tort et al., 2013)
alpha α	8-14 Hz	**9–12 Hz**	(Ahnaou et al., 2017; Amat-Foraster et al., 2019; Pothorszki et al., 2024)
bêta θ	12-32 Hz	**13–29 Hz**	(Ahnaou et al., 2017; Amat-Foraster et al., 2019; Pothorszki et al., 2024)
Gamma γ	25-125 Hz	**45–65 Hz**	(Cordon et al., 2015; Pal et al., 2015; Wróbel et al., 2020)
HFO	110–190 Hz	**130–180 Hz**	(Amat-Foraster et al., 2019; Cordon et al., 2015; Hunt et al., 2011; Tort et al., 2013; Wróbel et al., 2020)

Tableau 7 : Liste des types d'oscillations et gammes de fréquence étudiées. HFO = oscillations à haute fréquence (high frequency oscillations).

B) Analyses spectrales

1) Principe

Les spectres fréquentiels des LFP ont été déterminés par la transformée de Fourier rapide (fast fourier transform, FFT), permettant de convertir le signal LFP du domaine temporel au domaine fréquentiel en générant un spectre définissant ses composantes fréquentielles (**Figure 30**). La FFT est appliquée à une série temporelle (time series), c'est-à-dire une suite ordonnée de données mesurées successives dans le temps, correspondant à la valeur de l'amplitude du LFP dans le cas présent, échantillonnée à 1000 Hz. Elle permet de déterminer la densité spectrale de puissance (Power Spectral Density, PSD) ou spectre de puissance du signal, qui décrit la distribution de la puissance spectrale en fonction des fréquences. La puissance spectrale est déterminée par l'amplitude des différentes oscillations présentes dans le signal. Plus une population neuronale est synchronisée à une fréquence donnée, plus l'amplitude des oscillations à cette fréquence sera élevée, et plus la puissance spectrale dans cette bande est importante (**Figure 30**). La PSD reflète donc l'importance de la contribution des oscillations à chaque fréquence dans la composition globale du signal LFP enregistré.

$$X_k = \sum_{m=0}^{n-1} x_m e^{-i\frac{2\pi}{n}km} \qquad k = 0, 1, \ldots, n-1$$

Calcul de la Transformée de Fourier discrète (Discrete Fourier Transform), opérée séquentiellement sur une série temporelle discrète dans la FFT. X_k correspond à la représentation dans le domaine fréquentiel d'une série temporelle x_m de longueur n.

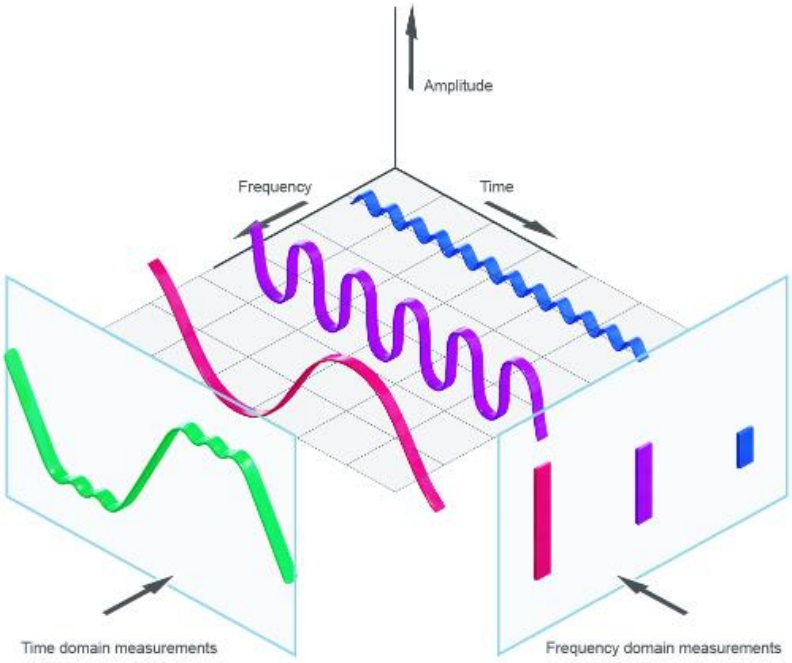

Figure 30 : Illustration de la décomposition d'un signal temporel en ses composantes fréquentielles. Le signal dans le domaine temporel est représenté en vert. Il est composé dans cet exemple de trois oscillations : une à faible fréquence et grande amplitude (en rose), une à fréquence et amplitude moyenne (en violet) et une à haute fréquence et faible amplitude (en bleu). L'oscillation rose, de la plus grande amplitude, contribue majoritairement à déterminer l'évolution de la valeur du signal dans le temps, et présente donc la plus grande puissance dans le domaine fréquentiel.

2) Calcul de la puissance spectrale relative

La PSD de chacun des signaux, organisés par état de vigilance pour chaque heure (**cf. p. 70**) a été calculée par transformée de Fourier rapide (FFT) *via* la toolbox MATLAB Chronux. La puissance absolue dans les bandes de fréquences d'intérêt (ex : thêta θ, 5-8 Hz), a été calculée comme l'aire sous la courbe (area under the curve, AUC) du spectre de puissance dans la bande considérée. Néanmoins, cette dernière présentant des grandes variations d'échelle entre les enregistrements, elle n'a pas pu être directement comparée entre eux. Ainsi, la puissance relative a été calculée, représentant la contribution relative d'une bande de fréquence donnée par rapport à l'ensemble de la PSD. La puissance relative

obtenue en divisant l'AUC (absolue) spécifique à chaque bande par l'AUC totale sur l'ensemble du spectre (**Figure 31**).

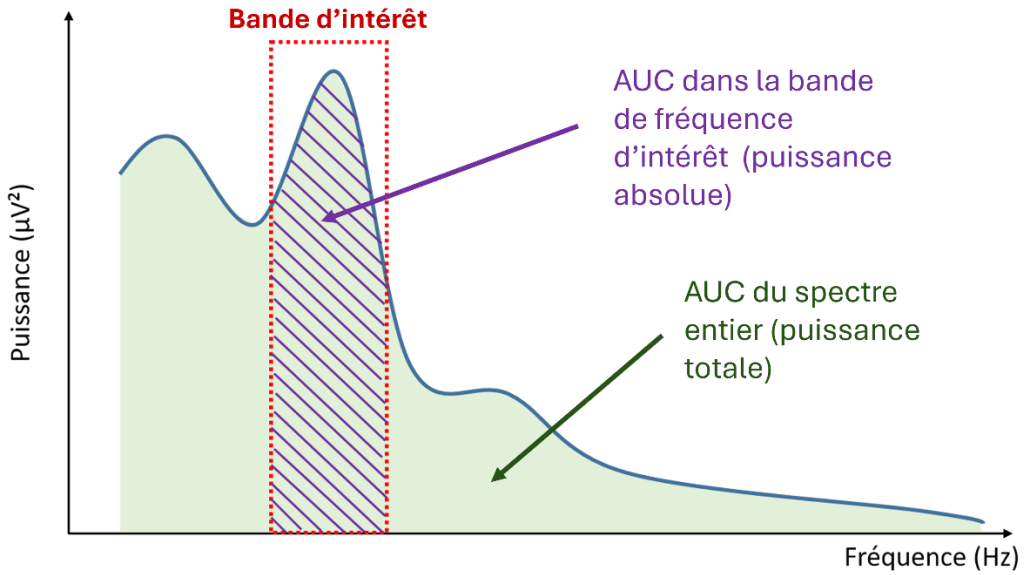

Figure 31 : Principe du calcul de la puissance relative. Pour une bande de fréquence donnée (pointillés rouges), l'aire sous la courbe (AUC) de la partie du spectre au sein de cette bande est calculée (hachures violettes), représentant la puissance absolue. L'AUC du spectre entier (en vert), représentant la puissance totale, est également déterminée. La puissance relative d'une bande de fréquence d'intérêt est obtenue en divisant sa puissance absolue par la puissance totale

Les valeurs de puissance relative de chaque bande de fréquence ont été déterminées pour chaque heure, puis moyennées en intervalles de 2 heures comme pour les données des états de vigilance sommeil. Il y a donc eu 5 intervalles au sein de la phase lumineuse, 6 au sein de la phase d'obscurité, et un intervalle suite au rallumage des lumières le lendemain.

3) Calcul de la cohérence corrigée

Afin de mesurer la connectivité fonctionnelle des trois structures d'intérêt une analyse de la cohérence quadratique de l'amplitude (magnitude-squared coherence, MSC) (Schneider et al., 2020) a été réalisée *via* la fonction *cohgramc* de Chronux. Cependant, même en l'absence de connectivité fonctionnelle, une cohérence aléatoire de « bruit » est générée par les fluctuations du signal enregistré dans chacune des structures étudiées. La cohérence « réelle » représente donc la valeur de cohérence totale à laquelle on soustrait la valeur de cohérence aléatoire, générant une cohérence corrigée. La cohérence dans chaque bande de fréquence a été calculée entre les dérivations bipolaires des signaux LFP de paires de structures (PRL–BLA, PRL–dHPC, BLA–dHPC). Cependant, l'analyse nécessitant

d'importantes ressources computationnelles, l'évaluation de la cohérence a été réalisée sur la première minute de signal de chaque heure d'enregistrement, pour chaque état de vigilance.

$$C_{(xy)}(f) = \frac{\left|G_{xy}(f)\right|^2}{G_{xx}(f)G_{yy}(f)}$$

Calcul mathématique de la MSC, avec $C_{(xy)}(f)$ la valeur de la MSC à la fréquence f entre deux signaux x et y, et $G_{xx}(f)G_{yy}(f)$ la PSD des signaux x et y à cette même fréquence f.

Afin d'estimer et de corriger la cohérence aléatoire, un bootstrap avec 1 000 itérations a été réalisé. A chaque itération, les points temporels d'un signal de la paire (60 s, avec une fréquence d'échantillonnage de 1 000 Hz, soit 60 000 points) ont été réarrangés aléatoirement à l'aide de la fonction randsample, mélangeant ainsi le signal. La valeur de cohérence entre ce signal randomisé et l'autre signal de la paire a ensuite été recalculée. La cohérence corrigée a été obtenue en soustrayant à la cohérence « réelle » le 95e percentile de la distribution issue du bootstrap (soit la 50ème plus grande valeur sur 1 000), représentant un seuil de significativité de 5 %, ce qui a permis de prendre en compte la cohérence « aléatoire » non propre au signal généré au sein des structures.

VII) Etude transcriptomique

A) Principe

La réaction en chaîne par polymérase quantitative après transcription inverse (Reverse Transcription quantitative Polymerase Chain Reaction, RT-qPCR), également appelée RT-PCR en temps réel (real time RT-PCR), est une méthode permettant de quantifier l'expression des gènes par la mesure de l'abondance relative de leur ARN messager (ARNm). Pour la RTq-PCR, l'ARN total, contenant les ARNm traduisant directement l'activité transcriptionnelle des gènes, est extrait d'échantillons biologiques étudiés. Comme la PCR ne peut être réalisée qu'à partir d'ADN, l'ARN est ensuite converti en ADN complémentaire (ADNc) par l'action de l'enzyme transcriptase inverse (reverse transcriptase, RT). Cet ADNc constitue la base utilisée pour l'amplification. L'amplification par qPCR est réalisée à l'aide d'amorces spécifiques au gène étudié et d'une ADN polymérase thermostable, qui peut polymériser et donc amplifier des séquences géniques d'intérêt délimitées par les amorces. L'accumulation quantitative du produit d'amplification, ou amplicon, est suivie à chaque cycle d'amplification grâce au signal fluorescent, obtenu par exemple grâce à un colorant intercalant de l'ADN comme le SYBR Green. Le signal fluorescent est comparé à un seuil de détection prédéfini

de manière à être situé au-dessus du bruit de fond pour ne pas entraîner de détection aspécifique. Le nombre de cycles d'amplification (cycle quantification, Cq) correspond au nombre de cycles nécessaires pour que la fluorescence franchisse ce seuil. Le nombre de Cq est inversement proportionnel à la quantité initiale d'ARNm : un gène fortement exprimé présentera un nombre de Cq faible, car nécessitera peu de cycles pour être détecté, tandis qu'un gène faiblement exprimé présentera un nombre de Cq élevé. La RT-qPCR peut être réalisée en duplicata ou triplicata, pour chaque gène étudié, c'est-à-dire en réalisant en parallèle deux ou trois fois la réaction pour un gène sur un échantillon donné. Afin de compenser de potentielles variations liées à la quantité initiale d'ARN, à l'efficacité de la transcription inverse ou celle de l'amplification, les résultats sont normalisés par rapport à un gène de référence, ou gène témoin, dont l'expression reste stable quelles que soient les conditions expérimentales. L'expression relative d'un gène cible est ainsi calculée par comparaison à ce gène témoin, souvent selon la méthode ΔΔCq, permettant d'estimer les différences d'expression selon les conditions expérimentales.

B) Validation des amorces

Pour les huit gènes dont l'expression a été analysée, incluant *36b4* (gène de référence), *Bdnf*, *FKBP5*, *Gr/Nr3c1*, *Gria1/Glua1*, *Grin2b/glun2b*, Mao_a et *Psd95/Dlg4*, les séquences d'amorces ont été conçues *via* l'outil bio-informatique PrimerBLAST afin de correspondre aux conditions de PCR utilisées dans l'équipe [température de fusion entre 59 et 65 °C, taille des amplicons entre 70 et 200 paires de bases (base pairs, bp), couvrant si possible une jonction exon-exon], ou bien sélectionnées dans la littérature lorsqu'adaptées à ces conditions (**Tableau 8**).

Nom du *gène* (protéine)	Séquence sens	Séquence antisens	Taille (bp)	source
Rplp0/36B4 **(RPLP0)**	CTGCCCGAGCCGGTGCCATC	TTCAATGGTACCTCTGGAG	131	PrimerBLAST
Bdnf **(BDNF)**	GGCCCAACGAAGAAAACCAT	CAGAAAGAGCAGAGGAGGCT	95	(Mingardi et al., 2023)
Fkbp5 **(FKPB5)**	AGTTATCTTCCCCGCTGCCC	GGAGACGGAGACGGAAGTCG	133	PrimerBLAST
Gr/Nr3c1 **(GR)**	AGCTCCCCCTGGTAGAGAC	GCGAAGATGCAGAAACCTTG	119	(Chen et al., 2021)
Gria1/Glua1 **(GRIA1/GLUA1)**	ACTGAACGAGCAGGGGCTTT	GTCCTTGGAGTCACCTCCCC	91	PrimerBLAST
Grin2b/glun2b **(GRIN2B/GLUN2B)**	GCATGATCGGTGAGGTGGTCA	CACAGTCCCATTGCTGCGAG	149	PrimerBLAST
Mao$_a$ **(MAO$_A$)**	TAGTCGTGATTGGAGGCGGC	CCCATTTGACATGCTCATTCCTCA	141	PrimerBLAST
Psd95/Dlg4 **(PSD95)**	CACCTCCCCAACCAGGCCAT	CCTCTGTTCCATTCACCTGCAAC	94	PrimerBLAST

Tableau 8 : Liste des amorces utilisées. A : adénine ; C : cytosine ; G : guanine ; T : thymine.

Ces séquences ont été testées sur des échantillons d'autres structures cérébrales prélevés en même temps que les échantillons d'intérêt, avec lesquels une qPCR a été réalisée (**cf. p. 78**). Une bonne amplification a été vérifiée par analyse visuelle des courbes de fusion (**Figure 32**) et par un nombre correct de cycles d'amplifications (seuil maximum Cq = 34, au-delà duquel la spécificité de l'amplification n'étant plus considérée comme fiable).

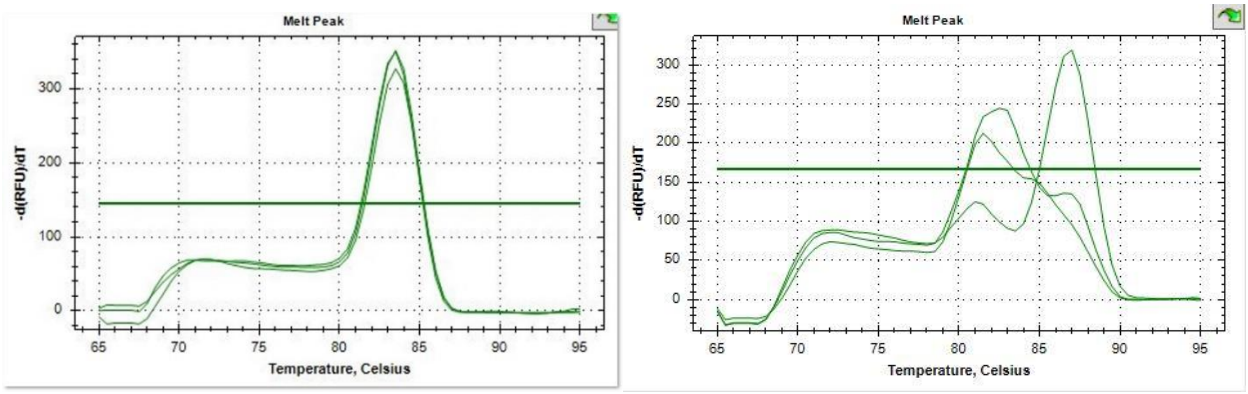

Figure 32 : Exemples de courbes de fusion. Le colorant (SYBR Green) est lié à l'ADN double brin (dsDNA) et émet de la fluorescence. En augmentant progressivement la température, l'ADN double brin se sépare en ADN simple brin, ce qui diminue la fluorescence. [−d(RFU)/dt] représente le taux de diminution de fluorescence, et les pics correspondent aux températures où la dissociation est la plus rapide. La présence d'un pic de fusion unique et similaire pour chacun des triplicatas (image de gauche) suggère une bonne amplification. Des pics décalés et peu alignés indiquent plusieurs produits d'amplicons ou des produits aspécifiques à la réaction ciblée (image de droite).

Afin de vérifier que la taille des amplicons correspondait à la taille attendue, une migration par électrophorèse a été réalisé sur un gel d'agarose. Le gel d'agarose (Euromedex, France) à 2 % a été préparé dans du tampon tris-acétate-acide éthylènediaminetétraacétique (TAE) 1× (solution stock 50X Euromedex, France), chauffé jusqu'à dissolution complète, puis refroidi avant l'ajout de bromure d'éthidium (BET) (Euromedex, France) (0,5 µg/mL), intercalant de l'ADN permettant le marquage des amplicons. Le gel a été coulé dans un moule comportant un peigne permettant de former des puits de dépôt. Une fois le gel polymérisé, ce dernier a été placé dans une cuve d'électrophorèse et recouvert de TAE 1×. Pour chaque échantillon, 5 µL d'ADNc ont été prélevés sur la plaque et mélangés avec 1 µL du tampon de charge, permettant d'alourdir les échantillons pour qu'ils s'enfoncent correctement dans les puits et de les colorer pour vérifier leur dépôt et migration. Les échantillons ont ensuite été déposés dans les puits. Quatre µL de marqueur de poids moléculaire (ladder), contenant des fragments d'ADN de tailles connues, ont été déposés dans les puits aux extrémités du gel. En migrant sur le gel, ces fragments créent une échelle de référence qui permet de déterminer la taille des amplicons. La migration a été effectuée en deux étapes : d'abord à 80 V jusqu'à la sortie des échantillons des puits, puis à 110 V jusqu'à séparation complète des fragments. Les gels ont ensuite été visualisés sur un système ChemiDoc (Bio-Rad, États-Unis) et analysés à l'aide du logiciel ImageLab (Bio-Rad, États-Unis) (**Figure 33**).



Figure 33 : Migration sur gel des amplicons de qPCR pour confirmation des tailles. Les échelles formées par la migration du marqueur de poids moléculaire sont situées à gauche et à droite. La migration des amplicons figure entre celles-ci, avec la taille attendue (en haut) et le nom correspondant (en bas) de chaque produit d'amplification.

C) Procédure de réaction en chaîne par polymérase quantitative

1) Prélèvement des échantillons

Les échantillons ont été prélevés chez 62 femelles et 59 mâles issus des cohortes 4 et 5. Après le phénotypage comportemental, les animaux exposés au CMS ont subi deux semaines additionnelles du protocole de stress chronique. Des groupes de traitement ont été constitués équilibrant, autant que possible, les performances comportementales et la masse corporelle au sein de chaque groupe. Ces groupes ont eu les effectifs suivant : mâles traités au NaCl (témoins, n = 10 ; CMS-non-vulnérables, n = 12 ; CMS-vulnérables, n = 11), mâles traités à la Ket (témoins, n = 8 ; CMS-non-vulnérables, n = 12 ; CMS-vulnérables, n = 6), femelles traitées au NaCl (témoins, n = 10 ; CMS-non-vulnérables, n = 12 ; CMS-vulnérables, n = 10) et femelles traitées à la Ket (témoins, n = 10 ; CMS-non-vulnérables, n = 10 ; CMS-vulnérables, n = 10). Le traitement a été administré au moins 24 heures après la fin du CMS et, deux heures avant le prélèvement, les rats ont reçu une injection intrapéritonéale de NaCl 0,9 % (1 mL/kg) ou de Ket (10 mg/kg, 1 mL/kg) selon leur groupe. Les animaux ont ensuite été anesthésiés avec de l'isoflurane (4 %) dans une chambre à induction en plexiglas pendant 4 minutes, et décapités. Les cerveaux ont été rapidement extraits, placés sur glace dans une matrice de punch, et des échantillons du mPFC (régions infralimbique, PRL et ACC), du dHPC et de l'AMY ont été prélevés par punch. Les échantillons ont été transférés dans des tubes Eppendorf de 1,5 mL et placés dans un congélateur à -80 °C jusqu'à extraction de l'ARN.

2) Extraction d'ARN par précipitation

Afin d'éviter les contaminations, toutes les surfaces de travail ont été nettoyées avec un agent de nettoyage éliminant les enzymes de dégradation de l'ARN (RNaseZAP™, Sigma-Aldrich, France). Afin de mesurer la masse des échantillons, des tubes Eppendorf 1,5 mL destinés à les recevoir ont été pesés *via* une balance de précision (Pioneer™ Précision, Ohaus, États-Unis) puis placés dans la glace pour être refroidis. Les échantillons ont été transférés dans ces nouveaux tubes, puis à nouveau pesés, la différence représentant la masse des échantillons.

Huit cents µL d'agent de lyse UptiZol™ (Interchim, France) ont été pipetés dans les tubes, et chaque échantillon broyé manuellement. Après 3-5 minutes, 160 µL de chloroforme-isoamyl (49:1) (Sigma-Aldrich, France) ont été ajoutés dans les tubes, qui ont été retournés 10 fois pour homogénéisation, puis laissés pendant 2 minutes au repos. Le chloroforme-isoamyl a permis de séparer la phase aqueuse, contenant l'ARN, et la phase organique contenant les protéines.

Les échantillons ont ensuite été centrifugés (centrifuge 5810R, Eppendorf, Allemagne) à 12 000 g pendant 15 minutes à 4 °C. La phase aqueuse contenant l'ARN (~500 µL par échantillon) a été transférée par pipetage dans un nouveau tube, dans lequel a été rajouté 400 µL d'isopropanol (Propanol-2 ≥99.9 %, HiPerSolv CHROMANORM®, Avantor Sciences, États-Unis). Les tubes ont ensuite été placés à -20 °C pendant 24 h pour précipitation de l'ARN.

Le lendemain, les échantillons ont été centrifugés à 12 000 g pendant 15 minutes à 4 °C. Le surnageant a été jeté et les échantillons ont été lavés par pipetage d'1 mL d'éthanol 75 %, préparé à partir d'éthanol anhydre. Le culot a été décollé du fond du tube par refoulements *via* la pipette, puis les échantillons ont été à nouveau centrifugés (7 500 g, 5 min à 4 °C). L'étape de lavage a été réalisée deux fois pour éliminer un maximum d'impuretés. Après élimination du surnageant, les culots ont séché à l'air libre pendant 45 minutes, puis ont été dissous dans 40 µL d'eau ultra pure stérile. Enfin, les échantillons ont été placés au bain-marie à 60°C pendant 5 minutes pour favoriser la dissolution de l'ARN, puis plongés dans la glace pendant 3 minutes et stockés au congélateur à -80°C.

3) Dosage des ARN

Les concentrations ainsi que la pureté de l'ARN (estimée par les rapports de densité optique A260/280, A260/230, permettant de vérifier la quantité relative d'acides nucléiques, absorbant à 260 nm vis-à-vis des protéine, absorbant à 280nm et de sels contaminants, absorbant à 230nm) ont été mesurées à l'aide d'un spectrophotomètre NanoDrop (Thermo Fisher, États-Unis). Deux µL d'eau ultra pure stérile ont été placés pour calibrage du spectrophotomètre, puis les mesures ont été réalisées

avec 2µL de chaque échantillon. La concentration d'ARN mesurée a permis de calculer le volume d'échantillon nécessaire pour obtenir 750 ng d'ARN, quantité utilisée pour la transcription inverse (reverse transcription, RT). Les échantillons ont été stockés à -80 °C.

4) Transcription inverse et dilution des ADNc

Pour chaque échantillon, un volume contenant 750 ng d'ARN a été pipeté dans des tubes 0.2 mL pour qPCR (Bio-Rad, États-Unis), complété avec de l'eau stérile jusqu'à 16 µL, puis avec 4µL d'enzyme de RT (iScript™ Reverse Transcription Supermix, Bio-Rad, États-Unis). La réaction a été réalisée par incubation dans un thermocycleur selon un protocole validé au sein de l'équipe en 4 étapes :

1. Pré-amorçage (25°C pendant 5 minutes)
2. Transcription inverse (42°C pendant 30 minutes)
3. Inactivation de l'enzyme (85°C pendant 5 minutes)
4. Refroidissement et stabilisation (température abaissée à 10°C)

Les échantillons d'ADNc ont été dilués par deux avec de l'eau stérile ; des vérifications préalables ont permis de vérifier que la concentration résultante était suffisante pour une bonne amplification de tous les gènes étudiés. Enfin, les échantillons ont été conservés à -20°C.

5) Réaction en chaîne par polymérase quantitative

Les solutions pour la qPCR, ou « mix de qPCR », ont été préparées dans des tubes Eppedorf 1,5 mL dans lesquels ont été pipetés 13 µL d'ADNc dilué par deux, 104 µL d'H_2O stérile et 195µL d'agent fluorescent SYBR green (SsoAdvanced Universal SYBR Green Supermix, Bio-Rad, États-Unis). Les mélanges ont été homogénéisés par application des tubes à un vortex. Pour chaque échantillon, 12µL de ce mix ont été déposés pour les 8 gènes ciblés en triplicata (lignes représentées **Figure 34**) pour chaque échantillon (24 puits par échantillon), permettant de préparer une qPCR pour quatre échantillons par plaque de 96 puits (Hard-Shell®, Bio-Rad, États-Unis). Trois µL d'une solution d'amorces (diluées 1:100 dans de l'eau ultra pure stérile) ont ensuite été ajoutés dans les puits. L'analyse de chacune des structures étudiées a donc nécessité au total 30 plaques de 96 puits et un quart d'une autre plaque de 96 puits.

	Echantillon 1			Echantillon 2			Echantillon 3			Echantillon 4		
	1	2	3	4	5	6	7	8	9	10	11	12
A (*36b4*)												
B (*Bdnf*)												
C (*Fkbp5*)												
D (*Gr*)												
E (*Gria1*)												
F (*Grin2b*)												
G (Mao_a)												
H (*Psd95*)												

Figure 34 : Organisation d'une plaque de qPCR de 96 puits.

Les réactions de qPCR ont été réalisées sur un thermocycleur en temps réel (CFX Connect, Bio-Rad, Etats-Unis ; CFX Opus 96, Bio-Rad, États-Unis) avec le programme thermique suivant :

1) Dénaturation initiale (95°C pendant 30s)
2) 40 cycles comprenant dénaturation (95°C, 5s) puis hybridation/élongation (60°C, 45s), étape au cours de laquelle la fluorescence est enregistrée
3) Génération de courbe de fusion (95°C, 10s ; 65°C, 5s puis augmentation progressive de la température jusqu'à 95°C par paliers de 0,5 °C) afin de vérifier la spécificité des produits amplifiés

6) Analyse des données

Les données générées par le thermocycleur ont été exportées du logiciel CFX Maestro™ (Bio-Rad, États-Unis) sous forme de fichiers excels .xlsx contenant les données relatives à la qPCR, incluant le nombre de Cq et l'écart type du nombre de Cq pour chaque triplicata.

L'expression des gènes a été normalisée au gène de référence *36b4*. Cette expression relative a été calculée selon la méthode $2^{(-\Delta\Delta Cq)}$, en utilisant les mâles témoins traités au NaCl comme groupe de référence, ce qui a permis d'évaluer les effets du sexe, de l'exposition et la vulnérabilité au CMS, et du traitement. Brièvement, le ΔCq a été calculé pour chaque gène au sein d'un échantillon par différence du nombre de Cq entre le gène en question et le gène témoin (*36b4*). Le ΔΔCq a ensuite été calculé par différence du ΔCq du gène/échantillon considéré par le ΔCq moyen du groupe de référence (témoins traités au NaCl). Afin d'approfondir ces résultats et révéler de potentielles signatures transcriptomiques en fonction des groupes, des matrices de corrélations entre l'expression des gènes des différentes structures ont été générées grâce à des scripts MATLAB.

RÉSULTATS

Cette section inclut les résultats de cette thèse présentés sous forme d'articles de recherche. Le premier article, consacré à l'étude comportementale, a été publié. L'article portant sur l'étude ex vivo de la plasticité hippocampique, ainsi que celui concernant l'étude transcriptomique, sont actuellement en phase de finalisation et pourront être soumis prochainement. En ce qui concerne l'étude d'électrophysiologie in vivo, si la collecte des données expérimentales est terminée, l'étude des potentiels de champ local est encore en cours. De ce fait, cet article n'a pas encore atteint sa forme définitive en vue d'une soumission. En revanche, l'étude du rythme veille-sommeil est terminée.

Behavioural characteristics and sex differences of a treatment-resistant depression model: Chronic mild stress in the Wistar-Kyoto rat

L'article suivant, préalablement publié, a été intégré dans ce manuscrit sans modification. Les pages correspondantes présentent leur propre numérotation, distincte de celle du manuscrit. La numérotation continue du manuscrit reprend à l'issue de cette insertion

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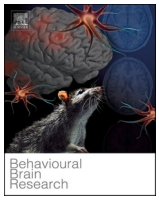

Research report



Behavioural characteristics and sex differences of a treatment-resistant depression model: Chronic mild stress in the Wistar-Kyoto rat

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ABSTRACT

Depression affects 20% of the general population and is a leading cause of disability worldwide, with a strong female prevalence. Current pharmacotherapies have significant limitations, and one third of patients are unresponsive. Male Wistar-Kyoto rats exposed to chronic mild stress (CMS) were recently proposed as a model to study antidepressant resistance. However, sex differences and interindividual vulnerability to stress are yet unexplored in this model. We aimed to investigate these in the context of the behavioural impact of CMS in the sucrose preference test, elevated plus maze (EPM), forced swim test (FST), open field test and daily locomotor activity rhythms, in male and female WKY rats exposed or not to a 4-week CMS protocol. CMS-exposed animals were clustered through K-means into subgroups based on the EPM and FST results. In both sexes, one subgroup behaved similarly to non-stressed animals and was labelled stress-non vulnerable; the second exhibited less open arms exploration in the EPM and higher immobility in the FST and was named stress-vulnerable. Vulnerable males presented phase delay in daily locomotor activity following CMS, but no significant rhythm could be determined in females. CMS-exposed males of both groups showed hyperlocomotion in reaction to novelty and slower weight gain through the course of CMS, while CMS-exposed females showed smaller sucrose intake. Unexpectedly, CMS did not affect sucrose preference. Our findings strengthen the view that in models of psychiatric pathologies based on stress exposure it is important to consider the effect of sex and to differentiate the non vulnerable and vulnerable subpopulations.

1. Introduction

Depression is a psychiatric disease which presents a major challenge to public health; according to the World Health Organisation, it is the primary cause of disability worldwide, with a prevalence of 10–20% of the general population [1]; notably, it is particularly prevalent in women, with a female/male ratio of 1.7:1 [2]. Depression is a disease significantly related to stress, considering that the experience of stressful events is an important risk factor for depressive episodes [3]. This is further amplified by genetic predispositions increasing vulnerability to stress [4]. Moreover, depressive patients show a dysregulation of the main stress response system, the hypothalamic-pituitary adrenal (HPA) axis, including weakened negative feedback processes, resulting in chronically elevated levels of blood cortisol [3]. A major concern regarding depression is that pharmacotherapies remain completely ineffective in about one third of the patients, whose disease is then labelled 'treatment-resistant depression' (TRD) [5]. One of the reasons why TRD remains difficult to overcome is poor knowledge of the underlying pathological brain processes, as well as the mechanisms through which other therapies such as deep brain stimulation or ketamine work. Nevertheless, a link between genetic mediated vulnerability to stress and resistance to conventional antidepressant treatment has been pointed out in humans, and experimentally observed in mice and non-human primates [6]. To further understand the physiological mechanisms of depression that ultimately lead to TRD, and study new therapeutic strategies, it is necessary to develop valid animal models [6].

As stress is a fundamental risk factor for depression, most valid animal depression-like models rely on stress exposure, based for example on social defeat [7]. The most commonly used stress-based model,

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however, relies on the exposure of rodents to a chronic mild stress (CMS) protocol, which includes several weeks of exposure to factors such as temporary restriction of access to water and/or food, modification of the light/dark cycle and wet bedding [8]. Recently, Paul Willner and collaborators [9] demonstrated that male Wistar-Kyoto (WKY) rats exposed to a CMS procedure showed impairments reminiscent of a depressive-like phenotype in comparison to non-stressed control animals [9]. These deficits were improved following subacute ketamine treatment or deep brain stimulation of the ventromedial prefrontal cortex, whereas the conventional antidepressant drugs imipramine, citalopram and venlafaxine were ineffective, conferring value of this model as one to study antidepressant resistant phenotype.

Nonetheless, important aspects of this model were left unexplored and are consequently addressed in the present study. First of all, numerous studies have raised the question of stress-related sex differences in the WKY strain, observing sex-dependent differences in tests evaluating anxiety-like behaviours when compared to other strains [10]; furthermore, a higher physiological response to stress in females has been indicated [11,12]. However, the question still remains overall unclear and poorly explored. Thus, the first aim of the present study was to focus on sex differences, an urgent need, as stated by Millard and colleagues [13]. Second of all, there exists strong interindividual vulnerability to stress due to genetic and epigenetic differences [14], so that in this type of experimental procedure there is the need to differentiate vulnerable from non vulnerable animals. Furthermore, although the WKY strain of rats is labelled as “inbred”, substrains with different characteristics can be bred from it [15], so that it is possible the remaining genetic heterogeneity in the strain may influence vulnerability to the CMS procedure. Therefore, the second aim of the study was to evaluate if interindividual differences in the response to CMS may be identified in male and female WKY rats. Finally, important behavioural aspects possibly influenced by chronic stress exposure remain yet unexplored, the investigation of which might increase our knowledge of the impact of CMS on WKY rats; therefore, in addition to the already performed exploration of anhedonia, body weight gain, and anxiety-like behaviour [9], our third goal was to explore new behavioural traits such as reaction to novelty, coping strategies in the face of an unescapable stressful situation, as maladaptive coping strategies to stressful life events correlate with depressive symptoms and are associated with more severe depression [16,17], as well as daily locomotor activity rhythm, as alterations of daily activity rhythm are important components of depression [18,19].

2. Materials and methods

2.1. Animals

This study, authorised by the French authorities (APAFIS #25270), was carried out with 70 WKY rats, 40 females and 30 males (Charles River, Sulzfeld, Germany). They arrived at the laboratory at the age of 4–5 weeks. They were housed in cages of 5 individuals for 1 week after their arrival, so they could acclimatise to the new housing conditions. They were then assigned to the different conditions; rats destined to the CMS protocol were housed individually while control animals were housed in pairs; males and females of the CMS condition were housed in the same room (3 m apart), and male and female controls were housed together (3 m apart) in a separate room. They were maintained on a 12 h light/dark cycle (lights on at 7:00 A.M.) with ad libitum access to food and water – except animals of the CMS condition according to the protocol (see below) –, with controlled temperature (23 ± 1 °C) and hygrometry of about 55%. One week after their arrival all rats were provided maintenance food (Altromin 1324) and remained on this diet during the entire study. Groups were composed as follows: males of the control condition (n = 6); males of the CMS condition (n = 24); females of the control condition (n = 8); females of the CMS condition (n = 32).

2.2. Experimental design

The timeline of the study is described in Fig. 1.

On week 3, rats were allowed to consume a 0.5% sucrose solution for 24 h in order to prevent neophobia during subsequent sucrose preference test (SPT). On week 4, they underwent home-cage locomotor activity testing (pre–CMS evaluation). On week 5, they were subjected to SPT and were weighed in order to obtain baseline measures. Then the stressors began to be applied in a sequence of events (see Table 1) which was repeated every week up until week 9, when the 4th and final intra-CMS SPT and weight measures were collected. Finally, animals underwent post–CMS behavioural testing during 3 weeks (week 10–12), including 24-h locomotor activity testing (post-CMS evaluation), then open field (OF) the day following the end of locomotor evaluation. The forced swim test (FST) sessions were conducted 48 h after the OF, and the elevated plus maze (EPM) the day after the second FST session; the three latter tests were performed between 9:00 AM and 1:00 PM (i.e., light phase) when CORT secretion, an indicator of the activity of the stress response HPA axis, is minimal and stable [20]. To fit this schedule, females were tested in two subgroups of 20 individuals, and males in two subgroups of 15 individuals.

2.3. CMS protocol

The CMS protocol lasted for four weeks and consisted of the following events (see Table 1): positioning of a metal plate in the bottom of the cage to isolate rats from the bedding; stroboscopic illumination (120 flashes/min) during the dark phase; food and/or water deprivation; reduction of space in the cage; intruder session; intermittent illumination (light on and off every 2 h) during the dark period; loud white noise (74 +/- 1 dB); soaking of the bedding (600 mL of water poured into the cage).

2.4. SPT

Sucrose preference was evaluated once weekly for 24 h. The regular water bottle was removed, and replaced by two new bottles, one containing 200 mL of water and one containing 200 mL of a 0.5% sucrose solution. Bottles were placed at 11:00 AM on Day 1 and removed at 11:00 AM the following day. The position of the water and sucrose bottles was alternated from one week to the other. Bottles were weighed before and after the test and the percentage of sucrose preference was assessed using the following formula [100 x (Weight difference of sucrose bottle / Weight difference of sucrose bottle + weight difference of water bottle)]. Then, sucrose preference measures obtained each week of the CMS procedure were indexed to the pre-CMS baseline measure. The consumption of sucrose and water were also calculated for each SPT in g consumed per g of body weight.

2.5. Body weight

Throughout the entire study, from their arrival until the end of the experimental procedure, animals were weighed once a week. After the start of the CMS protocol, the body weight was calculated as a percentage of the baseline (pre-CMS) measure to evaluate body weight gain during the 4 weeks of CMS.

2.6. Behavioural tests

2.6.1. Locomotor activity in the home cage

Locomotor activity was assessed in a novel home cage by means of two infrared light–beams perpendicular to the width of the cage, placed 4.5 cm above cage floor level and 28 cm apart along the length of the cage. The consecutive interruptions of both light beams were counted as one cage crossing. During both pre- and post-CMS sessions, rats were transported from the housing room to the test room, placed in their

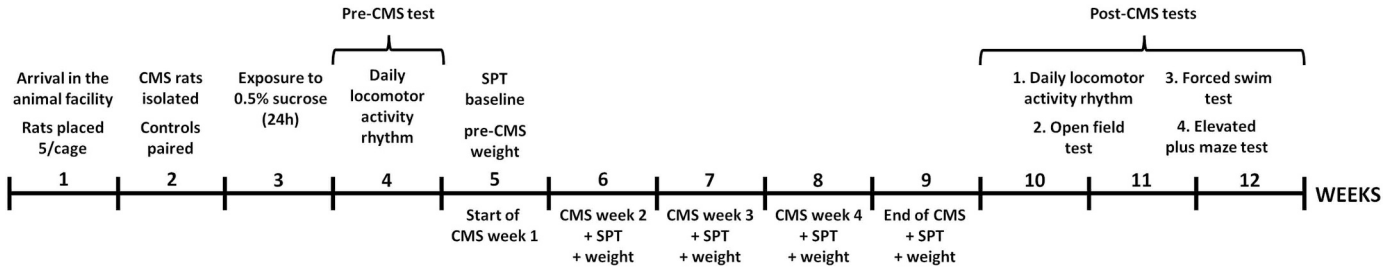

Fig. 1. Experimental design.

Table 1
Weekly schedule during the CMS protocol.

Week day	Morning	Afternoon
Day 1	New cage with clean bedding provided (**09 AM**). Start of SPT (**11 AM**).	Ongoing SPT
Day 2	End of SPT, weighing of the rats (**11 AM**). Regular water bottle returned, and metal plate placed on the bedding (**12 AM**).	Removal of metal plate (**6 PM**). Start of stroboscopic light (**8 PM**).
Day 3	End of stroboscopic light (**6 AM**). Food removed (**9 AM**).	Food returned (**5 PM**). Reduction of space (**6 PM**).
Day 4	Return to normal space (**9 AM**). Water removed (**10 AM**).	Water returned (**5 PM**). Start of intruder session (**6 PM**).
Day 5	End of intruder session (**9 AM**).	Start of intermittent illumination (**7PM**).
Day 6	Loud white noise ON (**9 AM**).	Loud white noise OFF (**5 PM**). Reduction of space (**6 PM**).
Day 7	Return to normal space (**9 AM**). Food and water removed (**10 AM**).	Food and water returned (**5 PM**). Soaking of the bedding (**6PM**).

respective testing cage, and recording was started at 10 AM for a 24-h duration. The activity during the first hour is influenced by some novelty due to the changes of room and cage, as well as the novel sawdust bedding [21] and is usually larger than the baseline diurnal activity. To assess locomotor reactivity to novelty, the first 10 min were analysed (identical duration to that used in the OF, which was also used to evaluate locomotor response to novelty; see 2.5.2). Moreover, we analysed the pattern of daily locomotor activity rhythm. Two-hour intervals of locomotor activity values were fitted by a non-linear least-squares COSINOR regression analysis [22] using the SigmaPlot Software (Systat Software Inc., version 12.5), with the following fitting equation

$$Y = A + B\left(2\pi * \frac{X - C}{24}\right)$$

where Y is the pooled number of crossings of the cage, A the MESOR (Midline Estimating Statistic Of Rhythm) or the mean level of the oscillation, B the amplitude of the oscillation, C the acrophase (time of the maximal value of the oscillation), X the time (h), with a period set at 24 h. Only significant best-fit parameters ($p < 0.05$) were included in this study if the residues of the fit respected the analysis of variance (ANOVA) rules.

2.6.2. OF

The test was performed in a square device (70 x 70 cm) comprising 50-cm high walls. A paper sheet comprising a mosaic of squares of equivalent sizes (14 x 14 cm, giving 5 x 5 squares) was placed under the clear Plexiglas floor of the OF in order to assess locomotor activity by numbering the crossings of the different squares. A crossing was counted when all 4 paws passed the delimitation between 2 adjacent squares. The number of rearings was also counted. The OF was cleaned with water and 70% ethanol between each rat. Rats were transported from their housing room to the room adjacent to the test room 1 h before the start of the test. The test started when the rat was gently placed facing the wall in one corner of the OF, and lasted for 10 min. The test session was recorded by means of a camera situated above the OF.

2.6.3. FST

The FST was performed as previously described by Gardner and colleagues [23] in a Perspex cylinder filled with water (21–23 °C) high enough so that the rats' tail could not touch the bottom of the cylinder. The test took place over two days; on each day rats were brought to the room and immediately tested. On day 1, the animals underwent a 15–minutes period of swimming as habituation, and the following day were tested over 6 min. The test session was recorded by means of a camera placed on the side of the cylinder, and the amount of time spent immobile (4 limbs immobile, with no swimming or struggling) was subsequently manually scored and expressed as the percentage of total time.

2.6.4. EPM

The apparatus was made of black Plexiglas, elevated 73 cm above the floor, and consisted of four arms (50 cm × 10 cm), with two opposing closed arms comprising 40 cm–high walls and two opposing open arms comprising 1.5 cm–high borders, fixed to a central platform (10 x 10 cm) forming a cross shape. Light intensity was 10 lx in open arms, 7 lx at the center of the maze, and 2.5 lx in closed arms. Rats were first brought to a room adjacent to the testing room; after an hour, they were placed in a new sawdust-free cage and brought into the testing room. The test began following 5 min of habituation to the room. Testing began when rats were placed on the central platform of the maze with their head facing one of the closed arms; it lasted for 5 min and was recorded by means of a camera situated above the maze. The maze was cleaned with water and 70% ethanol between each rat. The collected data included the number of entries and time spent in each of the four arms. Analysed data was the time spent in the open arms in percentage of the total time spent in the four arms.

2.7. Statistical analyses

First, on the basis of EPM and FST performances, CMS-exposed animals were subcategorized in clusters (see Supplementary Materials and Fig. S1). For statistical analyses, according to the EPM and FST, following clustering, performance of each cluster was compared to this of their respective control using pairwise Student's t-test. Other data were subjected to analysis of variance (ANOVA) with Group and Sex as between-subject factors and, when appropriate, with Week or Period (pre- vs post-CMS) as within-subject factors. In some cases, justified in the text, data for males and females were analysed separately in additional ANOVAs with Group as the only between-subject factor. The ANOVAs were completed by post hoc comparisons using the

Newman–Keuls multiple range test. To assess the impact of CMS on the rhythms of locomotor activity, amplitude, mean and acrophase of rhythms were compared between the pre- and post-CMS condition for each group using a paired t-test. The threshold for rejecting the null hypothesis was 0.05 throughout.

3. Results

3.1. Identification of "CMS-non vulnerable" and "CMS-vulnerable" animals

For each sex, the performance (% time in open arms in the EPM and % immobility in the FST) of clusters 1 and 2 revealed by the K-means algorithm (see Supplementary Materials and Fig. S2) were compared to that of control animals (Fig. 2). In both sexes, there was no significant difference between cluster 1 and controls in both tests [Females: EPM: $t(17) = -0.95$, $p > 0.3$; FST: $t(17) = 1.20$, $p > 0.2$; Males: EPM: $t(15) = 0.38$, $p > 0.7$; FST: $t(15) = 0.41$, $p > 0.6$], whereas there was a significant difference between cluster 2 and controls in both tests [Females: EPM: $t(24) = -5.22$, $p < 0.0001$; FST: $t(24) = 2.63$, $p = 0.01$; Males: EPM: $t(17) = -5.04$, $p < 0.001$; FST: $t(17) = 2.52$, $p = 0.02$]. Overall, these results indicated that, whatever the sex, performance of animals of cluster 1 was similar to that of control animals so that for each sex cluster 1 was hereafter referred to as "CMS-non vulnerable"; animals of cluster 2 presented a lower proportion of time in the open arms of the EPM and a higher proportion of time in immobility in the FST when compared to controls, so that for each sex cluster 2 was hereafter referred to as "CMS-vulnerable". The difference in proportion of vulnerable vs non vulnerable animals between males (46% vs 54%) and females (38% vs 62%) was not significant $X^2(1,53) = 0.34$, $p > 0.5$).

3.2. Sucrose preference test

3.2.1. Sucrose preference

At baseline (Fig. 3**A**), all groups showed preference measures significantly above 50% (at least $p < 0.00001$). The ANOVA indicated no significant effect of Sex ($F(1,61) = 0.035$, $p > 0.8$), no significant effect of Group ($F(2,61) = 1.59$, $p > 0.2$), and no significant Sex x Group interaction ($F(2,61) = 0.02$, $p > 0.9$). Overall, these analyses, indicated that at baseline, all groups presented similar preference for sucrose over water. In order to evaluate the evolution of the sucrose preference during the 4 weeks of the CMS procedure, data were indexed to baseline (Fig. 3**B-C**). Analysis indicated no significant effect of Group ($F(2,61) = 2.03$, $p > 0.1$), but a significant effect of Sex ($F(1,61) = 6.31$, $p = 0.01$) and a significant effect of Week ($F(3,183) = 13.94$, $p < 0.000001$). There was no significant interaction of Group x Sex ($F(2,61) = 0.12$, $p > 0.8$), Group x Week ($F(6,183) = 0.78$, $p > 0.5$), Week x Sex ($F(3,183) = 2.50$, $p > 0.05$) or Group x Sex x Week ($F(6,183) = 0.50$, $p > 0.8$). The post hoc conducted on the Week effect indicated significant differences between all weeks except between week 2 and week 3 ($p = 0.04$ for week 2 vs week 4, and $p < 0.01$ minimum for all other comparisons). Overall, these analyses indicated that the preference for sucrose (Fig. 3**B-C**) increased throughout the 4 weeks of CMS in control and CMS-exposed rats and that this increase was initially higher

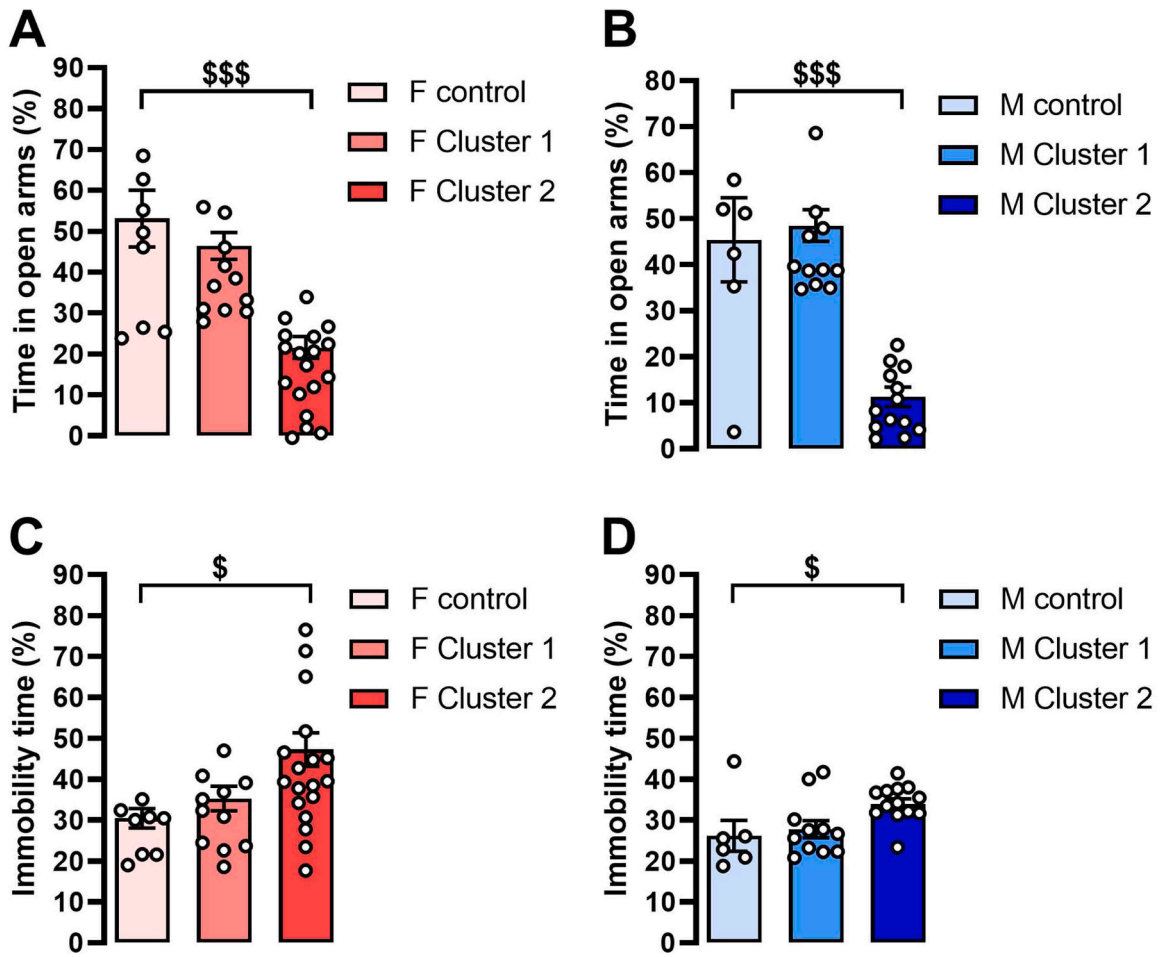

Fig. 2. Performance in the EPM (proportion of time spent in open arms) in females (**A**) and males (**B**), and performance in the FST (time spent immobile) in females (**C**) and males (**D**). Populations are as follows: females: control (n = 8), cluster 1 (n = 11), cluster 2 (n = 18); males: control (n = 6), cluster 1 (n = 11), cluster 2 (n = 13). $p < 0.05, $$$p < 0.001 control vs cluster 2 (t-tests).

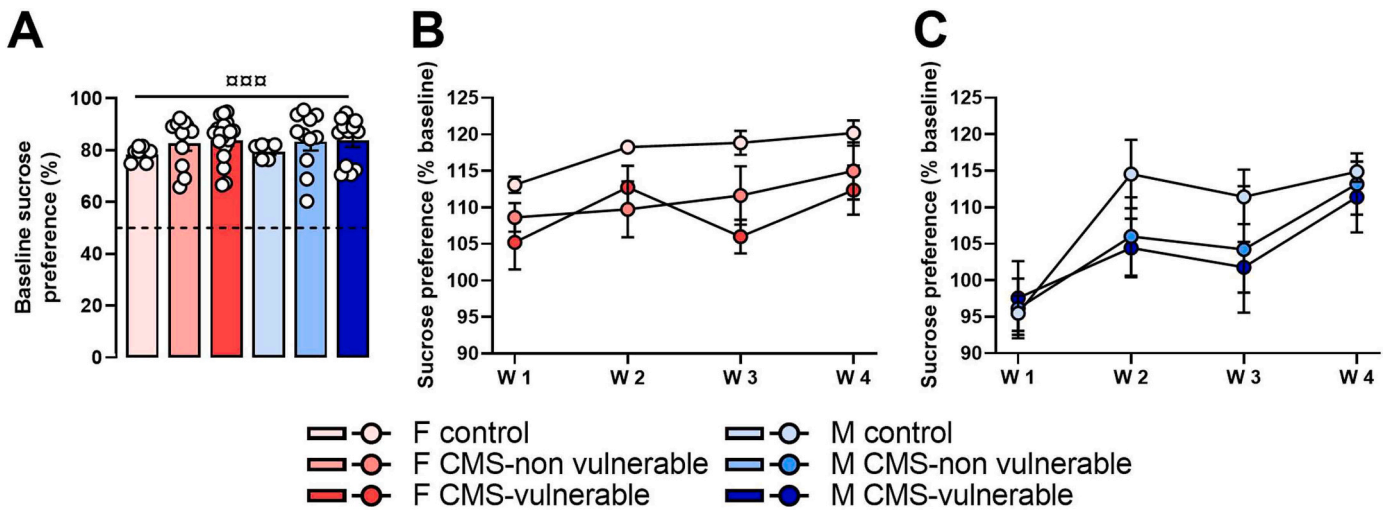

Fig. 3. Performance in the SPT for females of the control (n = 8), CMS-non vulnerable (n = 11) and CMS-vulnerable (n = 18) groups, and males of the control (n = 6), CMS-non vulnerable (n = 11) and CMS-vulnerable 2 (n = 13) groups, including baseline sucrose preference (**A**), and evolution of sucrose preference during the 4 weeks of CMS in females (**B**) and males (**C**). The dashed line marks the value of absence of preference (50%). ¤¤¤p < 0.0001 vs 50%. *Statistics for B and C*: main effect of sex and main effect of Week. For details, see text.

in female than in male.

3.2.2. Sucrose intake

Analysis indicated a significant effect of Sex ($F(1,61) = 83.64$, $p < 0.000001$), a significant effect of Group ($F(2,61) = 5.96$, $p < 0.01$) and a significant Sex x Group interaction ($F(2,61) = 6.72$, $p < 0.01$). In addition, there was a significant effect of Week ($F(4,244) = 5.32$, $p < 0.001$), and significant Week x Sex ($F(4,244) = 2.60$, $p = 0.04$), Week x Group ($F(8,244) = 2.64$, $p < 0.01$) interactions, whereas there was no significant Week x Sex x Group ($F(8,244) = 0.87$, $p > 0.5$) interaction. As the Sex X Group interaction was significant, analyses were performed separately by sex. In females (Fig. 4**A**), there was a significant effect of Group ($F(2,34) = 11.14$, $p < 0.001$) and of Week ($F(4,136) = 4.22$, $p < 0.01$), and a significant Group x Week interaction (F

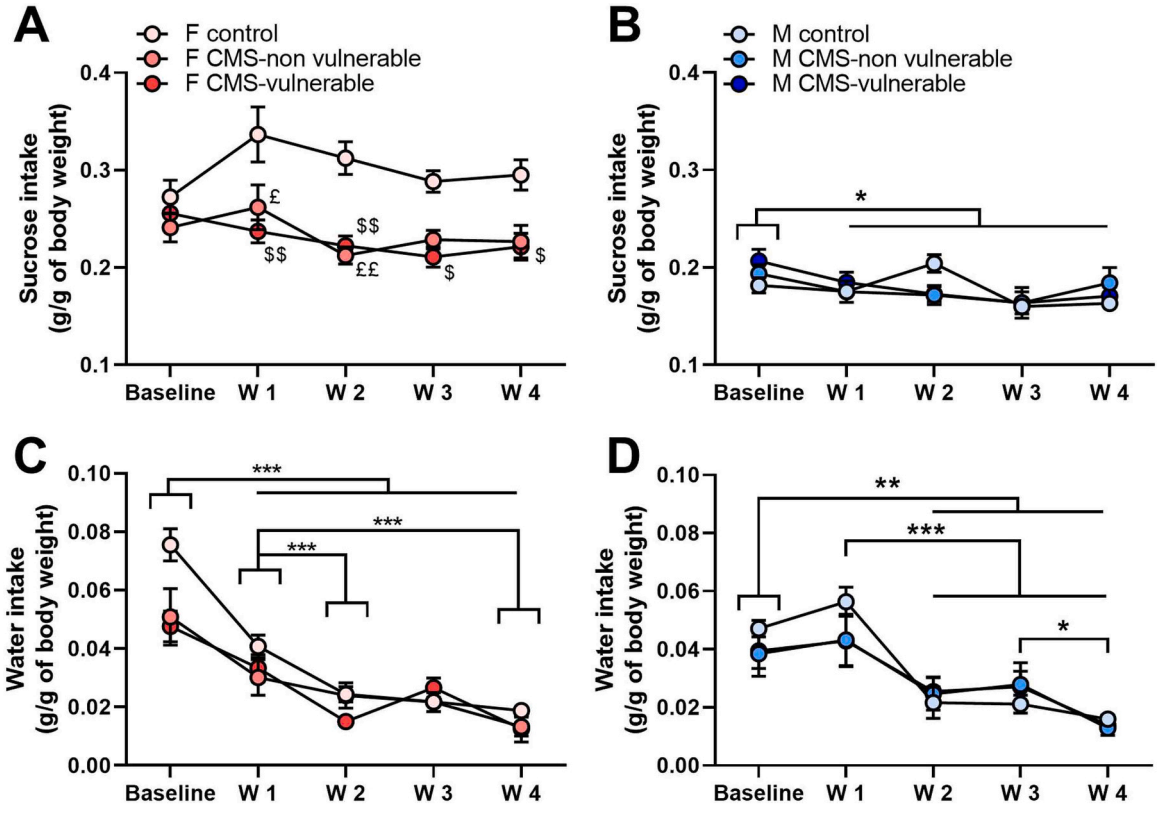

Fig. 4. Performance in the SPT for females of the control (n = 8), CMS-non vulnerable (n = 11) and CMS-vulnerable (n = 18) groups, and males of the control (n = 6), CMS-non vulnerable (n = 11) and CMS-vulnerable 2 (n = 13) groups, including the evolution of sucrose and water consumption in females (**A** and **C**, respectively) and males (**B** and **D**, respectively) from the baseline measure to week 4 of CMS. $p < 0.05, $$p < 0.01: CMS-vulnerable vs controls; £p < 0.05, £ £ p < 0.01: CMS-non vulnerable vs controls; *p < 0.05, * *p < 0.01, * **p < 0.0001 vs other time points.

(8,136) = 2.22, p = 0.03). Post hoc analysis indicated that while there was no between-Group difference in terms of baseline intake (0.48 < p < 0.73), sucrose intake of control animals significantly differed from that of both CMS groups during the first two weeks of CMS (week 1: p = 0.01 controls vs non vulnerable, p < 0.01 controls vs vulnerable; week 2: p < 0.01 controls vs non vulnerable, p < 0.01 controls vs vulnerable). While a significant difference was maintained with CMS-vulnerable animals throughout CMS (week 3: p = 0.03, week 4: p = 0.04), the difference was no longer significant with CMS-non vulnerable animals as from week 3 (0.059 < p < 0.1). In males (Fig. 4**B**), there was a significant effect of Week (F(4,108) = 4.23, p < 0.01) but no significant effect of Group (F(2,27) = 0.03, p > 0.9) and no significant Group x Week interaction (F(8,108) = 1.45, p > 0.1). Post hoc analysis of the Week effect indicated a significant decrease between baseline and all weeks of the CMS protocol (p = 0.02 for baseline vs week 1, p = 0.05 for baseline vs week 2, p < 0.01 for baseline vs week 3, p = 0.02 for baseline vs week 4), whereas there was no difference across the 4 weeks of CMS (0.10 < p < 0.93 among all comparisons).

3.2.3. Water intake

Analysis indicated a significant effect of Week (F(4,244) = 43.47, p < 0.000001) and a significant Week x Sex interaction (F(4,244) = 5.68, p < 0.001). However, there were no significant effect of Sex (F(1,61) = 0.002, p > 0.9), nor Group (F(2,61) = 1.66, p > 0.1), and no further significant interaction, whether it was Sex x Group (F(2,61) = 0.46, p > 0.6), Week x Group (F(8,244) = 1.52, p > 0.1), or Week x Sex x Group (F(8,244) = 0.55, p > 0.8). Post hoc on the Week x Sex interaction indicated that: i) In females (Fig. 4**C**) there was a significant difference between baseline intake and weekly intake during the CMS procedure (p < 0.0001), and between intake at week 1 vs week 2 (p < 0.01) and week 4 (p < 0.0001) of the CMS procedure, and, ii) in males (Fig. 4**D**), there was a significant difference between baseline intake and intake at week 2 (p < 0.001), week 3 (p < 0.01) and week 4 (p < 0.0001) of the CMS procedure, between intake at week 1 and each of the following weeks of the CMS procedure (p < 0.0001 for each comparison), and between week 3 and week 4 of the CMS procedure (p = 0.04). Overall, these analyses indicated that females consumed more sucrose than males. CMS-exposed females also consumed less sucrose than controls as from week 1 of CMS but this difference was no longer significant for CMS-non vulnerable females as from week 3. Males presented a slight decrease in their sucrose intake after the baseline, regardless of group. In both sexes, water intake decreased with time.

3.3. Weight gain

Analysis indicated a significant effect of Sex (F(1,61) = 13.47, p < 0.001) but no significant effect of Group (F(2,61) = 0.91, p > 0.4) and no significant Sex x Group interaction (F(2,61) = 1.46, p > 0.2). In addition, there was a significant effect of Week (F(3,183) = 822.60, p < 0.000001) and significant Group x Week (F(6,183) = 5.86, p < 0.0001), Sex x Week (F(3,183) = 12.57, p < 0.000001) and Group x Sex x Week (F(6,183) = 4.81, p < 0.001) interactions. Analyses were thus performed by sex. In females (Fig. 5**A**), the analysis showed no Group effect (F(2,34) = 1.5, p > 0.2), but a significant Week effect (F(3,102) = 292, p < 0.0001). Even if a significant interaction between Group and Week was present (F(6,102) = 2.78, p = 0.02), the subsequent post hoc showed no difference between groups at each week (0.182 < p < 0.972), though a significant difference between each week (p < 0.01 at least) within each group. In males (Fig. 5**B**), the analysis indicated no Group effect (F(2,27) = 0.87, p > 0.4) but both Week (F(3,81) = 638, p < 0.00001) and Week X Group (F(6,81) = 9.10, p < 0.00001) effects were significant. Post hoc analysis on the interaction showed a significant difference between controls and both CMS groups at week 4. It also indicated that in each group, all weeks were different from each other (p < 0.001 at least). Overall, these analyses indicated that while all groups naturally gained weight during the course of the experiment, CMS decreased weight gain at the end of the CMS procedure in males of both CMS-exposed groups whereas it did not affect weight gain in females.

3.4. Locomotor response to novelty

3.4.1. In a new home-cage (first ten minutes of recording during the pre- and the post-CMS tests)

Analysis of the number of cage crossings indicated a significant effect of Group (F(2,61) = 4.91, p = 0.01), no significant effect of Sex (F(1,61) = 2.28, p > 0.1), and no significant Group x Sex interaction (F(2,61) = 1.05, p > 0.3). There was no significant effect of Period (F(1,61) = 0.79, p > 0.3), no significant Period x Sex interaction (F(1,61) = 3.49, p > 0.06) and Period x Group (F(2,61) = 1.63, p > 0.2) interactions. However, there was a significant Period x Sex x Group interaction (F(2,61) = 4.65, p = 0.01). Therefore, analyses were performed in both sexes separately.

In females, analysis indicated a significant effect of Period (F(1,34)= 4.29, p = 0.05) but no significant effect of Group (F(2,34)= 0.85 p > 0.4) and no significant Period x Group interaction (F(2,34)= 0.47, p > 0.6). Visualisation of Fig. 6**A** indicates that female rats were generally less active during the post-CMS locomotor activity assessment, in all subgroups, when compared to the pre-CMS. In males, analysis

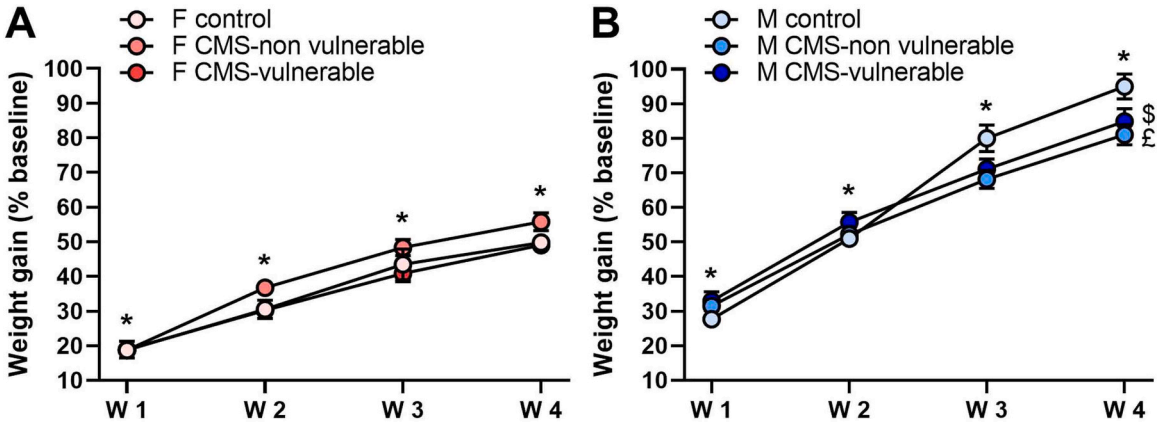

Fig. 5. Body weight gain in females of the control (n = 8), CMS-non vulnerable (n = 11) and CMS-vulnerable (n = 18) groups (**A**), and males of the control (n = 6), CMS-non vulnerable (n = 11) and CMS-vulnerable (n = 13) groups (**B**) during the 4 weeks of CMS. *p < 0.05 vs other time points; £p < 0.05 CMS-non vulnerable vs control, $p < 0.05 CMS-vulnerable vs control.

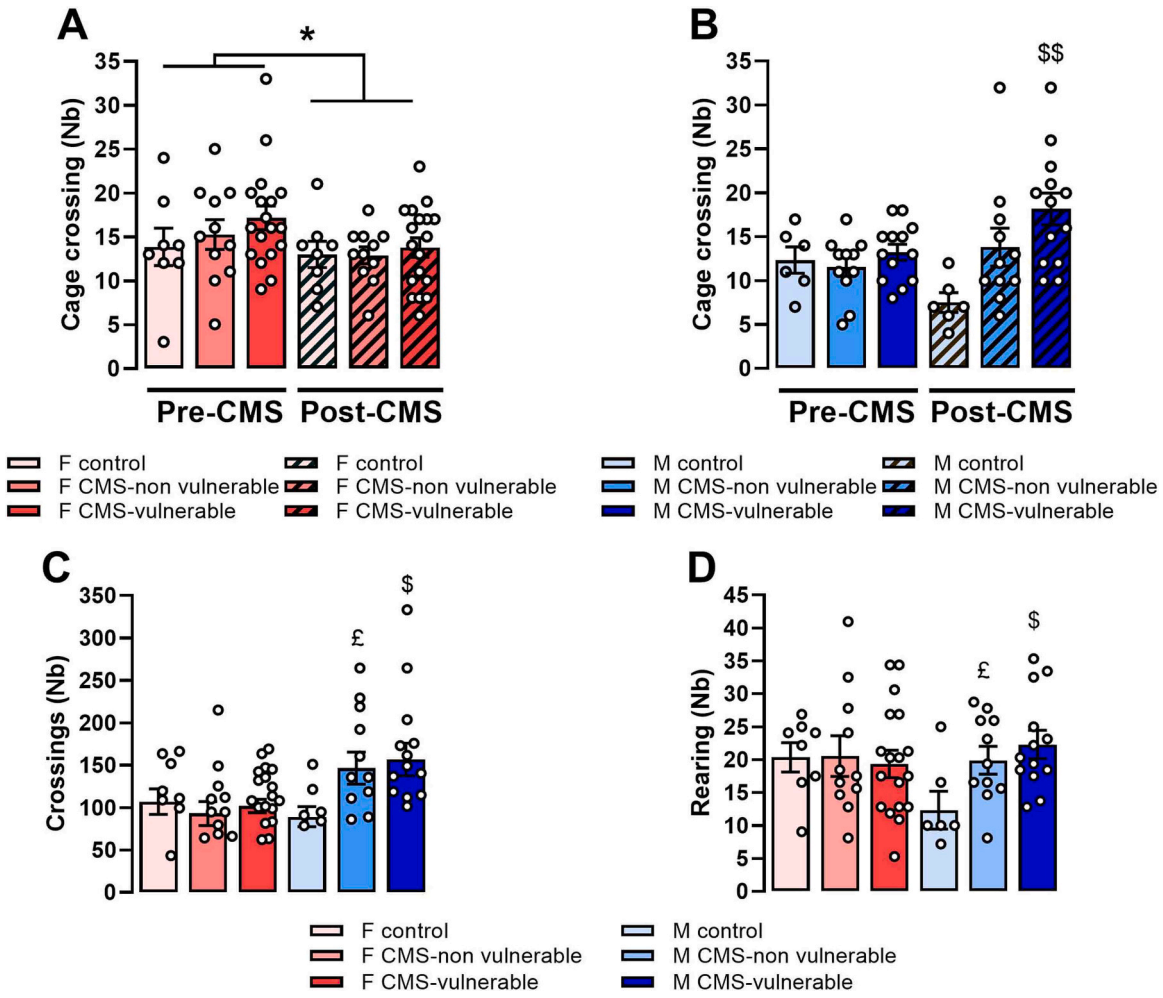

Fig. 6. Locomotor response to novelty for females of the control (n = 8), CMS-non vulnerable (n = 11) and CMS-vulnerable (n = 18) groups, and males of the control (n = 6), CMS-non vulnerable (n = 11) and CMS-vulnerable 2 (n = 13) groups. Number of cage crossings in the first 10 min of the locomotor activity test pre- and post-CMS in females (**A**) and males (**B**) either pre-CMS (plain bars) or post-CMS (striped bars). Total number of crossings (**C**) and total number of rearings (**D**) in the OF. *p < 0.05 pre- vs post-CMS; £p < 0.05 CMS-non vulnerable vs control; $p < 0.05, $$ p < 0.001 CMS-vulnerable vs control.

indicated no significant effect of Period ($F(1,27) = 0.43$, $p > 0.5$) but a significant effect of Group ($F(2,27) = 4.59$, $p = 0.02$) in addition to a significant Period x Group interaction ($F(2,27) = 5.05$, $p = 0.01$). Post hoc analysis indicated, at the post-CMS time point, a significant difference between animals of the control group and the CMS-vulnerable group ($p < 0.001$). At this time point, the differences between the CMS-non vulnerable and the two other groups did not reach significance ($p = 0.08$ for each comparison) (Fig. 6**B**). Overall, these analyses revealed in females a general decrease of reactivity during the post-CMS session; in males, they revealed that following CMS both CMS-exposed subgroups had an increased reactivity to novelty, which was exacerbated in CMS-vulnerable animals.

3.4.2. *In the OF*

Analyses of the numbers of crossings and rearings during the 10-min test indicated no significant effect of Group (crossings: $F(2,61) = 2.06$, $p > 0.1$; rearings: $F(2,61) = 1.46$, $p > 0.2$), no significant effect of Sex (crossings: $F(1,61) = 2.59$, $p > 0.1$; rearings: $F(1,61) = 0.80$, $p > 0.3$), and no significant Group x Sex interaction (crossings: $F(2,61) = 2.35$, $p = 0.06$ 0.1; rearings: $F(2,61) = 2.12$, $p > 0.1$). Visualisation of the figures nonetheless suggested the presence of significant differences in males, so that data of each sex were separately analysed. In females, no Group effect was evidenced for the number of crossings and rearings ($F(2,34) = 0.28$, $p > 0.7$; $F(2,34) = 0.08$, $p > 0.9$; Fig. 6**C-D**). In males, a significant Group effect was revealed for both variables (crossings: $F(2,27) = 3.62$, $p = 0.04$, Fig. 6**C**; rearings: $F(2,27) = 3.82$, $p = 0.03$, Fig. 6**D**). Post hoc analysis indicated a significant difference between CMS-exposed animals and controls (in crossings: $p = 0.04$ for controls vs non vulnerable, and $p = 0.02$ for controls vs vulnerable; in rearings: $p = 0.04$ for controls vs non vulnerable, and $p = 0.02$ for controls vs vulnerable), the former not differing from each other ($p > 0.4$ minimum). Overall, these analyses indicated that while females presented no between-group differences, CMS-exposed males of both subgroups presented a significantly higher number of rearings and crossings showing a greater exploratory response in a novel environment.

3.5. *Daily rhythms of locomotor activity*

3.5.1. *In females*

In the control (Fig. 7**A**) and CMS-non vulnerable (Fig. 7**B**) groups, there was a significant daily rhythm of locomotor activity before CMS exposure (cosinor, $p = 0.03$ and $p < 0.001$, respectively); however, no significant rhythmic activity was found after CMS exposure (cosinor, $p > 0.5$). In the CMS-vulnerable group (Fig. 7**C**) there was a significant daily rhythm of locomotion both in the pre-CMS (cosinor, $p < 0.001$) and post-CMS (cosinor, $p < 0.001$) periods. When we compared the rhythmic parameters between periods, only amplitude of rhythm was significantly different between the pre-CMS (9.8 ± 0.7) and post-CMS

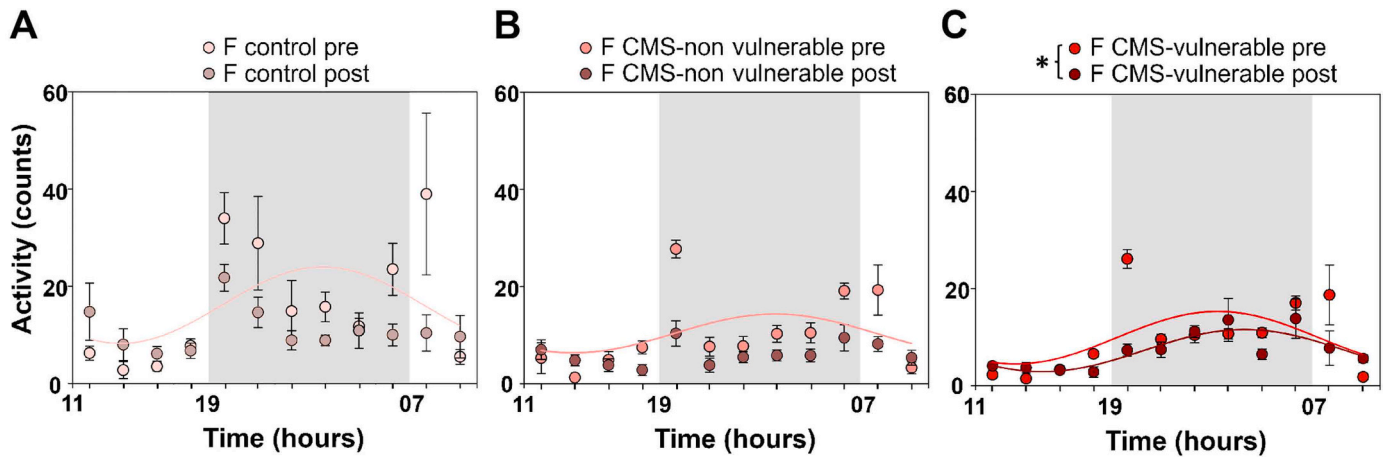

Fig. 7. Daily locomotor activity rhythm in females before and after the CMS period in the control group (n = 8) (**A**), the CMS-non vulnerable group (n = 11) (**B**), and the CMS-vulnerable group (n = 18) (**C**). The light and grey areas indicate the light and dark phase, respectively. For each graph, the curves represent the significant rhythms of daily locomotor activity. * $p < 0.05$ pre- vs post-CMS. (amplitude).

(7.2 ± 0.6) periods, indicating a reduced amplitude after CMS exposure ($t(34) = 2.74$; $p < 0.001$). No difference was found in the mean values of daily rhythms between pre-CMS (5.4 ± 1.0) and post-CMS (4.3 ± 0.9) ($t(34) = -0.78$; $p > 0.4$), nor in the acrophase (pre-CMS, 13.3 ± 0.7 vs. post-CMS, 14.9 ± 0.8; $t(34) = -1.48$, $p > 0.1$).

3.5.2. *In males*

In all three groups (Fig. 8**A-C**), there was a significant daily rhythm of locomotor activity at both the pre-CMS (cosinor $p = 0.01$ for controls, and $p < 0.001$ for CMS-exposed animals) and post-CMS (cosinor $p < 0.01$ for controls, and $p < 0.001$ for CMS-exposed animals) periods. There was a significant reduction in amplitude between the pre- and the post-CMS period in all groups (control: 8.6 ± 1.02 vs 5.1 ± 0.4; $t(10) = 3.11$; $p = 0.01$; CMS-non vulnerable: 8.3 ± 0.9 vs 4.3 ± 0.; $t(22) = -3.94$; $p < 0.001$; CMS-vulnerable: 7.7 ± 0.6 vs 4.7 ± 0.3; $t(22) = 3.92$; $p < 0.001$). In none of the groups were there significant differences concerning the mean value between the two time points (control: 4.3 ± 1.4 vs 2.1 ± 0.6; $t(10) = 1.42$; $p > 0.1$; CMS-non vulnerable: 3.4 ± 1.3 vs 2.5 ± 0.5; $t(22) = -0.63$; $p > 0.5$; CMS-vulnerable: 4.9 ± 0.94 vs 2.7 ± 0.5; $t(22) = 1.98$; $p > 0.05$). However, concerning the acrophase, whereas there was no difference between the two periods in the control (11.8 ± 1.2 vs 12.07 ± 1.1; $t(10) = -0.11$; $p > 0.9$) and CMS-non vulnerable (13.7 ± 1.4 vs 14.4 ± 0.8; $t(22) = 0.44$, $p > 0.6$) groups, a significant difference was found in the CMS-vulnerable group (13.2 ± 0.7 vs 15.8 ± 0.7; $t(22) = -2.52$; $p = 0.01$), indicating a phase delay (2.56 h) of the daily rhythm of locomotor activity after CMS exposure (Fig. 8**C**).

Overall, these analyses and observation of Figs. 7 and 8 indicated that all groups presented a significant rhythmic pattern of locomotor activity pre-CMS, but while such a pattern was still present in males during the second testing period, only females of the CMS-vulnerable group still had a significant rhythm of locomotor activity at the second period. Rhythms at the second testing period consistently presented a reduced amplitude regardless of the group, and CMS-vulnerable males showed a phase delay in their daily rhythm of locomotor activity.

4. Discussion

Our goal was to improve knowledge about the impact of CMS on WKY rats through the study of interindividual differences and the exploration of differences linked to sex on never studied behavioural features including the reaction to novelty, coping strategies in the face of an unescapable stressful situation, as well as daily activity rhythm.

Regarding inter-individual differences, k-means clustering based on behavioural results in the EPM and FST identified two distinct clusters in both male and female WKY rats exposed to CMS. Interestingly, in both tests, one behaved similarly to the control group and was thus called "CMS-non vulnerable". In contrast, the second cluster presented

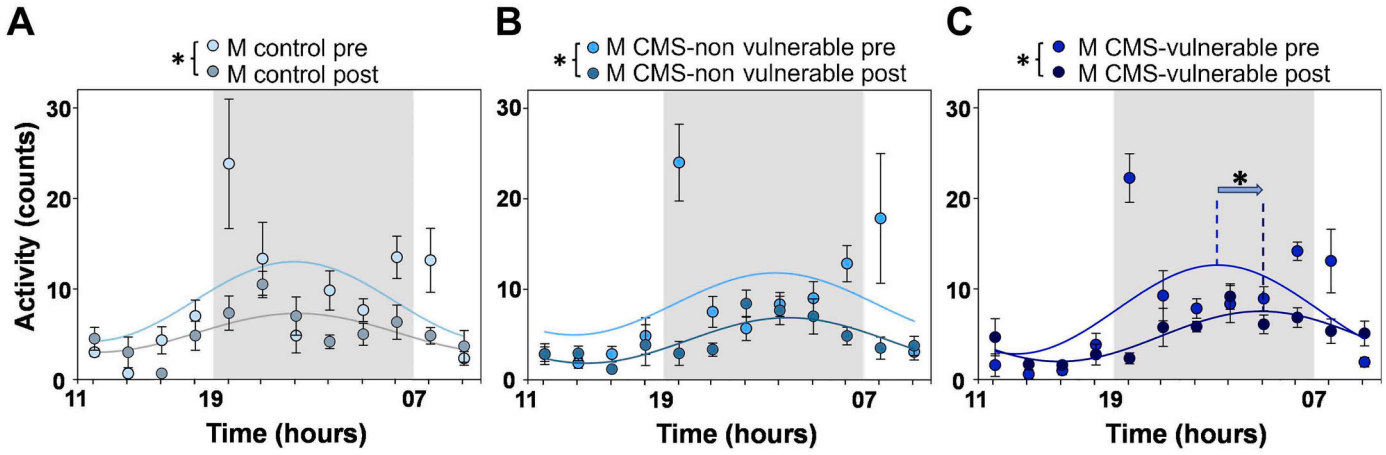

Fig. 8. Daily locomotor activity rhythm in males before and after the CMS period in the control group (n = 6) (**A**), the CMS-non vulnerable group (n = 11) (**B**), and the CMS-vulnerable group (n = 13) (**C**). The light and grey areas indicate the light and dark phase, respectively. For each graph, the curves represent the significant rhythms of daily locomotor activity. The dashed lines represent acrophase while the arrow indicates phase delay. *$p < 0.05$ pre- vs post-CMS period.

significant behavioural differences in both tests compared to the so-called "CMS-non vulnerable" group and to the control group. In both males and females, CMS-vulnerable animals presented a significantly smaller percentage of time in the open arms of the EPM, and a higher percentage of immobility in the FST, suggesting higher anxiety-like-related behaviour and a more passive coping strategy in the face of a stressful situation [24] in these animals compared to controls and CMS-non vulnerable rats. Higher anxiety-like behaviour in the EPM and immobility in the FST are often reported as a consequence of chronic stress in both male and female of different strains [25–30] including the WKY, at least in males [9]. However, these studies do not consider interindividual differences in vulnerability to stress. Our study shows that in both sexes, only some individuals were affected, while others showed a non vulnerable profile. Thus, even in this strain, considered as inbred, not all animals responded in the same way to CMS, raising the question of what factors influence individual vulnerability to stress. The two main causal factors may be genetic differences and/or environmental factors. Concerning the former, phenotypic and genotypic variability among WKY rats from the same colony have already been reported, to the point where selective breeding allows the generation of distinct WKY substrains [15]. Concerning environmental factors, it has been shown that rodent maternal care, such as pup licking and grooming, very often differs in amount between pups of the same litter [31], and leads to epigenetic modifications altering vulnerability to stress [31–33]. In parallel, clinical studies point out stressful life events [34–36], in particular early life events [35,36], as major risk factors for vulnerability to depression. As Strekalova and colleagues pointed out, interindividual variability in the susceptibility to stress is an important factor of any study exploring the behavioural and physiological consequences of stress exposure; some animals show vulnerability, i.e. present lasting consequences, while others are non vulnerable, so that a stratification of CMS-exposed animals into such categories is recommended [14]. Our study shows that using EPM and FST performance as segregation criteria, male and female WKY rats can be separated into CMS-non vulnerable and CMS-vulnerable groups. These behavioural characteristics are of great interest, as in humans, stressful life events are a particularly important causal factor for depression onset [37] in conjunction with dysfunctional processing and reaction to the situation, i.e. maladaptive coping, constituting an essential core of vulnerability [35]. Comorbid anxiety is equally relevant, as elevated anxiety is associated with greater severity of depression [38,39] and has been suggested to be core feature of depression [39].

Our data also indicated that only CMS-vulnerable males displayed a phase delay of their daily locomotor activity rhythms. It is a known fact that glucocorticoids can modulate rhythms and shift circadian phases [40], and phase shifts have previously been observed as a consequence of CMS in male Wistar rats, although as phase advances [41]. In parallel, depressed patients often present sleep and circadian rhythm disturbances [18] including phase delays of activity rhythms and hormonal release [19]. Unfortunately, as in females the daily rhythm of locomotor activity was not significant at the second time point for controls and CMS-non vulnerable animals, analysis is limited. The absence of a significant rhythm in those two groups may be due to the great variability of activity onset in females compared to males, as it is strongly modulated by the estrus cycle [42], the phase of which was not controlled for in the present study. Nonetheless, CMS-vulnerable females, who did present a post-CMS rhythm, did not display a phase delay. This difference with males could be the result of a differential impact of stress on clock genes, which regulate circadian rhythms, between females and males that would lead to greater impairments in the latter. A study conducted on Sprague-Dawley rats found sex specific effects of acute stress on the expression of clock genes, increasing the expression of the Per2 gene in the prefrontal cortex and hypothalamic paraventricular nucleus in females but not males [43]. However, this study did not consider locomotor rhythms and the authors emphasise that additional investigations of sex differences in the impact of acute and chronic stress on molecular clock rhythms are needed.

While the behaviour of CMS-vulnerable and non vulnerable animals differed in the EPM and FST, the chronic stress regimen affected other physiological or behavioural characteristics similarly in both groups, albeit in a sex-dependent manner. First, CMS impaired body weight gain in males only, in line with studies conducted in other strains such as Wistar [44,45], and Sprague-Dawley [46], and consistent with Willner's study on male WKY rats [9]. The fact that deficits in body weight gain were observed in male rats of both CMS subgroups suggests that despite presenting no impairments in terms of behavioural performance in the EPM, FST, and phase of the locomotor activity rhythm, CMS-non vulnerable male rats may still present CMS-induced metabolism impairments. This has previously been observed in the Wistar rat, in which the CMS-exposed group classified as "resilient" - albeit based on sucrose consumption or preference results - presented similar body weight gain deficits as the vulnerable group [47,48]. In contrast, CMS exposure did not affect body weight gain in females, which is coherent with previous studies in female Wistar [44,49] and Sprague-Dawley [46,50] rats. Furthermore, chronic administration of corticotropin releasing factor, which stimulates glucocorticoid release and which production is increased with CMS exposure [51], reduces body weight gain in male but not female Wistar rats, a differential metabolic response attributed to sex hormones, further suggesting a protective role against stress-induced metabolism impairments in females [52].

Secondly, we observed CMS-enhanced locomotor reaction to novelty in males, but not females. Indeed, only the former presented higher exploratory behaviour, in the OF and in a new home-cage during the first 10 min of actigraphy testing, although in the last case the CMS-non vulnerable group was below but near the significance threshold. The fact that locomotor activity during the remainder of the day, in both the light and dark periods, was similar in both CMS-exposed and control rats (data not shown), and that there was no inter-group difference prior to CMS, suggests that this enhanced activity is both provoked by the novelty of the situation, and is due to CMS exposure. Although some studies report a decrease in locomotor activity in the OF following CMS [53,54], impairments such as increased locomotor activity of CMS-exposed males has been observed previously in the OFT in Wistar [55] and Sprague-Dawley [56] rats. Furthermore, Strekalova and colleagues pointed out that rats exposed to CMS can display non-specific hyperlocomotion and general behavioural invigoration as a response to the slightly stressful procedure of behavioural testing [14] which may explain the difference observed in the present study. A similar sex-specific pattern of enhanced locomotion in males and no changes in females following CMS has been observed previously in Sprague Dawley rats [57].

Unexpectedly, CMS did not decrease the sucrose preference in either females or males. In fact, all groups, including controls, presented an increase of preference with time. According to Willner, several research groups have reported similar findings, which were branded as 'anomalous' [58]. However, studies exploring the consequences of CMS on the outcome of the SPT face significant reproducibility issues across laboratories [59]. Based on preliminary results, and recommendations of using the minimal concentration of sucrose in the solution [59], our study employed a 0.5% sucrose solution, rather than the usual 1%, but it is possible that further reduction of the concentration may lead to other results [59]. The length of the test may also play a role on behavioural results; although we also explored shorter test periods, i.e., 6 or 2 h (data not shown), a duration as short as a single hour may yield differences between groups [60]. It is also possible that longer periods of habituation to the sucrose solution could be necessary; although in the context of sucrose consumption, Mariusz Papp suggests as many as 7–9 weeks of weekly evaluation may be required before a stable and reliable consumption is reached [61]; in our case, preference, which gradually increased with time, may also only stabilize after several weeks of exposure. Nonetheless, regarding fluid *intake* per body weight, CMS-females did present a significantly smaller sucrose intake than

controls, which was not the case for males. If sucrose intake reflects a pleasurable activity, this may suggest that although CMS females did not exhibit anhedonia per se, i.e. a decrease in their preference for a palatable solution, CMS exposure could still impact their incentive for an appetitive behaviour. Additionally, we observed an overall higher sucrose intake in females compared to males; this has been previously observed in Wistar rats [44], in a study in which the authors proposed this phenomenon was not related to hedonistic behaviour but rather a sex steroid mediated difference in taste threshold of sucrose, decreased in females compared to males, therefore leading to increased intake [62].

Although both sexes seemed to respond differently to the CMS protocol depending on the test, as previously discussed, the proportion of CMS-non vulnerable and vulnerable individuals was similar in both sexes, therefore suggesting that females did not present increased vulnerability to chronic stress. While the sex-dependent response observed in the present study is consistent with previous findings [63], our results are not in line with a postulate that in rodents, males have better habituation than females to a chronic stress situation [63]. This hypothesis is based on observations that in chronically restrained Sprague Dawley rats, corticosterone release was observed for a longer period and in larger quantity in females [64], and by the observations by Paré and colleagues that female WKY rats exposed to chronic restraint stress, but not males, present impaired responses in a passive avoidance task and reduced feeding behaviour [12]. These studies however employed chronic restraint and not CMS as the stress regimen. In humans, the question of increased vulnerability to stress in women has been raised [65]. This “differential vulnerability hypothesis” could explain the higher prevalence of depression in women [2]. While some authors do suggest increased vulnerability to stressful life events in women [66], or increased heritability of resilience in men [67], the hypothesis of an increased vulnerability to stress in women is challenged [68], and such studies propose that higher female prevalence in stress related diseases is due to increased exposure rather than vulnerability to stress [65,68]. Nonetheless, further studies exploring the proportion of vulnerable vs non vulnerable animals between males and females are needed in the preclinical domain for a more in-depth comparison with clinical data.

5. Limitations of the study

The present study is not without limitations. Firstly, the absence of CMS effects on sucrose preference prevented vulnerable segregation based on this parameter. The estrous cycle was not controlled in female rats, which could have contributed to conclude on the absence of activity rhythm in females following CMS. Given the WKY’s spontaneous anxiety- and depressive-like behaviour, it would have been interesting to measure their basal anxiety-like and stress coping behaviours, and their progression with the ongoing CMS protocol. However, the EPM and FST cannot be performed several times, as repeated exposure in the former modifies behaviour, which no longer represents anxiety-like behaviour, through aversive learning [69,70], and given the stressful nature of the latter, repeated testing may in effect chronically stress the controls, as it has been shown to increase immobility in Wistar rats [71]. There is therefore a need for more depression-related behavioural tests that can be repeated to assess individual development.

6. Conclusion

Overall, our results show that CMS differentially impacts WKY rats depending on their vulnerability to stress and depending on their sex. The stress protocol leads to behavioural impairments relevant in the context of depression, such as anxiety-like behaviour and maladaptive coping strategy in a subset of animals of both sexes, and a phase delay of daily locomotor activity rhythms in vulnerable males. We also observed weight gain impairments and hyperlocomotion in reaction to novelty in all CMS-exposed males, and a reduction in sucrose intake in all CMS-exposed females. Our study suggests CMS-exposed WKY rats to be a good model of depression-like behavioural manifestations based on construct and face validity, and reinforce the importance of investigating differences of sex and inter-individual vulnerability to stress in such studies.

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Author statement

The authors declare that animals were handled in accordance with ethical requirements enacted by the French authorities and the Strasbourg university who approved the present project (APAFIS #25270).

Declaration of Competing Interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bbr.2023.114712.

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Chronic mild stress in the Wistar-Kyoto rat induces both short- and long-term hippocampal plasticity impairments differentially reversed by acute and chronic ketamine treatment

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A) Abstract

Chronic stress induces strong alteration of hippocampal synaptic plasticity, impairing long-term potentiation (LTP) and facilitating long-term depression (LTD), processes thought to be involved in the pathophysiology of depression. Ketamine (Ket), a noncompetitive NMDA receptor antagonist, has emerged as a fast-acting antidepressant whose action is believed to involve restoring impaired plasticity, though its sustained effects are elusive. Here, we investigated how chronic mild stress (CMS) and subsequent Ket treatment modulate CA3–CA1 synaptic plasticity in the dorsal hippocampus of CMS-vulnerable Wistar-Kyoto (WKY) rats. Adult male WKY rats were subjected to four weeks of CMS, and vulnerability was determined through behavioural performance in the forced swim test (FST) and elevated plus maze (EPM). CMS-vulnerable rats received either an acute or chronic (daily for one week) subanaesthetic dose of Ket (10 mg/kg, ip) or saline, and hippocampal slices were harvested for synaptic plasticity investigation. In saline-treated CMS-vulnerable animals, high-frequency stimulation (HFS)-induced LTP was impaired, and LTD could not be induced following low-frequency stimulation (LFS). Acute Ket restored normal LTP but led to potentiation rather than LTD following LFS, while chronic Ket prevented LTP yet enabled normal LTD induction. These findings indicate that Ket strongly modulates hippocampal plasticity in a treatment-dependent manner: acute administration seems to transiently depress the synapse before strongly promoting potentiation, whereas chronic exposure may drive homeostatic potentiation leading to saturation, but reenabeling depression. The results highlight complex temporal dynamics in Ket's modulation of hippocampal plasticity following chronic stress which involve an interplay between Hebbian and homeostatic mechanisms.

B) Introduction

Exposure to stressors or potential threats elicits physiological responses allowing the maintenance of homeostasis, which enables adaptive coping, essential for health and well-being. These responses include the activation of the rapid sympathetic adrenal medullary system and the slower-acting hypothalamic-pituitary-adrenal (HPA) axis [(Smith & Vale, 2006) (Joëls & Krugers, 2007) (Ulrich-Lai & Herman, 2009)]. When exposure to a stressor becomes repetitive, it can have deleterious consequences and represents a risk factor for developing psychiatric pathologies such as depression [(de Kloet et al., 2005) (Chrousos, 2009) (Lucassen et al., 2014) (Godoy et al., 2018)]. In animal models, chronic stress induces deleterious alterations in synaptic efficacy in the dorsal hippocampus (dHPC), particularly along critical communication pathways such as the Schaffer collaterals (SC) connecting cornu Ammonis (CA) regions CA3 and CA1. The HPC is of utmost importance in the response to stress, as it is a main regulator of the HPA axis, acting as a negative feedback control [(Lupien & Lepage, 2001) (Tasker & Herman, 2011)].

Synaptic plasticity mechanisms in regions such as the dHPC refer to the ability of synapses to modify their efficacy in response to specific patterns of activity. High frequency of activity typically leads to long-term potentiation (LTP), whereas low frequency of activity is associated with long-term depression (LTD). This ability is a hallmark of the Hebbian concept of associativity and represents the physiological basis of adaptation to one's environment (Albadawi, 2025). Chronic stress, including chronic mild stress (CMS), impairs SC LTP and facilitates SC LTD in the dHPC [(Kamal et al., 2014) (Logue et al., 2022) (Yang et al., 2018) (Alfarez et al., 2003)] (Von Frijtag et al., 2001) [see also (Marsden, 2013) (Chattarji et al., 2015) for review]. Rodents chronically exposed to stressors are routinely used to study the neurobiology of stress-related psychiatric pathologies such as major depressive disorder [(Willner & Belzung, 2015) (Slattery & Cryan, 2017)]. Such models help to understand cellular dysfunctions resulting from stress exposure. They also help to explore treatment strategies, as several types of antidepressants have been shown to reduce chronic stress-induced deficits in dHPC LTP and/or LTD, including the tricyclic imipramine (Von Frijtag et al., 2001), the selective serotonin (5-HT) reuptake inhibitor fluvoxamine (Holderbach et al., 2007) or the 5-HT/noradrenalin reuptake inhibitor milnacipran (Matsumoto et al., 2005).

A key issue concerning depression is that approximately 30 % of the patients remain unresponsive to standard antidepressants, a condition coined as treatment-resistant depression (TRD). The neurobiological bases of such resistance remain poorly understood [(Gaynes et al., 2020) (Klok et al., 2019) (Miola et al., 2023)]. Because of the rapid onset of its beneficial antidepressive effects,

ketamine (Ket), a noncompetitive inhibitor of the NMDA receptor, was first proposed and used to treat major depressive disorder [e.g. (Berman et al., 2000); see review (Averill et al., 2020)]. It was then discovered that acute Ket administration was the only pharmacotherapy improving TRD [(Walter et al., 2014) (Jelen & Stone, 2021) (Kim et al., 2024) (Meshkat et al., 2023)]. Interestingly, as pointed out in previous studies [e.g. (Walter et al., 2014) (Aleksandrova et al., 2019)], its peak efficacy occurs at a time when Ket is no longer present in an active form in the organism. This suggests that the beneficial effects may depend on long-term plasticity mechanisms such as hippocampal LTP [see review (Duman et al., 2016)]. Indeed, subanesthetic Ket doses restore chronic stress-induced impaired LTP [(Yang et al., 2018) (Logue et al., 2022)]. However, the beneficial effects of acute Ket are short-lasting; therefore, to prolong its efficacy, chronic treatments have been proposed and are currently under study [e.g. (Loo et al., 2023) (d'Andrea et al., 2024); see (McMullen et al., 2021) (Nikolin et al., 2023) for review]. Nonetheless, chronic Ket use carries the risk of addiction and can induce adverse effects, including cognitive impairments and psychomimetic symptoms [(Short et al., 2018) (Na & Kim, 2021) (Strong & Kabbaj, 2018)]. Therefore, more studies are needed to understand the physiological bases underlying the action of Ket, particularly under chronic administration. To better understand the neurobiology of TRD, CMS exposure in the Wistar-Kyoto (WKY) rat was proposed and shown to recapitulate key depressive-like behavioural deficits [(Willner et al., 2019) (Loizeau et al., 2024)]. Notably, acute Ket was the only pharmacotherapy shown to reverse these stress-induced deficits (Willner et al., 2019), making this model particularly relevant to study the physiological mechanisms underlying the action of Ket.

The aim of this study was to investigate dHPC SC LTP and LTD in WKY rats exposed to CMS and the impact of acute and chronic Ket administration on their induction. Only a subset of rats showing behavioural vulnerability to CMS exposure, as evidenced in our previous work (Loizeau et al., 2024), were used in this study to better represent a pathological state akin to depression.

C) Materials and methods

1) Animals

This study was conducted on male WKY rats (Charles River, Sulzfeld, Germany) from three different cohorts. For each cohort, rats arrived at the laboratory at the age of 4 to 5 weeks and were housed in groups of 5 for one week. They were then either assigned to the CMS group or to a non-stressed control group. Rats assigned to the CMS protocol were subsequently housed individually, while controls were housed in pairs in a separate room. Animals were maintained on a 12 h light/dark cycle (lights on at 7:00 A.M.), with ad libitum access to food (Altromin 1324) and water – except

animals undergoing CMS at specific periods of the protocol. Temperature was controlled throughout the study (23 ± 1 °C).

Experimental protocols and animal care were in compliance with the European Community Council Directive (2010/63/UE) and were approved by the regional ethical committee and the French Ministry of National Education, Higher Education and Research (APAFIS#25270).

2) Experimental design

Rats assigned to the CMS group underwent the stress protocol during four consecutive weeks. It was applied identically to our previous study (Loizeau et al., 2024). Behavioural testing was then conducted using the forced swim test (FST) and elevated plus maze (EPM) to identify CMS-vulnerable animals (Loizeau et al., 2024). Application of the CMS protocol was subsequently resumed for two additional weeks. Rats were then treated with either NaCl or Ket, according to group assignment, and were sacrificed for collection of brain slices including the dHPC. Finally, electrophysiological recordings were performed on the slices to investigate synaptic plasticity mechanisms.

3) Behavioural phenotyping

The FST was conducted over 2 consecutive sessions as previously described (Loizeau et al., 2024). Briefly, rats underwent a 15-minute habituation swim on day 1, followed by a 6-minute test on day 2, in a water filled plexiglass cylinder at controlled temperature (21 – 23°C). The amount of immobility time was manually assessed and expressed as a percentage of total time spent during the 6-minute test.

The EPM test was conducted as previously (Loizeau et al., 2024). Briefly, rats were tested in a black Plexiglas elevated plus maze with two open arms and two closed arms, under controlled lighting (10 lx in open arms, 7 lx at the centre of the apparatus, and 2.5 lx in closed arms). After 1 hour of habituation in an adjacent room, each rat was placed in the center of the maze facing one of the closed arms, and tested for 5 minutes. Time spent in the open arms was recorded and expressed as a percentage of total time in both open and closed arms.

Based on the percentage of immobility time in the FST, and the percentage of time spent in the open arms of the EPM, CMS-exposed rats of each cohort were clustered into two subpopulations using k-means (Loizeau et al., 2024) to distinguish CMS-vulnerable animals, i.e. displaying less time in the EPM's open arms and more time immobile in the FST, from non-vulnerable rats that presented control-like performance in those tests and were not included in this study. Three subgroups were constituted

from the CMS-vulnerable animals with as similar behavioural performance as possible, and destined to different treatment: NaCl (CMS-vulnerable-NaCl group), acute Ket treatment (CMS-vulnerable-Ket1 group), and chronic Ket treatment (CMS-vulnerable-Ket7 group).

4) Experimental groups and drug treatments

Control-NaCl (n = 8) and CMS-vulnerable-NaCl (n = 10) animals were administered a 0.9 % NaCl solution (1 ml/kg) intraperitoneally (ip). CMS-vulnerable-Ket1 (n = 10) rats received a single ip 10 mg/kg Ket injection (Imalgen 1000 ®, Boehringer Ingelheim, Germany; 100 mg/mL diluted 10 times in 0.9% NaCl to reach 10 mg/mL). CMS-vulnerable-Ket7 rats (n = 8) were treated daily with a 10 mg/kg Ket ip injection for 7 consecutive days. Injections were administered at 9 AM.

5) Acute slice preparation

Two hours after the acute injection or the last injection of the chronic treatment, animals were deeply anesthetised with an intraperitoneal injection of urethane (1.9 g/kg). When rats no longer presented reflexes (palpebral or of withdrawal upon gentle tail pinching), intracardiac perfusion was performed using oxygenated sucrose-based artificial cerebrospinal fluid (saCSF), kept at cold temperature (0–4 °C) and continuously bubbled with carbogen (95% O_2, 5% CO_2). The saCSF solution's composition was as follows: 248 mM sucrose, 11 mM glucose, 26 mM $NaHCO_3$, 2 mM KCl, 1.25 mM KH_2PO_4, 2 mM $CaCl_2$, 1.3 mM $MgSO_4$, and 2.5 mM kynurenic acid.

After 10 minutes of perfusion, rats were decapitated, and their brains extracted and mounted onto the stage of a vibratome (VT1200S; Leica, Nussloch, Germany). Transverse 450-µm thick slices including the dHPC region were collected and transferred to a chamber containing room temperature aCSF, bubbled continuously with 95% O_2 and 5% CO_2, containing 126 mM NaCl, 26 mM $NaHCO_3$, 2.5 mM KCl, 1.25 mM NaH_2PO_4, 2 mM $CaCl_2$, 2 mM $MgCl_2$, and 10 mM glucose (pH 7.3; ~310 mOsm, measured). When possible, both a slice destined for HFS and another for LFS were harvested per animal.

6) Field potential recordings

After at least 1 hour of recovery, individual slices were placed into a submerged recording chamber and continuously perfused with carbogen-bubbled aCSF at a rate of 2.5–3 mL/min. Stimulation and recording electrodes, made from borosilicate glass capillaries (1.20 mm inner diameter, 1.69 mm outer diameter; Warner Instruments, Harvard Apparatus) using a P-1000 horizontal

puller (Sutter Instruments), were placed in the *stratum radiatum* of the CA1 pyramidal region. The resistance of both electrodes was of approximately 3 MΩ when set up and filled with aCSF.

Field excitatory postsynaptic potentials (fEPSPs) were recorded in the CA1 region following stimulation of the SC. Test stimuli were delivered at a frequency of 0.033 Hz (one pulse every 30 seconds). Recordings were performed using a MultiClamp 700A amplifier (Molecular Devices). Signals were sampled at 20 kHz, digitised with a BNC-2110 data acquisition interface (National Instruments), and acquired using WinWCP software (Strathclyde Electrophysiology Software, John Dempster, University of Strathclyde, UK). Stimulation intensity was gradually increased to determine the level at which the fEPSP response reached a maximum. To avoid response saturation, subsequent stimulations were performed at the intensity that evoked an fEPSP amplitude at two-thirds of the maximum. The slope of each fEPSP was thereafter calculated as a measure of synaptic efficacy. Baseline synaptic transmission was monitored for 20 minutes. Either high-frequency stimulation (HFS, six 10-sec spaced trains of 5 pulses at 100Hz), or low-frequency stimulation (LFS, 900 pulses delivered continuously at 1 Hz during 15 minutes) was then applied to induce long-term synaptic plasticity. Following the induction protocol, fEPSPs were recorded over 50 minutes. For each slice, fEPSP slope values were then averaged in 10-minute blocks, including two pre-induction baseline blocks, which were all normalised to the average slope recorded during the final 5 minutes prior to induction.

7) Analyses

Normality was verified using Kolmogorov-Smirnov test. Behavioural data from the FST and EPM were analysed using one-way ANOVA or Kruskal-Wallis with group as a between-subject factor. fEPSP recordings including the two baseline blocks (labelled “block -1” and “block 0”) and five post-induction blocks (labelled “block 1-5”) were analysed independently for each group, using repeated measures ANOVAs with Block as a within-subject factor. When appropriate, the ANOVAs were followed by post hoc comparisons using Fisher's least significant difference (LSD) test to compare the data with those from the final pre-induction block (Block 0). A significance threshold of $p < 0.05$ was applied throughout the study.

D) Results

1) Behavioural tests

Our results revealed significant group differences in both the FST ($F_{3,32} = 9.78$, $p < 0.001$; **Figure 1A**) and the EPM ($\chi^2_{3,36} = 13.44$, $p = 0.004$; **Figure 1B**). Post hoc tests showed that, compared

to control-NaCl animals, CMS-vulnerable-NaCl, CMS-vulnerable-Ket1, and CMS-vulnerable-Ket7 animals displayed higher immobility in the FST ($p = 0.011$, $p = 0.001$, and $p < 0.001$, respectively) and lower time in the open arms of the EPM (all $p < 0.001$). The CMS-vulnerable-Ket-7 group slightly differed compared to CMS-vulnerable-NaCl in the FST ($p < 0.05$), but no other significant differences were found between the CMS-vulnerable groups.

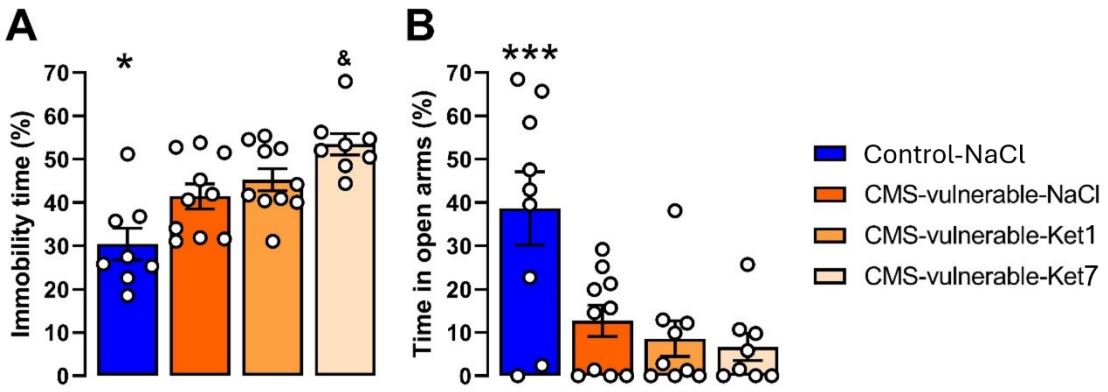

Figure 1. Behavioural performance of control and CMS-vulnerable groups in A) the forced swim test and B) the elevated plus maze. Note that behavioural testing was conducted prior to drug treatment. * and ***, difference with the other groups ($p < 0.05$ and $p < 0.001$ respectively). &, difference with CMS-NaCl group ($p < 0.05$). Data are represented as average ± SEM.

2) Induction of synaptic plasticity in the SC-CA1 pathway

In the Control-NaCl group, ANOVA performed on the fEPSP slope of the HFS recording revealed a significant effect of Block ($F_{6,42} = 3.90$, $p = 0.003$). Post hoc analyses indicated significant differences between the block 0 and the first four post-induction blocks ($p = 0.004$, $p = 0.009$, $p = 0.008$, $p = 0.005$, respectively); the difference with the last block being close to significance ($p = 0.063$). HFS induced a potentiation across the first four blocks following stimulation, with the mean slope during the final block reaching ~120% of baseline (**Figure 2A**). In this group, ANOVA on the data of the LFS recording indicated also a significant Block effect ($F_{6,30} = 4.54$, $p = 0.002$). Post hoc analyses revealed significant decrease across all five blocks following stimulation ($p = 0.025$, $p = 0.010$, $p = 0.002$, $p = 0.004$, $p = 0.004$, respectively), with the mean slope during the last block corresponding to ~60% of baseline (**Figure 2E**). These findings demonstrate that, in non-stressed control animals, HFS induced a robust, rapidly developing, though potentially unsustained LTP and

LFS induced a robust, rapidly developing, and persistent depression of synaptic efficacy, consistent with LTD.

In the CMS-vulnerable-NaCl group, ANOVA on the data of the HFS recording did not show a statistically significant Block effect, although it approached significance ($F_{6,54}$ = 2.10, p = 0.068); **Figure 2B**). Visual analysis of the data prompted post hoc analysis, which revealed a significant increase between Block 0 and the two last blocks (block 4: p = 0.034; block 5: p = 0.021), with slopes reaching ~120% of baseline (**Figure 2B**). ANOVA on the LFS recording data showed no significant effect of Block ($F_{6,54}$ = 0.60, p = 0.731; **Figure 2F**). Thus, in CMS-vulnerable animals, HFS induced slowly developing potentiation, consistent with a delayed and impaired LTP and LFS failed to induce LTD.

In the CMS-vulnerable-Ket1 group, ANOVA on the HFS recording data revealed a significant Block effect ($F_{6,42}$ = 18.25, p < 0.001). Post hoc analysis revealed that fEPSP slopes varied significantly during the pre-induction phase, with an increase between block -1 and block 0 (p = 0.015). In addition, there was significant potentiation at all post-induction blocks compared to block 0 (blocks 1: p = 0.003, blocks 2-5: all p < 0.001), reaching ~130% of baseline during the last block (**Figure 2C**). ANOVA on the LFS recording data also showed a significant Block effect ($F_{6,42}$ = 2.51, p = 0.036;). Post hoc analysis indicated a significant change in the final block only (p = 0.026), which however presented potentiation, rather than depression (**Figure 2G**). These findings indicate that following acute Ket treatment, HFS reliably induced robust LTP and LFS induced potentiation in CMS-vulnerable animals.

In the CMS-vulnerable-Ket7 group, ANOVA on the HFS recording data revealed no effect of Block ($F_{6,36}$ = 1.95, p = 0.100; **Figure 2D**). ANOVA on the LFS recording data showed a significant Block effect ($F_{6,30}$ = 6.02, p < 0.001; **Figure 2H**). Post hoc analyses indicated significant differences across all five blocks (p = 0.001, p = 0.007, p < 0.001, p < 0.001, p = 0.002, respectively) reflecting depression, with the mean slope during the last block corresponding to ~70% of baseline. These findings indicate that following chronic KET treatment, HFS failed to induce LTP, whereas LFS effectively elicited LTD in CMS-vulnerable animals.

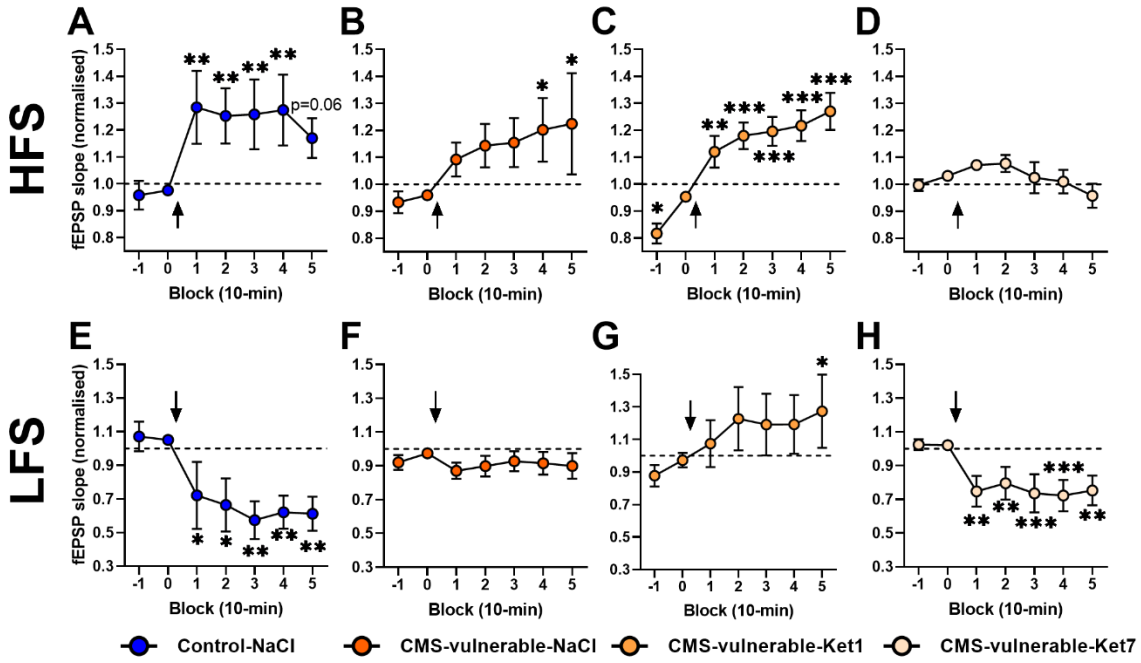

Figure 2. fEPSP slope values in CA1 of the different groups before and after high-frequency stimulation (A, B, C and D) or low-frequency stimulation (E, F, G and H) of the Schaffer collaterals. The arrow represents induction of HFS (A-D) or LFS (E-H). The dash line represents the norm to which data was normalised. *, ** and ***, difference with block 0 ($p < 0.05$, $p < 0.01$, $p < 0.001$ respectively). Data are represented as average ± SEM. Control-Nacl (2A: HFS, n=8, 2E: LFS, n=6), CMS-vulnerable-Nacl (2B: HFS, n=10, 2F: LFS, n=10), CMS-vulnerable-Ket1 (2C: HFS, n=8, 2G: LFS, n=8) and CMS-vulnerable-Ket7 (2D: HFS, n=7, 2H: LFS, n=6).

E) Discussion

Our study aimed to evaluate the long-term plasticity dynamics in the dHPC CA1-CA3 synapse of WKY rats vulnerable to CMS, and the effects of both acute and chronic Ket treatments in these animals. Exposure to CMS, and a subsequent acute or chronic Ket administration differentially modulated long-term plasticity capacities in the CA3-CA1 synapse of CMS-vulnerable animals.

Non-stressed controls, which only received NaCl, presented stable baseline fEPSP responses. Both plasticity mechanisms were functional, as HFS and LFS induced rapid-onset LTP and LTD, respectively, as from the first block. Of note, the final block during LTP was near, but slightly above the significance threshold, suggesting LTP may not have been further sustained. Interestingly, a previous study has observed a higher LTP decay speed in WKY rats compared to other strains, though still significant at 90 minutes post-HFS (Aleksandrova et al., 2020). However, absence of further data in the present study limits conclusion.

CMS-vulnerable animals treated with NaCl presented impairments in both LTP and LTD. While LTP was attained following HFS, it only reached statistical significance at the final two blocks, *i.e.* over 30 minutes after induction, while it was reached in the first block in controls. Our results concerning the effect of chronic stress on LTP are similar to previous findings [(Alfarez et al., 2003; Kamal et al., 2014; Logue et al., 2022; Yang et al., 2018)]. LTP impairment is a typical consequence of CMS exposure; while acute and transient stress can have adaptive pro-plasticity effects (De Kloet et al., 2008; Krugers et al., 2010), chronic stress impairs plasticity (McEwen, 2001), through glucocorticoid receptor-mediated (Krugers et al., 2006) dysregulation of intracellular signalling cascades necessary for LTP (Park et al., 2015) involving pro-LTP kinases (Gerges et al., 2004). These could involve calmodulin-dependent protein kinase II (CaMKII), a kinase critically involved in LTP (Lisman et al., 2012). While at baseline, compared to Wistar rats, WKY rats showed downregulation of CaMKII (Virijevic et al., 2025), CMS led to CaMKII overexpression in WKY rats (Virijevic et al., 2025). Such overexpression impaired LTP (Cho et al., 2007). Whereas previous studies have reported that CMS facilitated LTD (Jiang et al., 2020; Von Frijtag et al., 2001), LTD could not be induced at all with LFS in CMS-vulnerable animals while it was in non-stressed rats in our study. Some studies, however, also observed LTD to be abolished following chronic stress (Ma et al., 2007). Yang et al. also observed blocked LTD following chronic stress, although in the lateral habenula, which they attributed to impaired autophagy-mediated protein turnover (Yang et al., 2025), a mechanism which is essential for LTD (Kallergi et al., 2022). A similar impairment could explain the results of this study. Another possibility is a stress-induced impairment of the JAK2/STAT3 pathway, a pathway which is involved in the induction of LTD (Nicolas et al., 2012). While direct mechanisms linking chronic stress and JAK2/STAT3 dysregulation remain unclear, CMS has been shown to downregulate hippocampal expression of the active forms of both JAK2 and STAT3 in the WKY rat (Virijevic et al., 2025). Such effects may prevent the development of LTD.

The synaptic profile of CMS-vulnerable animals after acute Ket treatment, displaying gradual potentiation during pre-induction baseline stimulations evidenced in the HFS recording, rapid LTP, but no LTD, suggest that the synapses are in a depotentiated state at the start of the recording. Such a state could be explained given the pharmacological properties of Ket. During the 2 h delay between drug administration and slice collection, Ket's blockade of NMDA-R likely reduced the influx of Ca^{2+} in the postsynaptic neurons (Hansen et al., 2017), favouring a depression of the synapse while simultaneously preventing potentiation (Lüscher & Malenka, 2012). Synaptic efficacy may thus have been initially reduced. However, by the time slices were collected, NMDA-R blockade was likely

lifted, as Ket has a ~0.9 h half-life in the rat brain (Zou et al., 2009). The lifting of NMDA-R blockade may have allowed the hypothetically depressed synapse to be once more potentiated, by re-enabling normal calcium influx (Hansen et al., 2017). Subsequent HFS indeed allowed for rapid LTP induction. Such temporal dynamics of Ket's effects on LTP have been observed in a previous study in the WKY rat ; although not in the context of stress, Ket treatment prevented significant LTP compared to non-treated animals when evaluated 0.5 h post-administration, but allowed a robust and sustained LTP when evaluated 3.5 h post-administration (Aleksandrova et al., 2020). Additionally, the rapid LTP observed here contrasts with the results in the CMS-vulnerable animals treated with NaCl, in which LTP induction appeared delayed. Ket has been previously shown to restore chronic stress induced LTP impairments (Yang et al., 2018). This effect seems independent of stress-related mechanisms, as Ket-enhanced LTP is also observed in non-stressed animals and in animals in which chronic glucocorticoid treatment did not affect LTP, although this study applied the drug to the slices directly (Piazza et al., 2024). On the other hand, LTD could not be induced by LFS, which in fact showed eventual potentiation rather than depression. These results may suggest the synapse to either be in a saturated state of depression before the stimulation protocol (O'Connor et al., 2005), where further depression cannot be induced, and/or that the synaptic plasticity stimulation threshold has shifted favourably for potentiation and disfavourably for depression by the time of the recording's start. This is supported by the increasing fEPSP slope values at baseline, despite stimulation at the standard 0.033 Hz, *i.e.* every 30 seconds, designed not to induce any plasticity mechanism. These hypotheses are consistent with the observation of a two-phase action of Ket on enzymes critical in plasticity mechanisms; the drug initially leads to kinase auto-inhibition, possibly through NMDA-R blockade mediated reduced influx of Ca^{2+}, before it shifts to kinase auto-activation (Adaikkan et al., 2018). Ket is known to strongly influence both the kinase and phosphatase systems. It disinhibits pyramidal neurons (Jang & MacIver, 2021; Widman & McMahon, 2018), activates Ca^{2+}-permeable AMPA-Rs (Zaytseva et al., 2023), and promotes kinase auto-activation (Adaikkan et al., 2018), while suppressing calcineurin (Huang et al., 2020) and possibly Protein phosphatase 1 (Hou et al., 2013). A recent study notably observed that a subanaesthetic dose of Ket restores hippocampal-dependent mechanisms impaired in a chronic restraint stress model of depression through Ca^{2+}-permeable AMPA-R upregulation in the CA1 region (Flowers et al., 2025). Such effects would lead to a shift in the synaptic plasticity threshold in favour of potentiation (Jedlicka, 2002), allowing low-frequency stimulation to evoke LTP. Impairments in HFS-induced LTP following chronic stress may thus be countered by stress-independent effects of acute Ket administration, which, however, do not appear to affect the LTD impairments observed in CMS-vulnerable animals treated with NaCl, at least at the evaluated time point.

Ket is known to trigger homeostatic plasticity mechanisms (Kavalali & Monteggia, 2020). We may therefore hypothesise that repeated daily NMDA-R blockade caused by its chronic administration in CMS-vulnerable animals could trigger NMDA and/or AMPA-R upregulation, strengthening the synapse (Turrigiano, 2012). In CMS-vulnerable animals treated with chronic Ket, HFS failed to induce LTP, while it did in acutely-treated animals, suggesting a saturated state of potentiation (O'Connor et al., 2005). As baseline stimulation after chronic treatment did not lead to a gradual increase in fEPSP slopes, such potentiation saturation may have been reached in vivo, prior to recording. In such a synaptic state, further potentiation would not be possible, and only depression could occur. We indeed observed LTD after LFS application. Therefore, potential dysregulation of mechanisms necessary to LTD in CMS-vulnerable animals such as impaired autophagy-mediated protein turnover and/or impaired JAK2/STAT3 signalling may no longer be in effect. Consistent with this observation, Ket has been shown to upregulate such impaired autophagy (Yang et al., 2025), as well as JAK2/STAT3 signalling (Ho et al., 2019; Patton et al., 2017).

Plasticity mechanisms at the CA3–CA1 synapse are regulated by activity-driven Ca^{2+} influx, whose amplitude and temporal profile determine the facilitated mechanism, potentiation or depression (Franks & Sejnowski, 2002; Yang et al., 1999). Typically, high-frequency stimulation strongly and transiently increases $[Ca^{2+}]i$, activating kinases which lead to both increased AMPA-R trafficking to the membrane and increased conductance of these receptors (Lisman et al., 2012). Conversely, low-frequency stimulation leads to moderate increases in $[Ca^{2+}]i$, which stimulates phosphatases such as calcineurin, promoting LTD (Kemp & Bashir, 2001). These plasticity mechanisms appear impaired in CMS-vulnerable WKY rats, but Ket treatment may counter them to an extent, with acute or chronic delivery being a key parameter to avoid saturation.

F) Conclusion

Overall, our study demonstrated that synaptic plasticity in the hippocampal CA3-CA1 synapse is impaired in CMS-vulnerable animals through disruption of both LTP and LTD induction. Ket administration in such animals strongly modulates synaptic function, depending on treatment modality. Acute Ket may transiently and mildly depotentiate synapses, before facilitating LTP, possibly due to a delayed activation of plasticity-related kinases. Conversely, chronic Ket treatment may potentiate synapses over time through homeostatic plasticity mechanisms, to the point of saturation. These findings highlight complex temporal dynamics of Ket's effects on impaired hippocampal plasticity in

CMS-vulnerable animals and suggest that its therapeutic effects may involve interplay between classical Hebbian and homeostatic plasticity mechanisms. A better understanding of this balance could allow for optimisation of antidepressant Ket treatment. Other studies investigating different doses, treatment frequencies, and durations, would be welcome to deepen comprehension of this balance.

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Consequences of chronic mild stress and subsequent acute and chronic Ketamine treatment on sleep-wake pattern in the Wistar-Kyoto rat

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A) Abstract

Sleep disturbances are a core feature of major depressive disorder (MDD) and are particularly pronounced in treatment-resistant depression (TRD) for which ketamine (Ket) treatment has therapeutic efficacy. Chronic stress is a central factor in the pathophysiology of the pathology, and the stress-sensitive Wistar-Kyoto (WKY) rat subjected to chronic mild stress (CMS) has emerged as a putative model of TRD, although only a subset of animals present vulnerability to CMS. In this study, we examined the impact of CMS on sleep-wake patterns in CMS-vulnerable WKY rats, and assessed the effects of acute and repeated Ket treatment within this group. Following CMS exposure, male WKY rats were phenotyped using the forced swim test and elevated plus maze test to identify CMS-vulnerable individuals. Animals were implanted with electrocorticogram electrodes, and 24-hour sleep recordings were conducted in three sessions: post-CMS, after a single Ket injection (10 mg/kg, s.c.), and following chronic Ket treatment involving seven daily injections. Compared to non-stressed controls, CMS-vulnerable rats exhibited prolonged NREM sleep onset latency, tended to have reduced REM sleep onset latency, displayed disrupted sleep-wake rhythm characterised by flatter light and dark period patterns, and increased dark-period vigilance state fragmentation. During the light period, both acute and chronic Ket treatment initially suppressed sleep before leading to NREM sleep rebounds. Chronic treatment also led to REM sleep rebounds later in the light period. Chronic Ket further improved disrupted dark period sleep-wake patterns, but neither treatment improved fragmentation. These results suggest that CMS-vulnerable WKY rats present sleep deficits relevant to TRD, and that Ket, depending on treatment modality, may partially restore these.

B) Introduction

Major depressive disorder (MDD) is an increasingly diagnosed psychiatric disease with a point prevalence of about 13 % (Lim et al., 2018) and a growing socioeconomic burden (Greenberg et al., 2015). Because chronic stress is a central causal factor in depression, chronic stress protocols, particularly chronic mild stress (CMS) in rodents, are the most frequently used and validated paradigms to generate preclinical models of depression (Strekalova et al., 2022; Willner, 2017; Willner & Belzung, 2015). However, there is strong interindividual variability to vulnerability to chronic stress in both humans and animal models (Harro, 2012). The pathology is strongly linked to sleep disturbances, as over 90 % of MDD patients report sleep impairments (Geoffroy et al., 2018), which are one of its nine symptoms according to DSM-V (American Psychiatric Association, 2013). Sleep is a fundamental mechanism governed by homeostatic processes and circadian rhythms involving two main states: non-rapid eye movement (NREM) sleep, and REM sleep (Schwartz & Klerman, 2019) which is dominated by theta oscillations. Depressed patients present delayed NREM sleep onset, reduced NREM sleep duration, reduced REM sleep onset latency and increased REM sleep duration, and overall frequent awakenings with poor sleep efficiency (Lam, 2006). Additionally, sleep disturbances can be worsened by the side effects of classical antidepressant treatments (Lam, 2006).

Classical antidepressants are ineffective in about 30 % of MDD patients, who thus suffer from treatment-resistant depression (TRD) (McIntyre et al., 2023), in which sleep impairments are considered a key feature (Xu et al., 2024). Since the 2000s, rapid-acting antidepressants, such as ketamine (Ket), have shown therapeutic efficacy including in TRD (Berman et al., 2000; Corriger & Pickering, 2019). However, optimal treatment frequency as well as the drug's exact therapeutic effects remain unclear (Fountoulakis et al., 2025; Reddy et al., 2024), prompting the need for additional studies. The Wistar-Kyoto rat exposed to CMS has been proposed as a model of TRD: this model shows depression-like behavioural impairments and resistance to classical antidepressants, but not to Ket (Loizeau et al., 2024; Willner et al., 2019). Not all animals are vulnerable to CMS (Strekalova et al., 2022), and only a subset of WKY rats can be thus labelled (Loizeau et al., 2024), constituting a more accurate model of depression (Scharf & Schmidt, 2012). We have previously shown delayed day/night activity rhythm in male CMS-vulnerable WKY rats, suggesting potential sleep impairments. While baseline sleep abnormalities, including increased REM sleep time and increased fragmentation have been observed in the WKY rat compared to its control strain, the Wistar rat (Dugovic et al., 2000; Willner & Belzung, 2015), from which it is derived (Okamoto & Aoki, 1963), sleep impairments induced by chronic stress remain unexplored in this model. Few rodent studies have investigated the

consequences of CMS on sleep architecture, but they have shown that it induces sleep disturbances similar to MDD patients: increased sleep fragmentation (Grønli et al., 2004), increased NREM sleep onset latency and reduced NREM sleep time (Dalanon et al., 2021; Zhang et al., 2023), reduced REM sleep onset latency (Cheeta et al., 1997) and increased total REM sleep time (Cheeta et al., 1997; Grønli et al., 2004).

The purpose of this study was to investigate stress-induced impairments in WKY rats displaying behavioural vulnerability to CMS, and the effects of acute and chronic Ket treatment on various sleep parameters over a light/dark cycle.

C) Materials and Methods

1) Animals

This study was conducted on male WKY rats (Charles River, Sulzfeld, Germany) from three different cohorts. Two of these cohorts included rats exposed to CMS, with their controls serving to select the animals vulnerable to CMS used in this study. The third provided the non-stressed animals used in the present study. Rats arrived at the laboratory at the age of 4-5 weeks and were housed in groups of five for one week. They were then either assigned to the CMS group and housed individually, or to a control group not subjected to CMS, and housed by pairs. They were maintained on a 12-hour light/dark cycle (lights on at 7:00 A.M.), with ad libitum access to food (Altromin 1324) and water – except animals undergoing CMS at specific periods of the protocol. Temperature was controlled throughout the study (23 ± 1 °C). Experimental protocols and animal care were in compliance with the European Community Council Directive (2010/63/UE) and were approved by the regional ethical committee and the French Ministry of National Education, Higher Education and Research (APAFIS #25270).

2) Experimental design

CMS-assigned rats underwent 4 weeks of the stress protocol, conducted as described in our previous study (Loizeau et al., 2024). They were then subjected to behavioural testing using the forced swim test (FST) and elevated plus maze (EPM) to identify CMS-vulnerable animals (Loizeau et al., 2024). Those animals underwent surgery required for the recording of the electrocorticogram (ECoG). After recovery, CMS-vulnerable animals underwent two additional weeks of the CMS protocol. ECoG recordings were then conducted. Eight age-matched non-stressed control animals (NS) of another cohort were similarly operated and their ECoG recorded; they were not exposed to any stressors,

including FST and EPM, which represent potential stressors for rats (Bernal-Morales et al., 2018; de Kloet & Molendijk, 2016).

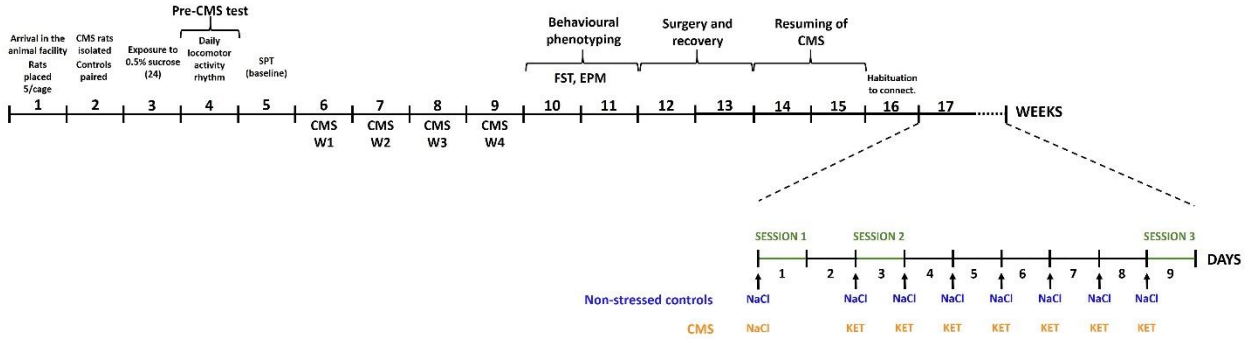

Figure 1: Experimental design timeline.

3) Identification of CMS-vulnerable animals

To determine vulnerability or non-vulnerability to CMS, rats were tested in the FST and the EPM such as described in our previous study (Loizeau et al., 2024). CMS-exposed rats were clustered into two subpopulations using k-means (Loizeau et al., 2024). This allowed distinction between CMS-vulnerable and CMS-non-vulnerable animals. In contrast to CMS-non-vulnerable animals, CMS-vulnerable animals displayed increased immobility in the FST and reduced open-arm exploration in the EPM when compared to control animals not subjected to CMS. Nine CMS-vulnerable animals were therefore selected for the present study. The FST and EPM data of those animals and of the control animals not subjected to CMS are presented in **Supplementary Figure 1**.

4) Surgery

The surgical procedure consisted of the implantation of an electrode to record the ECoG, as well as electrodes to record local field potentials (LFP) in different cerebral regions. Data extracted from the ECoG recordings served to study vigilance states and were analysed in the present article. Data extracted from LFP electrodes are the subject of another publication (**see page 137**).

Recording electrodes were implanted under isoflurane anaesthesia (IsoFlo® 100 %, Zoetis, USA ; 4 % for induction, 1.5 % for maintenance). Following induction, the top of their head was quickly shaved and they were placed on a heating pad, to prevent hypothermia, within a stereotaxic frame with their head in a horizontal « flat-skull » position. Their nose was placed in a breathing mask allowing isoflurane anaesthesia maintenance during surgery. Prior to positioning in the frame, the tip of each ear bar was coated with a cream containing a mixture of lidocaine and prilocaine (Biogaran, France). An ophthalmic ointment (Lubrithal, Dechra, UK) was applied on their eyes to protect the

cornea from desiccation. After disinfecting the incision site on the head with iodine solution (Vetedine Solution®, Vetoquinol, Magny-Vernois, France), a subcutaneous injection of local anesthetics including lidocaine (2 mg/kg; Lidor, Axience, France) and bupivacaine (2 mg/kg; Mylan, USA) was administered. After 5 minutes, the skin was incised in the longitudinal plane to expose the cranial surface, and the bone surface was gently scraped with sterile cotton-tips; a saline solution was used to remove bone debris ; the skull was subsequently dried with sterile cotton tips. Electrode positions were marked on the skull surface with a pencil, and burr-holes were drilled accordingly. The screws were then placed in sequence, beginning with the electrocorticogram (ECoG) screw, positioned frontally above the right hemisphere, followed by the ground screw (located caudally above the cerebellum), the reference screw (positioned frontally above the left hemisphere), and the three additional screws serving as anchors. The ECoG, reference and ground screws had been pre-soldered to a 5-cm segment of tungsten wire (0,004 inches, PFA-coated, A-M Systems, USA). Subsequently, the LFP electrodes were lowered into the right hemisphere at their designated locations, one at a time, after carefully piercing the dura. Electrode placement was based on the following anteroposterior (AP), lateral (L), and dorsoventral (DV) coordinates [in mm from Bregma, according to a standard rat brain atlas (Paxinos & Watson, 2013)]: PRL (AP, +2.6; L, - 0.7; DV, -3), BLA (AP, -2.5; L, -4.9; DV, -6.9), dHPC (AP, -3.8; L, -2.7; DV, -2.2). Each electrode was composed of a bundle of three 5-cm long sections of tungsten wire (0,004 inches, PFA-coated, A-M Systems, USA). Before insertion, the tip of each electrode was covered with DiI stain (Invitrogen Molecular probes, USA) for *post mortem* histological verification of their position. Each electrode was lowered individually and secured to a nearby screw using UV-curable glue (Metafil FLO, SUN Medical Co., Moriyama-shi, Japan) before inserting the next. Once all three LFP electrodes were positioned, an adhesive resin (Super-Bond C&B®, Sun Medical Co., Moriyama-shi, Japan) was applied to embed and firmly fix the LFP electrodes along with all screws (ECoG, reference, ground, and anchor) to the skull. Subsequently, each tungsten wire (ECoG, reference, ground, and the three LFP electrodes for each structure) was connected to an electronic interface board (EIB-16, Neuralynx, USA). The entire assembly was then embedded in dental acrylic cement (Paladur®, Kulzer, Germany). The rats were gently removed from the stereotaxic frame and administered a subcutaneous injection of the non-steroidal anti-inflammatory drug meloxicam (1 mg/kg; Metacam, Boehringer Ingelheim) to minimise discomfort during recovery. Animals were returned to their home cage on clean bedding under a heating lamp until fully awake. They were subsequently returned to their housing room and allowed a two-week recovery period, during which they were monitored and weighed daily. NS rats housed by pairs were operated on the same day and put back together once they had awakened.

5) Recordings

Animals were habituated to the recording device for three consecutive days (3 hours/day). During recording sessions, each rat was placed individually in a home cage within a Faraday cage. Signals were acquired with an Intan system (model C3334, Intan Technologies, USA) connected to the EIB via a cable and swivel connector, allowing free movement. Data were sampled at 1,000 Hz, band-pass filtered (1.26–500 Hz), and saved in consecutive 15-min files (.rhd format).

Three 24-hour recording sessions (onset at 09:00; 2 hours after the start of the light period) were performed. Before each recording session, animals received a subcutaneous injection (1 ml/kg). The first recording session (Session 1) was performed after injection of 0.9 % NaCl solution. The second recording session (Session 2), performed 24 hours after the end of the first one, was started after injection of 0.9 % NaCl for NS animals and Ket (10 mg/kg; Imalgen 1000®, Boehringer Ingelheim, Germany) for CMS-vulnerable animals. The animals were then given a daily injection of the same solution, and the third recording session (Session 3) was started immediately after the seventh dose.

6) Hypnogram determination

ECoG signals were converted from .rhd to .edf format using custom-written scripts in MATLAB (version 2022b; MathWorks, Natick, USA) and uploaded to the Profusion Sleep software (version 4.0, Compumedics, USA). Each 24-hour recording was segmented into 4-second epochs, an approach allowing high resolution of sleep microarchitecture (McShane et al., 2010). For each epoch, the vigilance state was identified based on visual analysis of the ECoG pattern (Hoshino et al., 2016): Wake was characterised by fast and irregular waves, NREM sleep by slow, high-amplitude delta (1.26-4 Hz) waves, and REM sleep by fast, highly regular oscillations dominated by the theta (4-12 Hz) band.

7) Data analysis

Hypnogram data were exported from Profusion Sleep as .xlsx files, which were imported into MATLAB for analysis with custom-written scripts. For each session, the total duration of each vigilance state, the number and duration of wake, NREM sleep and REM sleep episodes, as well as the total number of transitions between states, were quantified for each of the three 24-hour recording sessions. Data were subsequently pooled in 2-hour blocks for statistical analysis. As the start of recording did not coincide with the onset of the light period, the light period that was analysed was composed of five 2-hour blocks. The dark period was analysed in its entirety (six 2-hour blocks).

NREM sleep latency, defined as the time elapsed from the start of the recording (while rats were awake) to the onset of the first NREM sleep episode, and REM sleep latency, defined as the time elapsed from the first NREM sleep episode to the first REM sleep episode, were also quantified (McKenna et al., 2008).

In order to highlight differences between NS and CMS-vulnerable animals, data from Session 1 were compared between the two groups. For each lighting period, data (except latencies) were subjected to analysis of variance (ANOVA), with Block as within-subject factor and Group as between-subject factor. For latencies, unpaired Student's t-tests were used. In order to assess the stability of the data from session to session, the three recordings obtained in NS animals were compared. For each period, data (except latencies) were subjected to ANOVA with Session and Block as within-subject factors. Latencies were analysed using ANOVA with Session as within-subject factor. Finally, data of CMS-vulnerable animals from all three sessions were compared in the same way to evaluate the effects of acute and chronic Ket treatments. ANOVAs were completed by Fisher's Least Significant Difference (LSD) post hoc tests when appropriate. The threshold for rejecting the null hypothesis was 0.05 throughout.

D) Results

1) Sleep-wake pattern in non-stressed and CMS-vulnerable animals

Data from session 1 were analysed to compare the sleep-wake pattern between the two groups; the results of the ANOVAs performed on all parameters are displayed in **Supplementary Table 1**. An illustrative hypnogram of a rat from each group is presented in **Supplementary Figure 2.**

Sleep-wake pattern during the light period

The latency of the first NREM sleep episode was different between groups ($t_{15} = 3.727$, $p < 0.01$). CMS-vulnerable animals were awake longer than NS animals (**Figure 2A**). Analysis of REM sleep onset latency (**Figure 2B**) revealed a trend towards a difference between groups ($t_{15} = 1.947$, $p = 0.07$).

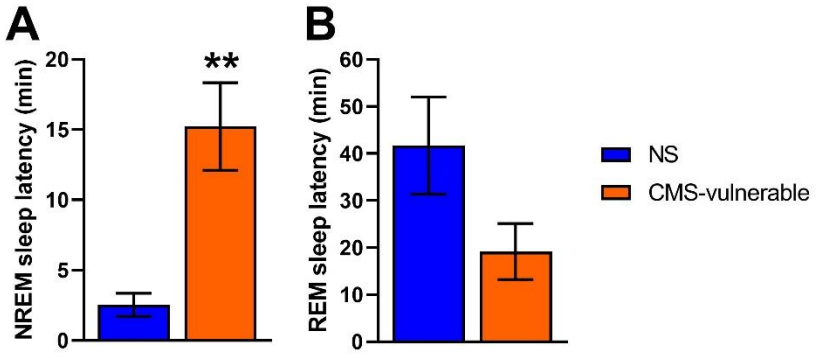

Figure 2. Latency of NREM sleep (A) and REM sleep (B) onsets in NS and CMS-vulnerable animals during recording session 1. **, difference between groups ($p < 0.01$). Data are represented as mean ± SEM.

During the light period, the total duration of the different states was similar in both groups, as the ANOVAs showed no main effect of Group. However, their sleep-wake pattern was different as the ANOVAs revealed significant Block x Group interactions for Wake and REM $_{sleep}$ (wake, $F_{4,60} = 3.28$, $p < 0.02$; NREM sleep, $F_{4,60} = 2.17$, $p = 0.08$; REM sleep, $F_{4,60} = 4.21$, $p < 0.01$). Post hoc test on these interactions indicated, regarding Wake, a significant group difference during the second and the last blocks, and concerning REM sleep, a significant group difference during the last block (LSD: $p < 0.05$ at least; **Figure 3**). Whereas Wake duration was greater in CMS-vulnerable animals during block 2, the opposite was observed during block 5 (less Wake time). Greater Wake duration at the beginning of the light period (block 2) led to shorter duration of both sleep states ($p = 0.05$ for NREM sleep; $p = 0.19$ for REM sleep), whereas decreased Wake time at the end of the period was mostly the result of increased REM sleep duration (LSD: $p < 0.05$). Furthermore, in NS animals, Wake duration decreased from the first to the second block (LSD: $p < 0.001$) and increased between the fourth and fifth block (LSD: $p < 0.01$), to reach a duration identical to that of the first block. In CMS-vulnerable animals, decreased Wake duration at the beginning of the light period was only apparent from block 3 onwards (LSD: $p < 0.001$ for block 1 vs block 3, and $p < 0.01$ for block 2 vs block 3). While Wake duration subsequently increased (LSD: $p < 0.02$ for block 3 vs block 4), it remained lower at the end of the light period compared to the beginning (LSD: $p < 0.05$ for block 1 vs block 5).

Variation in total Wake duration during the light period was associated with variation in mean episode duration but not of the number of episodes. Indeed, only the ANOVA on the mean Wake episode duration revealed a significant Group x Block interaction ($F_{4,60} = 2.74$, $p < 0.05$). Post hoc indicated a significant group difference during the last block ($p < 0.05$), where mean Wake episode

duration was higher in NS than in CMS-vulnerable (**Figure 3**). In addition, within NS animals, mean Wake episode duration was greater during the first and last blocks than during other blocks, whereas within CMS-vulnerable animals, it was greater during blocks 1, 2 and 4 than during the others. In contrast, light period variation in total REM sleep duration was associated with variations in the number of episodes, but not with that of their mean duration. Indeed, only the ANOVA on the number of episodes revealed a significant Group x Block interaction ($F_{4,60} = 2.88$, $p < 0.05$). Post hoc indicated a significant difference during the last block (LSD: $p < 0.01$), during which this number was higher in CMS-vulnerable (**Figure 3**). The analysis of the total number of transitions during the light period indicated neither Group nor Block effects, nor any interaction between these factors (**Supplementary Figure 3**).

In summary, CMS-vulnerable animals experienced delayed NREM sleep onset during the light period, and a tendency for delayed REM sleep onset. Wake time was greater in the second block, but lower at the end of the light period, during which CMS-vulnerable animals spent more time in REM sleep. At the beginning and end of the light period, the variation in Wake time of the CMS-vulnerable animals was accompanied with a variation in the mean duration of wake episodes, which were longer at the beginning and shorter at the end.

Sleep-wake pattern during the dark period

During the dark period, the ANOVAs performed on the total duration of the different states indicated significant Block x Group interactions (Wake: $F_{5,75} = 2.89$, $p < 0.02$; NREM sleep: $F_{5,75} = 2.63$, $p < 0.05$; REM sleep: $F_{5,75} = 2.95$, $p < 0.02$). Post hoc on those interactions revealed a significant group difference for each state during the first block (LSD: at least $p < 0.02$). During this block, total NREM sleep and REM sleep durations were higher and total Wake duration was lower in CMS-vulnerable compared to NS animals (**Figure 3**). Furthermore, while the total duration for each state varied in NS across the period (blocks 1 and 3 *vs* blocks 2, 4 and 5; LSD: at least $p < 0.05$), it did not in CMS-vulnerable. In these animals, only total REM sleep duration was greater during the first block when compared to the others, which did not differ between each other (LSD: at least $p < 0.05$ except block 1 vs block 3, $p = 0.07$).

The analyses of the number of episodes also indicated significant Block x Group interactions (Wake: $F_{5,75} = 3.28$, $p < 0.01$; NREM sleep: $F_{5,75} = 3.51$, $p < 0.01$; REM sleep: $F_{5,75} = 3.95$, $p < 0.01$). Post hoc revealed significant group differences for each state during the first and the third blocks (LSD: at least $p < 0.01$). For each state, the number of episodes was higher in CMS-vulnerable (**Figure 3**). Post hoc tests also showed in NS animals only that the number of Wake and NREM sleep episodes

fluctuated during the dark period (block 1 > blocks 2, 4, 5 and 6 and block 3 > block 4; LSD: at least $p < 0.05$). Concerning the number of REM sleep episodes, it was higher during the first block than during blocks 2, 5 and 6 in CMS-vulnerable (LSD: at least $p < 0.05$), whereas in NS animals it fluctuated throughout the period (block 2 > blocks 1 and 3; block 5 > blocks 1, 3, 4 and 6; LSD: at least $p < 0.05$). The analyses of the mean duration of episodes showed a significant interaction between the Group and Block factors but only concerning Wake. Post hoc revealed group differences during the first (LSD: $p < 0.01$) and third (LSD: $p < 0.01$) blocks, during which mean duration of episodes for each state was lower in CMS-vulnerable. They also indicated variations in mean Wake duration but only within the NS group (greater duration during blocks 1 and 3 than during the others; LSD: at least $p < 0.02$). Analysis of the total number of transitions revealed a significant Block x Group interaction. Post hoc indicated significant group differences during the first and third blocks (LSD: $p < 0.01$) in which it was higher in CMS-vulnerable. This number varied during the period but only within the NS group (block 1 > blocks 2, 4, 5 and 6; block 3 > blocks 2, 4 and 5; LSD: at least $p < 0.05$) (see **Supplementary Figure 3**).

In summary, CMS-vulnerable animals exhibited less total Wake time at the beginning of the dark period, characterised by more numerous, but shorter episodes. At this moment, the increased total duration of both NREM sleep and REM sleep was driven by a higher number of episodes, as mean episode duration did not differ compared to NS animals. Furthermore, in contrast to NS animals, that showed typical sleep-wake pattern during the dark period, with peaks of Wake at the beginning, middle and end of the period, CMS-vulnerable animals did not display an evident sleep-wake pattern. Of note, during moments of consolidated wakefulness, characterised by longer-lasting episodes in NS animals (blocks 1, 3), CMS-vulnerable animals exhibited a more fragmented pattern.

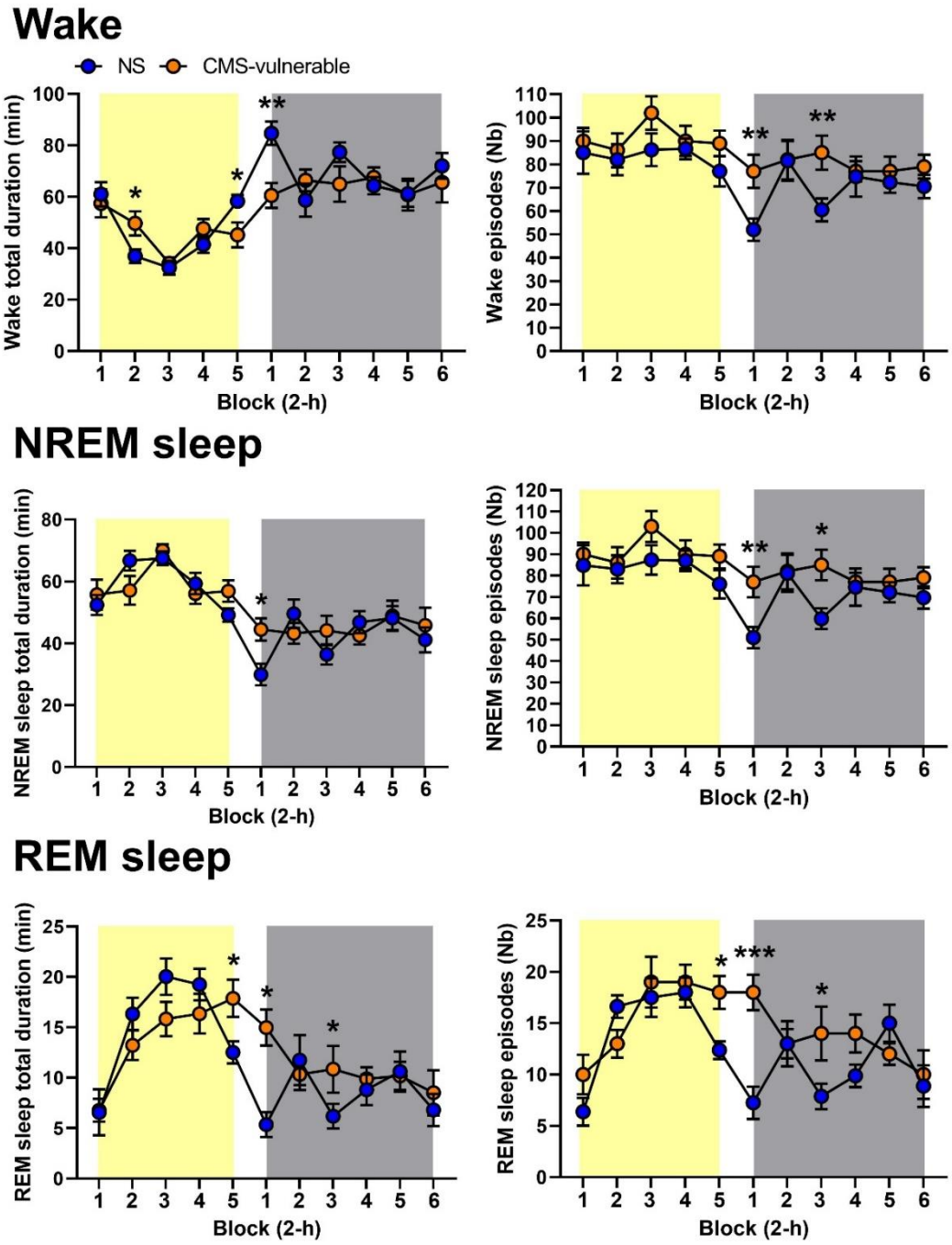

Figure 3. Total duration (left) and total number of episodes (right) of Wake (top), NREM sleep (middle) and REM sleep (bottom) in NS and CMS-vulnerable animals during the light (yellow) and dark (grey) period of Session 1. *, **, ***, difference between groups at the same block ($p < 0.05$, $p < 0.01$, $p < 0.001$ respectively). For figure legibility reasons, statistics concerning between-block differences within each group are not shown and are described only in the text. Data are represented as mean ± SEM.

2) Sleep-wake pattern stability between recordings in non-stressed animals

Data of the three recording sessions of NS animals were compared to evaluate stability between recording sessions. The results of the ANOVAs performed on all parameters are displayed in

Supplementary Table 2. Even if some ANOVAs revealed a significant Session effect, none of them showed a significant interaction between Session and Block, prompting the conclusion that the sleep-wake patterns of NS animals during both the light and dark periods were similar between sessions (See **Figure 4** for NREM sleep and REM sleep onset latencies, **Figure 5** for the total duration and total number of episodes, and **Supplementary Figure 4** for the total number of transitions). This stability suggests no bias of session in the comparison of sleep-wake patterns from multiple recordings in a within-subject design to evaluate the effects of Ket treatment in CMS-vulnerable animals.

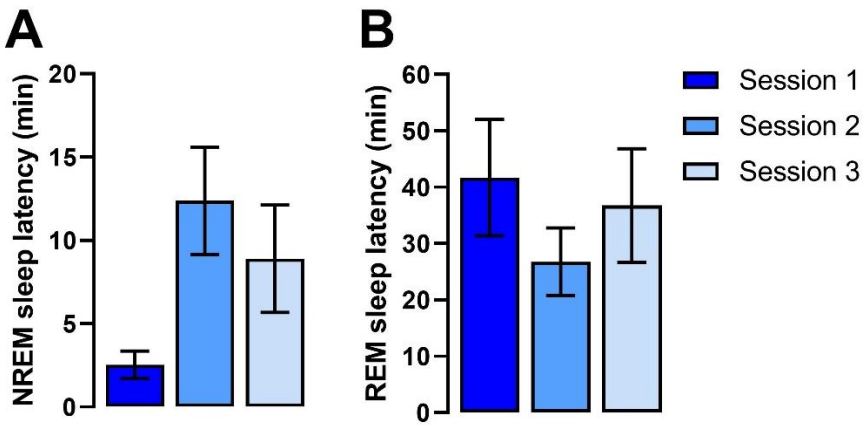

Figure 4. Latency of NREM sleep (A) and REM sleep (B) onsets in NS animals during the three recording sessions. Data are represented as mean ± SEM

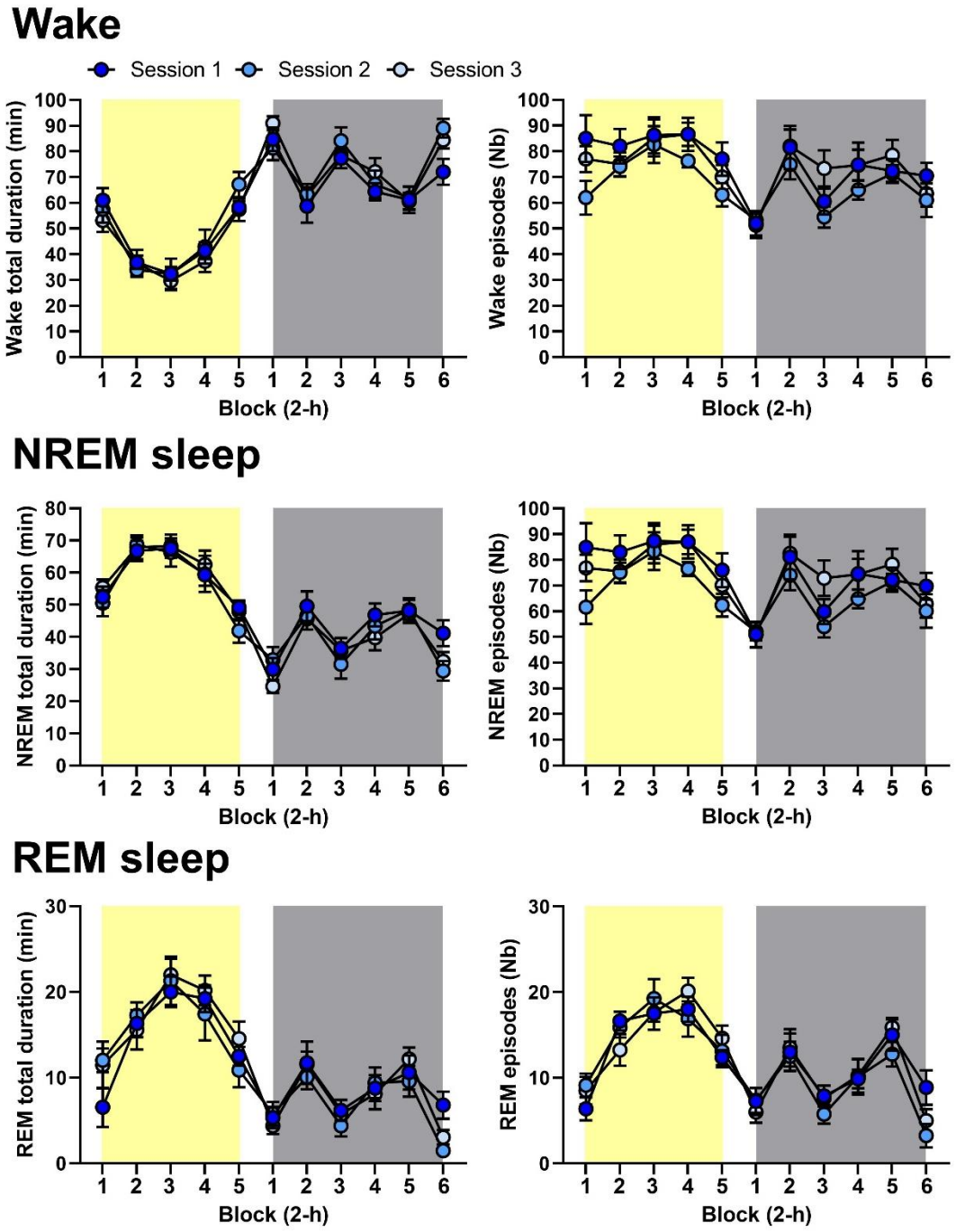

Figure 5. Total duration (left) and total number of episodes (right) of Wake (top), NREM sleep (middle) and REM sleep (bottom) in NS animals during the three recording sessions during the light (yellow) and dark (grey) periods. Data are represented as mean ± SEM

3) Impact of acute and chronic ketamine treatment on sleep-wake pattern in CMS-vulnerable

Data from the three recording sessions obtained in CMS-vulnerable animals were analysed to evaluate the effects of acute (Session 2) and chronic (Session 3) Ket treatments. The results of the ANOVAs performed on all parameters, except for latencies which are described below, are displayed in **Supplementary Table 3**. An illustrative hypnogram of a rat for all three sessions is presented in **Supplementary Figure 5.**

Impact on sleep-wake pattern during the light period

Analysis of NREM sleep and REM sleep onset latencies revealed a significant Session effect (NREM sleep, $F_{2,16} = 51.295$, $p < 0.001$; REM sleep $F_{2,16} = 5.795$, $p < 0.02$). Post hoc revealed significant differences between the first and the other two sessions (LSD: at least $p < 0.02$). Both acute and chronic Ket treatment increased NREM sleep and REM sleep onset latencies (**Figure 6**).

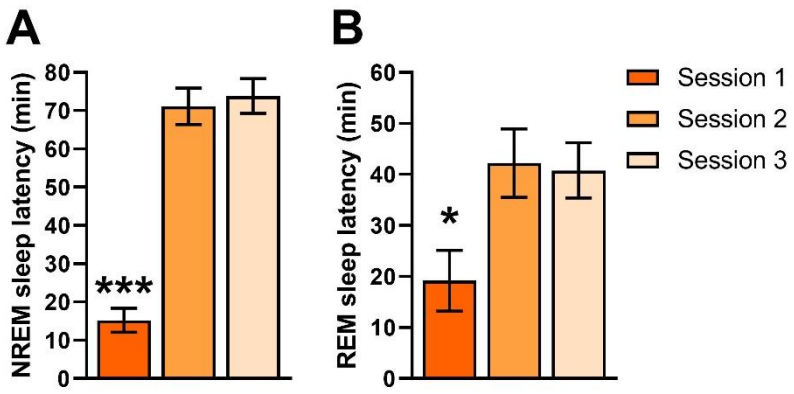

Figure 6. Latencies of NREM sleep (A) and REM sleep (B) onset in CMS-vulnerable animals during the three recording sessions. * and ***, difference between session 1 and the two other sessions ($p < 0.05$ and $p < 0.001$ respectively). Data are represented as mean ± SEM.

During the light period, total duration of each state was similar during the three sessions as the ANOVAs showed no main effect of Session. However, sleep-wake patterns differed as the ANOVAs revealed significant Session x Block interactions for each vigilance state (Wake: $F_{8,64} = 10.30$, $p < 0.001$; NREM sleep: $F_{8,64} = 9.82$, $p < 0.01$; REM sleep: $F_{8,64} = 4.29$, $p < 0.001$). Post hoc on these interactions revealed a significant difference between the first and both the second and third sessions during blocks 1 and 2 (LSD: at least $p < 0.01$) for both Wake and NREM sleep total durations, and only during block 1 (LSD: $p < 0.01$) for REM sleep total duration (**Figure 7**). At the beginning of the light period (block 1), total Wake duration was increased after both acute and chronic Ket treatments,

this increase occurring at the expense of both NREM sleep and REM sleep duration (LSD: at least $p < 0.001$). During block 2, total Wake duration was, in contrast, decreased after acute and chronic Ket treatments (LSD: at least $p < 0.001$); the increased sleep duration only concerned NREM sleep duration (LSD: at least $p < 0.001$). Post hoc revealed significant differences in total Wake duration between the first and third sessions during block 4 (LSD: $p < 0.02$), and a trend toward significance between the second and third sessions (LSD: $p = 0.06$). Total Wake duration was decreased after chronic Ket treatment during this block, while REM sleep duration was increased (LSD: at least $p < 0.01$).

ANOVAs performed on the number (**Figure 7**) and mean duration of episodes (not shown) for the three vigilance states also revealed significant Session x Block interactions (Wake: number, $F_{8,64} = 5.29$, $p < 0.001$; mean duration, $F_{8,64} = 27.21$, $p < 0.001$; NREM sleep: number, $F_{8,64} = 5.39$, $p < 0.001$; mean duration, $F_{8,64} = 2.20$, $p < 0.001$; REM sleep: number, $F_{8,64} = 3.55$, $p < 0.01$; mean duration, $F_{8,64} = 1.81$, $p = 0.12$). During block 1, post hoc indicated significant differences between the first and other two sessions for both the number and mean duration of Wake episodes, and only for the number of episodes of NREM sleep and REM sleep (LSD: at least $p < 0.001$). The number of Wake, NREM sleep and REM sleep episodes decreased after acute and chronic Ket treatments, while only the mean duration of wake episodes increased. During block 3, post hoc indicated significant difference between the first session and the other two in the number of episodes of both Wake and NREM sleep, and regarding mean duration of NREM sleep only (LSD: at least $p < 0.05$). The number of Wake and NREM episodes decreased after acute and chronic Ket treatments, while only the mean duration of NREM sleep episodes increased. Analysis of the total number of transitions also revealed a significant Session x Block interaction ($F_{8,64} = 5.70$, $p < 0.01$). Post hoc indicated significant differences between sessions 1-2 and session 3 during blocks 1 and 3 (LSD: at least $p < 0.05$). Acute and chronic Ket treatments decreased the number of transitions during these two blocks (**Supplementary Figure 6)**.

In summary, acute and chronic Ket treatments delayed NREM sleep and REM sleep onset latency during the light period. At the beginning of the light period, Ket-induced increased Wake duration was accompanied by fewer, but longer Wake episodes. Decreased sleep duration was accompanied by both fewer NREM sleep and REM sleep episodes. The delayed sleep onset was compensated for by a subsequent increase in NREM sleep. Chronic Ket treatment also prolonged sleep by increasing total REM sleep duration towards the end of the light period.

Impact on sleep-wake pattern during the dark period

During the dark period, only ANOVAs performed on total Wake and REM sleep durations (**Figure 7**) revealed a significant Session x Block interaction (wake, $F_{10,80} = 2.27$, $p < 0.05$; REM sleep,

$F_{10,80} = 3.25$, $p < 0.01$). Post hoc tests on these interactions indicated no differences between the first and second sessions; however, they revealed significant differences between the first and third sessions, during the first and last blocks (LSD: at least $p < 0.05$). At the beginning and the end of the dark period, chronic treatment increased total Wake and decreased total REM sleep durations. Furthermore, while during the first session, total Wake duration was similar between blocks, it was greater after chronic treatment during the first and the last blocks compared to the others (LSD: at least $p < 0.05$). In addition, while during the first session, total REM sleep duration decreased between the first and second blocks, and remained stable thereafter, chronic treatment lowered total REM sleep duration during the first and the last blocks compared to the other blocks (LSD: at least $p < 0.05$).

ANOVAs performed on the number of episodes (**Figure 7**) and their mean duration (not shown) revealed a significant Session x Block interaction regarding the number of REM sleep episodes ($F_{10,80} = 2.47$, $p < 0.02$) and a trend towards a significant interaction for mean Wake duration ($F_{10,80} = 1.75$, $p = 0.08$). Post hoc on the interaction only indicated significant differences between the first and third sessions during the first and last blocks. At the beginning and the end of the dark period, chronic, but not acute Ket treatment increased mean Wake duration (LSD: block 1, $p = 0.07$; block 5, $p < 0.05$) and decreased the number of REM sleep episodes (LSD: block 1, $p < 0.01$; block 6, $p = 0.05$). The ANOVA performed on the total number of transitions revealed no significant session x Block interaction ($F_{10,80} = 1.65$, $p = 0.1$) (see **Supplementary Figure 6**).

In summary, during the dark period, chronic but not acute Ket treatment induced alterations of the sleep-wake pattern in CMS-vulnerable animals. Chronic treatment increased Wake duration at the beginning and at the end of the period by increasing the mean duration of wake episodes. At these moments, decreased sleep duration was due to a decrease in REM sleep duration and number of episodes. Furthermore, whereas an NS-like sleep-wake pattern was absent prior to treatment, chronic treatment partially restored such a pattern with more consolidated wake, characterised by longer episodes, at the beginning and at the end of the period.

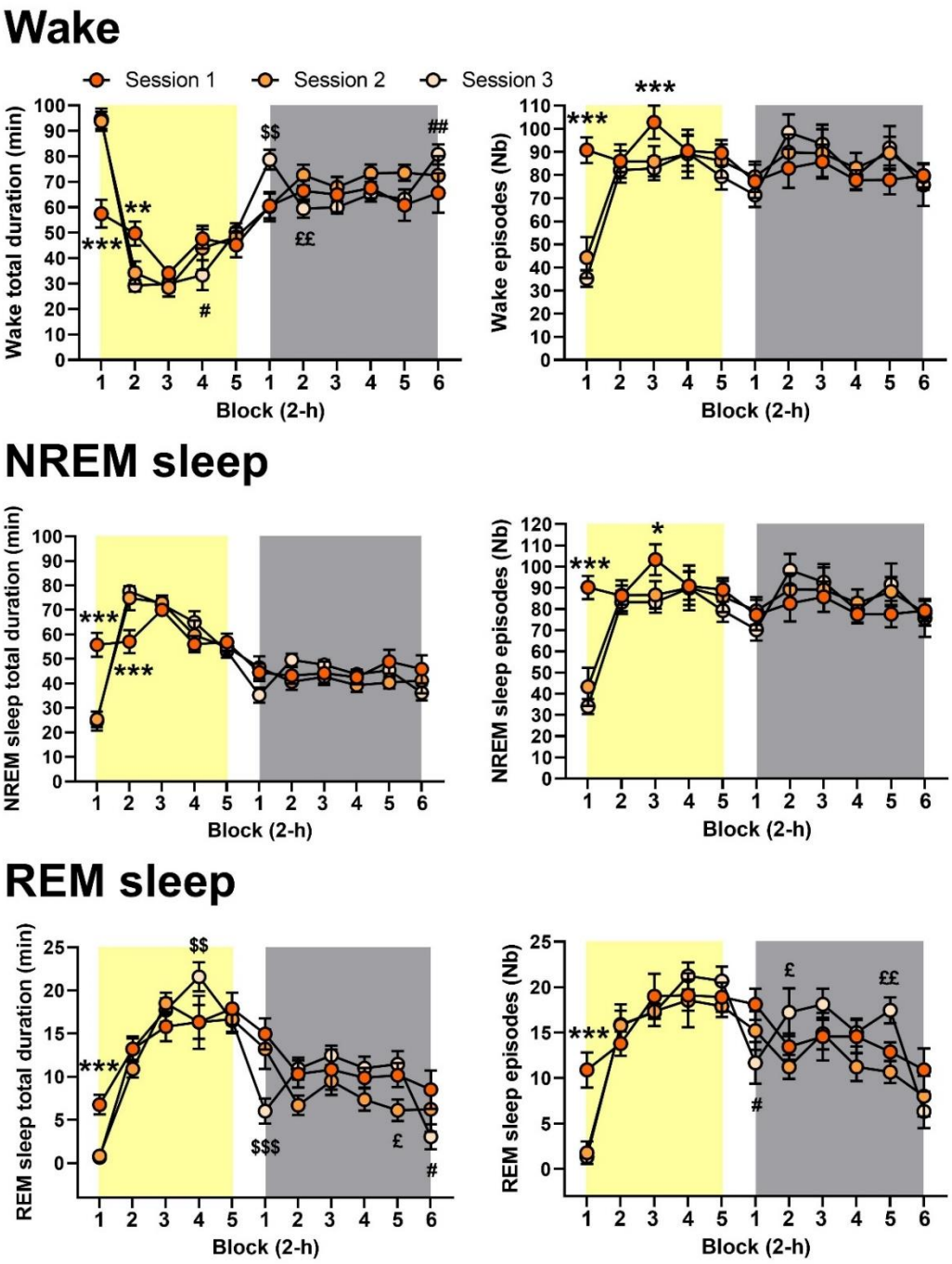

Figure 7. Total duration (left) and total number of episodes (right) of Wake (top), NREM sleep (middle) and REM sleep (bottom) in CMS-vulnerable animals during the three recording sessions during the light (yellow) and dark (grey) periods. *, ** and ***, difference between session 1 and the two other sessions at the same block ($p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively). $$, difference between session 3 and the two other sessions at the same block ($p < 0.01$). # difference between session 3 and session 1 at the same block ($p < 0.05$). £ and ££, difference between session 3 and session 2 at the same block ($p < 0.05$ and $p < 0.01$). For reasons of legibility, statistics concerning differences between blocks within each session are not shown and are described only in the text. Data are represented as mean ± SEM.

E) Discussion

The first aim of this study was to describe the sleep-wake pattern in WKY rats vulnerable to CMS compared to NS animals. Secondly, the effects of acute and chronic Ket treatment on different sleep/wake parameters were evaluated within CMS-vulnerable animals. These animals showed several impairments, which appeared to be partially countered by chronic Ket treatment.

NREM sleep onset latency was increased in CMS-vulnerable animals compared to controls in line with previous CMS studies (Zhang et al., 2023). Delayed NREM sleep onset is expected as a result of chronic stress. Corticosterone (CORT), the stress hormone in rodents, inhibits sleep and promotes wakefulness in rodents for up to several days after chronic stress exposure (Yao et al., 2023). This phenomenon may underlie the delayed NREM sleep onset observed here. CMS-vulnerable rats also tended to have shorter REM sleep onset latency compared to controls, also in line with previous work involving CMS exposure (Cheeta et al., 1997) or other chronic stress paradigms such as repeated social defeat (Matsuda et al., 2021; Xia et al., 2023). Although a direct relationship between facilitated REM sleep onset latency and chronic stress remains unclear, a study using CMS has demonstrated that impaired CORT regulation and enhancement of REM sleep are closely associated (Nollet et al., 2019). The initiation and maintenance of REM sleep involve a fine interplay between REM sleep-promoting cholinergic neurons in the subcoeruleus nucleus and REM sleep-suppressing monoaminergic inputs from the locus coeruleus, dorsal raphe, and tuberomammillary nucleus (Fraigne et al., 2015; Wang et al., 2015). Exact mechanisms underpinning how chronic stress could shift this balance *e.g.* by favouring REM sleep-promoting neurons or disfavouring REM sleep-inhibiting neurons are unclear. However, chronic stress has been shown to downregulate serotonergic activity within, and projecting from, the dorsal raphe ; such changes, which have been linked to depressive-like behavior in the FST (Camargo et al., 2025; Natarajan et al., 2017), could weaken REM sleep-suppressing influences and thereby facilitate earlier REM sleep onset.

NS animals presented a marked circadian sleep-wake pattern. During the light period, it was characterised by a U-shaped pattern of Wake time, and inversely for NREM sleep and REM sleep. In contrast, CMS-vulnerable rats exhibited a flatter total wake time profile during the light period, with greater wake time in the second block, consistent with delayed NREM sleep onset, and reduced Wake time in the final block, accompanied by greater REM sleep time. During the dark period, NS animals displayed a peak of Wake time at the beginning, early-mid, and end of the period, and likewise a trough of NREM sleep and REM sleep time, a trimodal pattern consistent with previous studies (Stephenson et al., 2012). In contrast, CMS animals displayed a flatter dark period pattern characterised by reduced

early period Wake time, mirrored by increased NREM sleep and REM sleep time, as well as increased early-mid period REM sleep time. Together, these findings suggest impairments in circadian rhythmic sleep-wake organisation within CMS-vulnerable animals. In mice, CMS has been shown to decrease the amplitude of, *i.e.,* 'flatten' circadian rhythmic variations of parameters such as locomotor activity or body temperature, though sleep was not explored in this study (Logan et al., 2015). Our previous work has shown that CMS-vulnerable WKY rats present abnormal rhythmic activity, evidenced by a phase delay (Loizeau et al., 2024). Circadian sleep-wake patterns are regulated by the coordinated expression of clock genes such as Cry1, Cry2, Per2, and Bmal1 (Sochal et al., 2024). These genes appear to be dysregulated as a consequence of CMS: a literature review describes evidence from several CMS studies showing that such a paradigm "disrupts the regulated gene expression of several clock genes" both peripherally and centrally, leading to circadian misalignment (Agorastos et al., 2020). Similar findings have been reported in other chronic stress paradigms, such as repeated social defeat (Kong et al., 2022), chronic corticosterone treatment (Ma et al., 2018), or repeated exposure to forced swimming (Sarrazin et al., 2024). Such circadian misalignment may underlie the rhythmic abnormalities observed here. During the early dark phase, CMS-vulnerable rats also showed greater vigilance state fragmentation, in line with previous studies (Grønli et al., 2004), evidenced during the early dark period by greater number of episodes and shorter average Wake time, and greater total number of transitions. Although the light period represents the primary resting period for nocturnal rodents, their polyphasic sleep structure means they still spend approximately one-third of the dark period asleep (Vivaldi et al., 1994), making dark period fragmentation functionally relevant. Of note, the dark period, and particularly early dark period, coincides with peak rhythmic CORT concentration in rats (Birnie et al., 2022; Mohawk et al., 2007), which as stated above, inhibits sleep and promotes wakefulness. These natural variations coupled with increased overall CORT elicited by CMS could explain why fragmentation impairments appeared in the early dark period. Of note, CMS-vulnerable rats also showed a trend toward elevated total REM sleep time during the dark period. Overall, REM sleep time increases are typically reported in chronic stress and depression models such as repeated social defeat (Cheeta et al., 1997; Grønli et al., 2004; Matsuda et al., 2021) although acute social defeat can shorten REM sleep episodes (Chouvaeff et al., 2025). The absence of statistical significance in our data may reflect a ceiling effect, as WKY rats naturally exhibit elevated REM sleep, approximately 50 % higher than its control strain, the Wistar rat (Dugovic et al., 2000), or other strains, like the Sprague-Dawley rat (Kantor et al., 2023). Such high baseline REM sleep could therefore cap further stress-induced increases. Overall, the pattern we observed remains consistent with the broader literature linking chronic stress exposure to REM sleep facilitation.

The second aim was to evaluate the effects of acute and chronic Ket treatments within the CMS-vulnerable group. As suggested by the analyses in NS animals, repeated recording itself had no impact on sleep-wake patterns. In CMS-vulnerable animals, both acute and chronic Ket treatment increased NREM sleep onset latency as well as subsequent REM sleep latency. Such observations are consistent with previous studies (Burgdorf et al., 2019), including in the WKY rat (Kantor et al., 2023), and this effect appears to be dose-dependent (Ahnaou et al., 2017; Kantor et al., 2023). Classical antidepressants, which target monoamine systems, often increase REM sleep latency, although have heterogeneous effects of NREM sleep latency (Thase, 2006; Wichniak et al., 2012). Ket's effects on these parameters likely depends on its antagonism of NMDA-R, since other NMDA-R antagonists, such as MK-801 or NPC 12626, have been shown to suppress sleep, affecting both NREM sleep and REM sleep (Campbell & Feinberg, 1996; Stone et al., 1992). Conversely, NMDA-R agonism has been shown to facilitate NREM sleep onset, although not REM sleep onset (Burgdorf et al., 2019). Furthermore, (S)-Ket, one of two enantiomers present in racemic Ket, which has a four-fold greater affinity for NMDA-R, has a greater effect in increasing sleep onset latency (Koncz et al., 2023). Anatomically, NMDA-R within the hypothalamus, particularly in the lateral preoptic nucleus which is a region rich in sleep-promoting neurons (Gallopin et al., 2000), contribute to the initiation of sleep and maintenance of NREM sleep, and to some extent of REM sleep (Miracca et al., 2022). Therefore, antagonism of these receptors could contribute to suppressing sleep onset. Additionally, glycine has been shown to promote sleep through NMDA-R activation in the suprachiasmatic nuclei, an effect blocked by NMDA-R antagonism (Kawai et al., 2015). Taken together, these findings suggest that intact NMDA-R function may be necessary for normal sleep initiation, and that its blockade by Ket likely underlies the observed delays in both NREM sleep and REM sleep onset.

After this initial suppression of sleep, both acute and chronic Ket treatment increased NREM sleep time in the second light period block, and chronic treatment also increased REM sleep time in the 4th light period block, compared to session 1, indicating sleep rebounds. Rebounds in NREM sleep and REM sleep have been previously observed after approximately 2 h-long Ket-induced sleep suppression, consistent with our results (Ahnaou et al., 2017; Burgdorf et al., 2019) . These suggest implications of compensatory homeostatic sleep mechanisms following the suppression of sleep, similar to sleep deprivation. Sleep deprivation is a therapeutic approach to depression, and convergent transcriptomic signatures have been reported following either sleep deprivation or Ket administration (Orozco-Solis et al., 2017). Furthermore, a study using a high dose of Ket (80 mg/kg) demonstrated NREM sleep rebound effects comparable to sleep deprivation, and independent of circadian influences (Tóth et al., 2023), reinforcing the hypothesis of homeostatic mechanisms explaining these.

Following these rebounds, the chronic Ket treatment appeared to normalise dark period sleep-wake pattern. Compared to the pattern observed in session 1, it led to Wake time peaks, and corresponding troughs of NREM sleep and REM sleep time, at the beginning and end of the dark period. This pattern is closer to the pattern observed in NS animals. If, as hypothesised above, such impairments were due to circadian misalignment caused by CMS, these results suggest that Ket may induce circadian realignment. Consistent with this hypothesis, Ket has been shown to modulate clock genes: in vitro, it can downregulate the circadian expression of Bmal1, Per2 and Cry1 of cultured fibroblasts (Bellet et al., 2011) ; in vivo, Ket counteracts stress-induced disruptions of clock genes by normalising the expression of Per2, Cry1, Cry2, and Rorα in the prefrontal cortex (Sarrazin et al., 2024). Together, these findings suggest that the normalisation of sleep-wake patterns after chronic stress may involve a restoration of clock gene function. In our study, such effects emerged only with chronic, not acute, Ket treatment, raising the possibility that repeated modulation of clock gene expression is required to impact circadian sleep-wake rhythms.

Finally, dark period vigilance state fragmentation did not appear to improve with Ket treatment. Studies examining the long-term effects of Ket on this parameter are lacking. If stress-induced elevated CORT is responsible for increased fragmentation during the dark period, and CORT levels remain high (Yao et al., 2023), it would be expected that dark-period remains fragmented. Notably, a study reported that Ket did not produce sustained effects on CORT regulation in WKY rats during the light period (Birnie et al., 2022), although that study did not specifically investigate chronic stress-related changes. Thus, the persistence of fragmentation may reflect ongoing stress-related neuroendocrine dysregulation that Ket does not fully address.

F) Conclusion

Overall, we observed that CMS-vulnerable WKY rats, a putative model of TRD, present sleep impairments consistent with those observed in patients, such as delayed sleep, a trend toward faster REM sleep onset, a flattened sleep/wake pattern, and increased fragmentation. While both acute and chronic Ket initially suppress sleep, only chronic Ket seemed to improve dark period sleep/wake pattern, while neither kind of treatment seemed to ameliorate fragmentation. Limitations

While the present study provides evidence on stress-induced sleep disturbances and Ket's partially beneficial effects on these in WKY rats, several limitations should be acknowledged. Vigilance state scoring was based solely on the ECoG, as movement data were not available. The present findings are limited to male CMS-vulnerable WKY rats, a single Ket dose, and mechanistic underpinnings were not directly examined. Future studies incorporating both sexes, additional dosing

regimens, and multimodal neurobiological analyses would be helpful to better understand the mechanisms through which Ket may improve disturbed sleep architecture following chronic stress.

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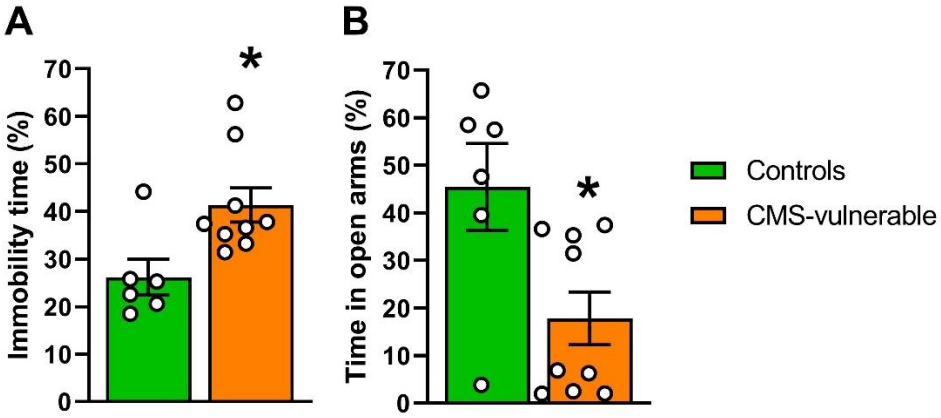
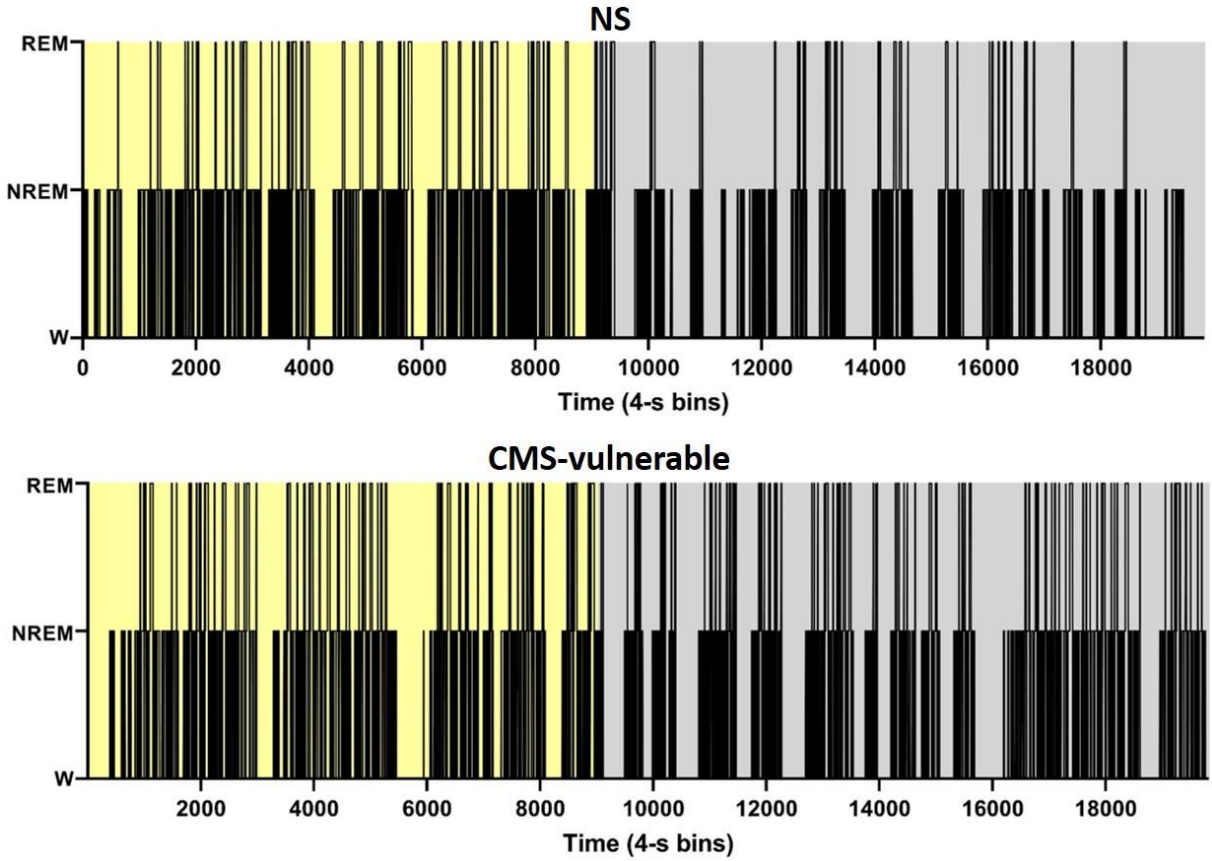
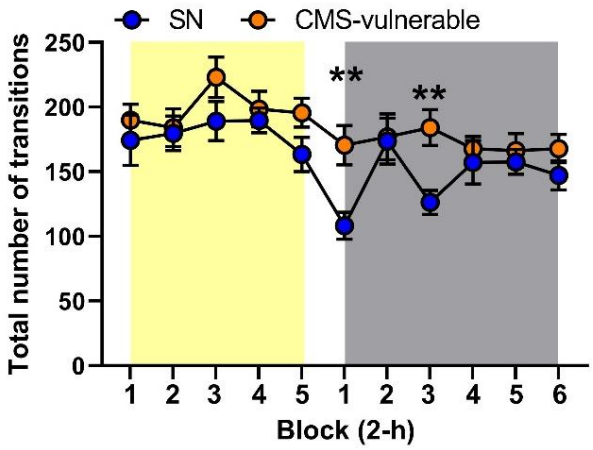
G) Supplementary information



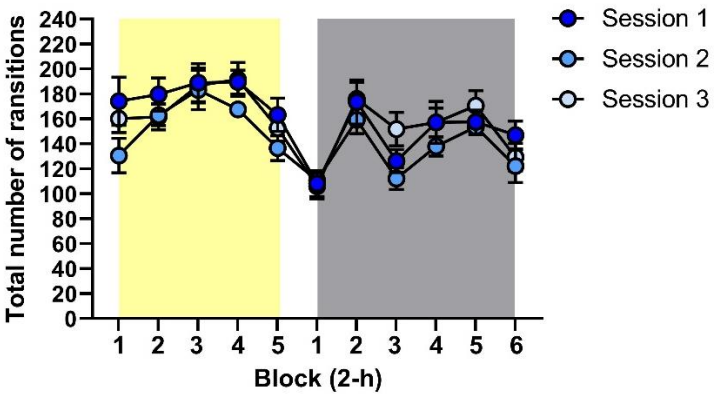
Supplementary Figure 1. Behavioural performance of controls and CMS-vulnerable animals in A) the forced swim test and B) the elevated plus maze. *, p < 0.05. Data are represented as mean ± SEM.



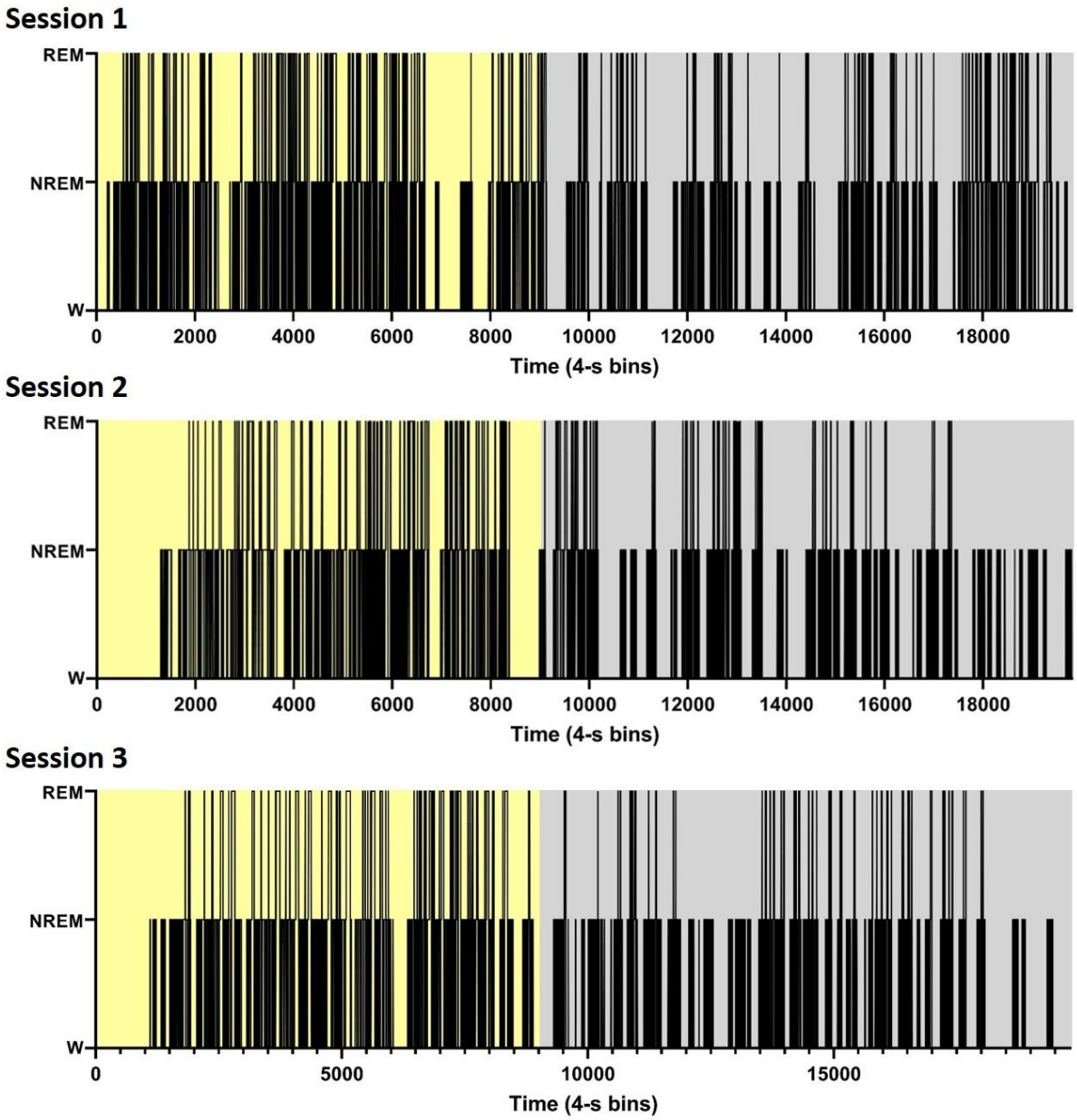
Supplementary Figure 2. Examples of hypnograms from a NS (top) and CMS-vulnerable (bottom) animal during session 1. For the CMS-vulnerable animal, one can particularly notice, for example, the increased NREM sleep latency at the beginning of the light period and the increased number of REM sleep episodes during the dark period.



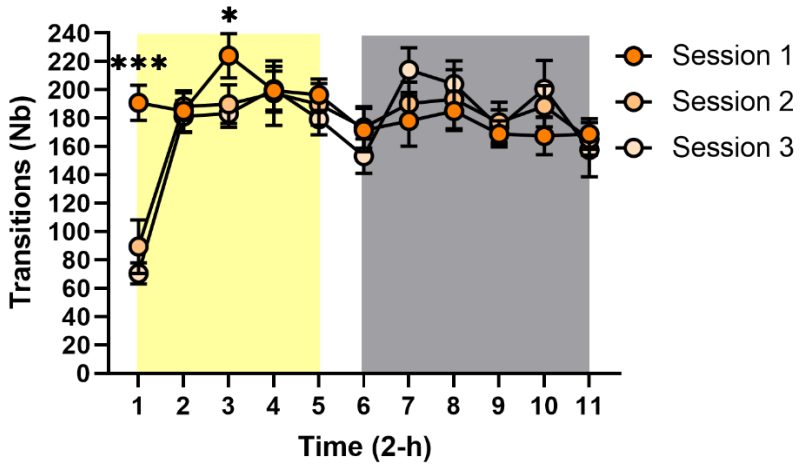
Supplementary Figure 3. Total number of transitions in NS and CMS-vulnerable animals during the light (yellow) and dark (grey) periods in Session 1. **, difference between groups at the same block ($p < 0.01$). Data are represented as mean ± SEM



Supplementary Figure 4. Total number of transitions during the three recording sessions in NS animals during the light (yellow) and dark (grey) periods. Data are represented as mean ± SEM



Supplementary Figure 5. Examples of hypnograms from a CMS-vulnerable animal during session 1 (top), session 2 (middle), and session 3 (bottom). For both sessions 2 and 3, increased NREM sleep onset latency at the beginning of the light period, and decreased REM sleep occurrence at the end of the dark period are particularly evident.



Supplementary Figure 6. Total number of transitions during the three recording sessions in CMS-vulnerable animals during the light (yellow) and dark (grey) periods. *and ***, difference between session 1 and the two other sessions at the same block ($p < 0.05$ and $p < 0.001$ respectively). For reasons of legibility, statistics concerning differences between blocks within each session are not shown and are described only in the text. Data are represented as mean ± SEM.

	Light period	Dark period
Total time		
wake	Group: $F_{1,15}$ = 0.08, p = 0.77 **Block: $F_{4,60}$ = 12.93, p < 0.001** **G x B: $F_{4,60}$ = 3.28, p < 0.02**	Group: $F_{1,15}$ = 1.50, p = 0.24 Block: $F_{5,75}$ = 1.87, p = 0.11 **G x B: $F_{5,75}$ = 2.89, p < 0.02**
NREM sleep	Group: $F_{1,15}$ = 0.003, p = 0.96 **Block: $F_{4,60}$ = 7.96, p < 0.001** G x B: $F_{4,60}$ = 2.17, p = 0.08	Group: $F_{1,15}$ = 0.55, p = 0.47 **Block: $F_{5,75}$ = 2.95, p < 0.02** **G x B: $F_{5,75}$ = 2.63, p < 0.05**
REM sleep	Group: $F_{1,15}$ = 0.31, p = 0.59 **Block: $F_{4,60}$ = 23.62, p < 0.001** **G x B: $F_{4,60}$ = 4.21, p < 0.01**	Group: $F_{1,15}$ = 3.96, p = 0.06 Block: $F_{5,75}$ = 1.15, p = 0.34 **G x B: $F_{5,75}$ = 2.95, p < 0.02**
Number of episodes		
wake	Group: $F_{1,15}$ = 1.63, p = 0.22 Block: $F_{4,60}$ = 1,46, p = 0.2 G x B: $F_{4,60}$ = 0.59, p = 0.67	Group: $F_{1,15}$ = 2.51, p = 0.13 **Block: $F_{5,75}$ = 3.59, p < 0.01** **G x B: $F_{5,75}$ = 3.28, p < 0.01**
NREM sleep	Group: $F_{1,15}$ = 1.52, p = 0.24 Block: $F_{4,60}$ = 1,70, p = 0.16 G x B: $F_{4,60}$ = 0.60, p = 0.66	Group: $F_{1,15}$ = 2.60, p = 0.13 **Block: $F_{5,75}$ = 3.68, p < 0.01** **G x B: $F_{5,75}$ = 3.51, p < 0.01**
REM sleep	Group: $F_{1,15}$ = 2.36, p = 0.15 **Block: $F_{4,60}$ = 14.39 p < 0.001** **G x B: $F_{4,60}$ = 2.88, p < 0.05**	**Group: $F_{1,15}$ = 6.67, p < 0.05** Block: $F_{5,75}$ = 1.55, p = 0.18 **G x B: $F_{5,75}$ = 3.95, p < 0.01**
Mean episode duration		
wake	Group: $F_{1,15}$ = 0.06, p = 0.81 **Block: $F_{4,60}$ = 4.33, p < 0.01** **G x B: $F_{4,60}$ = 2.74, p < 0.05**	Group: $F_{1,15}$ = 4.39, p = 0.05 **Block: $F_{5,75}$ = 3.94, p < 0.01** **G x B: $F_{5,75}$ = 4.19, p < 0.01**
NREM sleep	Group: $F_{1,15}$ = 1.2, p = 0.29 **Block: $F_{4,60}$ = 5.29, p < 0.001** G x B: $F_{4,60}$ = 1.47, p = 0.22	Group: $F_{1,15}$ = 0.69, p = 0.42 Block: $F_{5,75}$ = 0.89, p = 0.49 G x B: $F_{5,75}$ = 0.47, p = 0.79
REM sleep	Group: $F_{1,15}$ = 3.08, p = 0.10 Block: $F_{4,60}$ = 0.95, p = 0.44 G x B: $F_{4,60}$ = 1.62, p = 0.18	Group: $F_{1,15}$ = 0.52, p = 0.48 Block: $F_{5,75}$ = 0.06, p = 0.99 G x B: $F_{5,75}$ = 0.35, p = 0.88
Number of transitions	Group: $F_{1,15}$ = 1.89, p = 0.19 Block: $F_{4,60}$ = 2.01, p = 0.11 G x B: $F_{4,60}$ = 0.71, p = 0.58	Group: $F_{1,15}$ = 3.50, p = 0.08 **Block: $F_{5,75}$ = 3.16, p < 0.02** **G x B: $F_{5,75}$ = 3.90, p < 0.01**

Supplementary Table 1. Results of the ANOVA performed on the data from the first recording session. G, Group factor and B, Block factor.

	Light period	Dark period
Total time		
wake	Session: $F_{2,14}$ = 1.16, p = 0.34 **Block: $F_{4,28}$ = 18.41, p < 0.001** S x B: $F_{8,56}$ = 0.58, p = 0.79	**Session: $F_{2,14}$ = 4.65, p < 0.05** **Block: $F_{5,35}$ = 29.29, p < 0.001** S x B: $F_{10,70}$ = 1.12, p = 0.36
NREM sleep	Session: $F_{2,14}$ = 1.14, p = 0.35 **Block: $F_{4,28}$ = 16.55, p < 0.001** S x B: $F_{8,56}$ = 0.44, p = 0.89	**Session: $F_{2,14}$ = 4.42, p < 0.05** **Block: $F_{5,35}$ = 38.31, p < 0.001** S x B: $F_{10,70}$ = 1.01, p = 0.44
REM sleep	Session: $F_{2,14}$ = 0.68, p = 0.52 **Block: $F_{4,28}$ = 17.57, p < 0.001** S x B: $F_{8,56}$ = 1.27, p = 0.28	Session: $F_{2,14}$ = 1.52, p = 0.25 **Block: $F_{5,35}$ = 12.61, p < 0.001** S x B: $F_{10,70}$ = 1.25, p = 0.27
Number of episodes		
wake	**Session: $F_{2,14}$ = 6.63, p < 0.02** **Block: $F_{4,28}$ = 3.89, p < 0.02** S x B: $F_{8,56}$ = 0.90, p = 0.52	Session: $F_{2,14}$ = 1.65, p = 0.23 **Block: $F_{5,35}$ = 16.80, p < 0.001** S x B: $F_{10,70}$ = 1.14, p = 0.35
NREM sleep	**Session: $F_{2,14}$ = 6.38, p < 0.02** **Block: $F_{4,28}$ = 4.48, p < 0.01** S x B: $F_{8,56}$ = 0.90, p = 0.54	Session: $F_{2,14}$ = 1.69, p = 0.22 **Block: $F_{5,35}$ = 17.76, p < 0.001** S x B: $F_{10,70}$ = 1.09, p = 0.38
REM sleep	Session: $F_{2,14}$ = 0.16, p = 0.85 **Block: $F_{4,28}$ = 32.81, p < 0.001** S x B: $F_{8,56}$ = 1.27, p = 0.27	Session: $F_{2,14}$ = 2.08, p = 0.16 **Block: $F_{5,35}$ = 14.33, p < 0.001** S x B: $F_{10,70}$ = 1.45, p = 0.18
Mean episode duration		
wake	**Session: $F_{2,14}$ = 5.24, p < 0.02** **Block: $F_{4,28}$ = 5.56, p < 0.01** S x B: $F_{8,56}$ = 0.70, p = 0.69	Session: $F_{2,14}$ = 1.35, p = 0.29 **Block: $F_{5,35}$ = 12.86, p < 0.001** S x B: $F_{10,70}$ = 0.59, p = 0.82
NREM sleep	Session: $F_{2,14}$ = 2.44, p = 0.12 **Block: $F_{4,28}$ = 10.96, p < 0.001** S x B: $F_{8,56}$ = 0.88, p = 0.54	**Session: $F_{2,14}$ = 3.91, p < 0.05** **Block: $F_{5,35}$ = 5.85, p < 0.001** S x B: $F_{10,70}$ = 0.60, p = 0.81
REM sleep	Session: $F_{2,14}$ = 0.51, p = 0.61 **Block: $F_{4,28}$ = 4.19, p < 0.01** S x B: $F_{8,56}$ = 1.54, p = 0.16	*#Session: $F_{2,8}$ = 0.84, p = 0.46* *Block: $F_{5,20}$ = 0.94, p = 0.47* *S x B: $F_{10,40}$ = 0.78, p = 0.65*
Number of transitions	**Session: $F_{2,14}$ = 4.28, p < 0.05** **Block: $F_{4,28}$ = 5.79, p < 0.01** S x B: $F_{8,56}$ = 0.84, p = 0.57	Session: $F_{2,14}$ = 1.81, p = 0.20 **Block: $F_{5,35}$ = 19.62, p < 0.001** S x B: $F_{10,70}$ = 1.20, p = 0.31

Supplementary Table 2. Results of the ANOVA performed on the data obtained in the three recordings obtained in the control animals. S, Session factor and B, Block factor. *# As some animals did not exhibit any REM sleep episodes during certain blocks, the ANOVA only took into account animals that did exhibit them (in this analysis n = 5).*

	Light period	Dark period
Total time		
wake	Session: $F_{2,16} = 2.25$, p = 0.14 **Block: $F_{4,32} = 57.56$, p < 0.001** **S x B: $F_{8,64} = 10.30$, p < 0.001**	Session: $F_{2,16} = 2.26$, p = 0.14 Block: $F_{5,40} = 1.65$, p = 0.17 **S x B: $F_{10,80} = 2.27$, p < 0.05**
NREM sleep	Session: $F_{2,16} = 0.69$, p = 0.52 **Block: $F_{4,32} = 48.48$, p < 0.001** **S x B: $F_{8,64} = 9.82$, p < 0.001**	Session: $F_{2,16} = 1.21$, p = 0.32 Block: $F_{5,40} = 1.05$, p = 0.40 S x B: $F_{10,80} = 1.68$, p = 0.10
REM sleep	Session: $F_{2,16} = 1.38$, p = 0.28 **Block: $F_{4,32} = 40.40$, p < 0.001** **S x B: $F_{8,64} = 4.29$, p < 0.001**	**Session: $F_{2,16} = 4.01$, p < 0.05** **Block: $F_{5,40} = 4.09$, p < 0.01** **S x B: $F_{10,80} = 3.25$, p < 0.01**
Number of episodes		
wake	**Session: $F_{2,16} = 10.32$, p < 0.01** **Block: $F_{4,32} = 19.35$, p < 0.001** **S x B: $F_{8,64} = 5.29$, p < 0.001**	Session: $F_{2,16} = 1.05$, p = 0.37 **Block: $F_{5,40} = 3.82$, p < 0.01** S x B: $F_{10,80} = 1.24$, p = 0.28
NREM sleep	**Session: $F_{2,16} = 10.42$, p < 0.01** **Block: $F_{4,32} = 20.17$, p < 0.001** **S x B: $F_{8,64} = 5.39$, p < 0.001**	Session: $F_{2,16} = 0.99$, p = 0.39 **Block: $F_{5,40} = 3.79$, p <0.01** S x B: $F_{10,80} = 1.30$, p = 0.26
REM sleep	Session: $F_{2,16} = 1.87$, p = 0.19 **Block: $F_{4,32} = 35.31$, p < 0.001** **S x B: $F_{8,64} = 3.55$, p < 0.01**	Session: $F_{2,16} = 2.79$, p = 0.09 **Block: $F_{5,40} = 6.04$, p < 0.001** **S x B: $F_{10,80} = 2.47$, p < 0.02**
Mean episode duration		
wake	**Session: $F_{2,16} = 24.31$, p < 0.001** **Block: $F_{4,32} = 103.31$, p < 0.001** **S x B: $F_{8,64} = 27.21$, p < 0.001**	Session: $F_{2,16} = 0.02$, p = 0.98 **Block: $F_{5,40} = 2.66$, p < 0.05** S x B: $F_{10,80} = 1.75$, p = 0.08
NREM sleep	**Session: $F_{2,16} = 5.14$, p < 0.02** **Block: $F_{4,32} = 14.96$, p < 0.001** **S x B: $F_{8,64} = 2.20$, p < 0.05**	Session: $F_{2,16} = 1.69$, p = 0.21 Block: $F_{5,40} = 0.69$, p = 0.64 S x B: $F_{10,80} = 1.27$, p = 0.26
REM sleep	**Session: $F_{2,14} = 0.48$, p = 0.62* *Block: $F_{3,21} = 2.14$, p = 0.12* *S x B: $F_{6,42} = 1.81$, p = 0.12*	***#Session: $F_{2,12} = 5.46$, p < 0.05*** *Block: $F_{5,30} = 1.42$, p = 0.25* *S x B: $F_{10,60} = 1.75$, p = 0.09*
Number of transitions	**Session: $F_{2,16} = 9.75$, p < 0.01** **Block: $F_{4,32} = 25.58$, p < 0.001** **S x B: $F_{8,64} = 5.70$, p < 0.001**	Session: $F_{2,16} = 1.03$, p = 0.38 **Block: $F_{5,40} = 4.12$, p < 0.01** S x B: $F_{10,80} = 1.65$, p = 0.11

Table 3. Results of the ANOVA performed on the data obtained in the three recordings in CMS-vulnerable animals. S, Session factor and B, Block factor. **As only few animals exhibited REM sleep episodes during the first block of the light period following acute and chronic Ket treatments, the ANOVA was only performed on the blocks 2 - 5. Furthermore, as some animals did not exhibit any REM sleep episodes during certain blocks, the ANOVA only took into account animals that did exhibit them (n = 8). # As some animals did not exhibit any REM sleep episodes during certain blocks, the ANOVA only took into account animals that did exhibit them (n = 7).*

Consequences of chronic mild stress and subsequent acute and chronic ketamine treatment on a corticolimbic network in the Wistar-Kyoto rat

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Rappel : *Les données présentées dans cet article sont encore en cours d'analyse. Si les résultats sont présentés en format « article » comme pour les autres études dans cette thèse, les résultats de cette étude sont voués à être modifiés et l'article n'est donc pas dans sa forme finale.*

A) Abstract

Depression is associated with disrupted functional connectivity within corticolimbic networks including the medial prefrontal cortex (mPFC), hippocampus (HPC), and amygdala (AMY). Ketamine (Ket), a rapid-acting antidepressant, modulates these networks, yet its electrophysiological effects on their dynamics remain insufficiently characterised. The Wistar-Kyoto (WKY) rat exposed to chronic mild stress (CMS) has been proposed as a model of treatment-resistant depression (TRD), exhibiting depressive-like behaviour and reduced responsiveness to classical antidepressants. Here, we recorded local field potentials (LFPs) from the prelimbic cortex (PRL), dorsal HPC (dHPC), and basolateral AMY (BLA) across 24 hours, including successive light and dark period, to examine neural activity through spectral power, and inter-regional communication, through coherence, during wakefulness (Wake), non-rapid eye movement (NREM) sleep, and REM sleep in non-stressed (NS) and CMS-vulnerable WKY rats. Effects of acute or chronic (daily for a week) Ket treatment were then evaluated within the CMS-vulnerable group. Compared to NS, CMS-vulnerable animals displayed impairments in neural activity suggestive of altered aperiodic components of the LFP power spectrum, predominantly during late dark-period wakefulness. These alterations were most pronounced in the PRL, less pronounced in the BLA, while sparing the dHPC. CMS-vulnerable animals also showed

disrupted dark-period variation of alpha and gamma band power, resulting in a pattern opposite to that of NS. Ket administration, independent of regimen, transiently modulated cortical oscillations during Wake by decreasing theta and beta power while enhancing gamma power, consistent with NMDA receptor-mediated disruption of interneuron-pyramidal cell interactions. Ket subsequently increased NREM delta power, likely reflecting sleep pressure following sleep suppression. In the dHPC, acute Ket increased Wake low-frequency power and decreased high-frequency power after the initial effects, possibly reflecting aperiodic component modulation as well. The absence of this effect with chronic Ket suggests adaptation with repeated administration. Conversely, only chronic Ket reduced BLA Wake beta power during the beginning and end of the dark phase, possibly indicating more calm Wake. Coherence measures could not yet be reliably interpreted.

B) Introduction

Depression is a leading cause of disability worldwide (Chen et al., 2025; Friedrich, 2017). A consistent pathophysiological observation in depressed patients, using neuroimaging methods such as fMRI, is altered functional connectivity (FC) within a network of regions including, among others, the medial prefrontal cortex (mPFC), the amygdala (AMY), and the hippocampus (HPC) (e.g. Hossein et al., 2023; Tahmasian et al., 2013). A meta-analysis conducted in 2015 showed that different networks were affected in depressed patients, including the default mode (DMN), salience (SN) and dorsal attention networks (Kaiser et al., 2015). Moreover, these alterations correlated with poor emotional regulation and hyperresponsiveness to negative stimuli (Hossein et al., 2023; Kaiser et al., 2015; Tang et al., 2018).

A main concern of the pathology is that approximately 30 % of depressed patients do not respond to classical antidepressants (McIntyre et al., 2023). Ketamine (Ket), an antagonist of the glutamatergic N-methyl-D-aspartic acid receptor (NMDA-R), has been proposed as an alternative therapeutic strategy and has shown rapid improvement in clinical symptoms including in treatment-resistant depressed patients (TRD) (Berman et al., 2000; Corriger & Pickering, 2019). TRD patients show specific FC alterations within the DMN, involving the mPFC, HPC, and AMY (Runia et al., 2022), and Ket has been shown to produce a large range of FC modifications in such patients, including between the above-cited regions (Alario & Niciu, 2021; Kotoula et al., 2023; Yun & Kim, 2024). Additionally, Ket normalises communication within the DMN and the SN in TRD (Evans et al., 2018). Nonetheless, optimal treatment frequency as well as the exact consequences of Ket on physiological parameters such as FC in networks affected in the pathology remain poorly understood and require preclinical investigations using suitable models (Fountoulakis et al., 2025; Reddy et al., 2024).

As chronic exposure to stressful situations is a central factor in developing psychiatric pathologies such as depression (Krasner et al., 2025; Pittenger & Duman, 2008), a model recently proposed is the stress-sensitive Wistar-Kyoto (WKY) rat exposed to chronic mild stress (CMS) (Willner et al., 2019). This model shows depressive-like behavioural manifestations including anhedonia, anxiety, cognitive deficits, and passive coping response during an inescapable stressful experience (Loizeau et al., 2024; Willner et al., 2019). Interestingly, we have shown that only a sub-population of WKY rats subjected to CMS, labelled CMS-vulnerable, present a depressive-like phenotype (Loizeau et al., 2024), bringing finer delineation in the study of the model. Finally, WKY rats subjected to CMS show resistance to classical antidepressants, but not to Ket (Willner et al., 2019), making it a suitable TRD model. While our understanding of the behavioural and physiological features of such models has recently increased, knowledge concerning the potential modifications of brain regional activity and FC, that could help improve its face validity, is still lacking. To investigate these, methods such as *in vivo* recording of the extracellular local field potential (LFP) are of great use. The LFP represents the superposition of transmembrane currents of a large population of neurons located in the vicinity of the electrode; they reflect the synchronisation of local synaptic activity (Berens et al., 2008; Buzsáki et al., 2012; Friston et al., 2015). Various frequency bands can be studied, *e.g.* delta, theta, alpha, beta, and gamma (Ahnaou et al., 2017; Amat-Foraster et al., 2019; Cordon et al., 2015; Hinman et al., 2013; Kittelberger et al., 2012; Pal et al., 2015; Pothorszki et al., 2024; Tort et al., 2013; Wróbel et al., 2020). These define various internal states, from high alertness to sleep, and therefore differ according to vigilance state such as wakefulness (Wake), non-rapid eye movement sleep (NREM sleep) and REM sleep (Steriade, 2000). Several analytical aspects of brain oscillations help understanding how cerebral regions are impacted and how they communicate, when individuals are exposed to stressful situations. One such aspect is oscillatory spectral power, which varies according to the degree of synchrony of the activity of the recorded neuronal population, high and low spectral power reflecting high and low synchrony, respectively. This analysis is of particular interest as CMS alters delta, theta, alpha, beta and gamma power in the prelimbic cortex (PRL) and dorsal HPC (dHPC) Thériault et al., 2021). Another key analytical aspect of oscillatory activity is coherence. The greater the coherence between the oscillatory activity of various brain regions, the more likely they are to exchange information (Buzsáki et al., 2012; Fries, 2015). CMS has been shown to alter coherence among a network of structures including the PRL, the HPC, the BLA, the lateral habenula, the nucleus accumbens and the lateral dorsal nucleus of the thalamus Zheng et al., 2015; Mirbolouk et al., 2023; Cao et al., 2023; Qi et al., 2020).

The first aim of this study was to explore neural regional activity and inter-regional communication within a network composed of the PRL, BLA and dHPC through LFP recordings in order to investigate possible dysfunctions in CMS-vulnerable animals compared to non-stressed animals. The second aim was to evaluate the consequences of both acute and chronic Ket treatments on such potential dysfunctions. For this purpose, a within-subject design was chosen to investigate possible therapeutic mechanisms; therefore, the effects of Ket were only evaluated in the CMS-vulnerable animals. As spectral power strongly depends on the vigilance states (Adamantidis et al., 2019), and since the effects of Ket on spectral power may also depend on them (Kantor et al., 2023), spectral power was determined for each vigilance state, *i.e.* Wake, NREM sleep and REM sleep. Furthermore, studies most often explore the functional effects of Ket over a limited amount of time following administration, leaving a gap in our understanding of possible variations with prolonged evaluation, and nycthemeral differences according to light and dark periods. Therefore, LFP recordings were performed during 24 hours, and data were separately analysed according to both periods.

C) Materials and methods

1) Animals

This study was conducted on male WKY rats (Charles River, Sulzfeld, Germany). The CMS-vulnerable animals studied here (n=9) were those whose electrocorticogram (ECoG) signals were analysed during our third study (article 3, page 103), ECoG and LFP signals having been acquired simultaneously. The age-matched non-stressed control animals (NS; n=8), also included in our third study, were from a different cohort and were not exposed to any stressor.

2) Experimental design

The design was identical to our third study (page 103). CMS-assigned rats underwent 4 weeks of the stress protocol as described in our previous study (Loizeau et al., 2024) ; article 1, page 83). They then underwent behavioural testing using the forced swim test (FST) and elevated plus maze (EPM) to identify CMS-vulnerable animals (Loizeau et al., 2024 and article 3). Such animals underwent electrode implantation surgery in the PRL, the CA1 region of the dHPC, and the BLA as described previously (article 3, page 103). After recovery, CMS-vulnerable animals underwent two additional weeks of the CMS protocol. Following habituation to being connected to the data acquisition system, LFP recordings were then conducted. Age-matched NS were similarly operated on, and their LFPs recorded. Three 24-hour recording sessions were performed in the following conditions: ***i***)

Session 1 was conducted following NaCl injection in both groups; ***ii***) Session 2 was conducted following a first Ket injection in CMS-vulnerable animals (or first NaCl injection for NS animals), and ***iii***) Session 3 was conducted following the 7th daily Ket injection in CMS-vulnerable animals (or 7th NaCl daily injection for NS animals). The dose of Ket was 10 mg/kg (1 ml/kg) at each injection and NaCl (0.9 %) was also injected at a volume of 1 ml/kg.

3) Data analyses

LFP preprocessing

LFP signals, recorded with three channels for each structure, were analysed using a pipeline of MATLAB scripts. In order to eliminate potential volume-conducted signals from surrounding sources, for each implanted region, bipolar derivations were computed thusly: two of the three channels with optimal signal quality (presenting least noise and/or artefacts on the signal itself and its computed power spectrum) were visually identified; their signal was then subtracted from each other. Data were filtered using a Butterworth filter in the passband domain (1.26 – 200 Hz, applied with the filtfilt function for zero-phase filtering), and artefacts were removed from the signal using an algorithm from Islam et al. (2014) based on stationary wavelet transform. Any remaining line noise at 50 Hz was removed using a Butterworth notch filter tailored to each signal's contamination.

After filtering, the signal was segmented into one-hour bins according to the three different vigilance states determined during the previous study, namely Wake, NREM sleep, and REM sleep. Briefly, hypnogram data of each recording session was used as an index on the temporally corresponding LFP data. Thus, for the 24 hours of each recording, signal segments were concatenated according to vigilance state. To avoid transition signal between states, two epochs (8 seconds of signal) at both the beginning and end of each segment were removed. Only the first minute of signal of each hourly concatenated segment was processed for coherence analysis.

Spectral power analyses

Spectral power was computed using Fast Fourier Transform (FFT) via the Chronux Toolbox. Power spectra were generated using the *mtspecgramc* function with a 4-second sliding window in 0.2-second steps, 9 tapers and a time-half bandwidth product set to 5. For each hour, absolute power within the frequency bands of interest [delta (1.26-4 Hz), theta (5-8 Hz), alpha (9-12 Hz), beta (13-29 Hz), gamma (45-65 Hz)] was calculated as the area under the curve (AUC; computed with the *cumtrapz* function) of the power spectrum in that band. For session 1, power in higher frequencies, namely high

frequency oscillations (HFO; 130-180 Hz) were later also investigated to better characterise spectral changes between NS and CMS-vulnerable animals considering the broader power spectrum. Due to important differences in scale concerning power values within and between recordings, relative rather than absolute power was compared. Relative power was obtained by dividing the band-specific absolute AUC by the total AUC across the entire frequency range.

Coherence analyses

Magnitude-squared coherence (Schneider et al., 2020) was computed via the Chronux *cohgramc* function between bipolar LFP signals in pairs of structures (PRL-BLA, PRL-dHPC, BLA-dHPC) over the first minute of each vigilance state within each hour of each recording session. Coherence values were extracted for the same frequency bands used in the power analysis. To estimate and correct for chance-level synchrony, bootstrapping with 1,000 iterations was performed by temporally reshuffling the signal within each analysed 1-minute segment, and calculating the new coherence values each time. Corrected coherence was obtained by subtracting, from the original coherence, the 95th percentile of the bootstrapped distribution (50th highest value out of 1,000), representing a 5 % significance threshold, thereby accounting for random coherence arising from nonstationary or correlated noise components.

4) Histology

At the end of the experiment, rats were deeply anaesthetised [ketamine (100 mg/kg; Imalgen 1000 ®, Boehringer Ingelheim, Germany) / xylazine (10 mg/kg; Rompun ® , Bayer, Germany)] and transcardially perfused with 60 ml of 0.1 M PBS (4°C) followed by 60 ml of 4 % paraformaldehyde [PFA (Carl Roth, Germany); pH 7.4; 4°C]. Brains were extracted and post-fixed in the same fixative for 4 hours, then stored for 48 hours at 4°C in a 20 % sucrose solution in 0.1 M PBS for cryoprotection. They were then snap-frozen by immersion in isopentane (Carl Roth, Germany) at - 35°C for 1 minute. Coronal 40 µm-thick sections were cut using a cryostat (Cryostar NX70, Epredia via Microm Mictotech, France), and collected onto gelatin-coated microscope slides. Sections were coverslipped with a DAPI-fluoromount medium. Electrode tracks, labelled with DiI, were visualised using an Apotome microscope (Zeiss Axio Imager M2, Zeiss®, Germany) with the software Zen (Blue Edition) to confirm placement within the intended brain regions by comparison with a standard rat brain atlas (Paxinos and Watson, 7th edition, 2014).

5) Statistical analyses

LFP signals were processed according to the three vigilance states of interest (Wake, NREM sleep, REM sleep). Data were analysed in 2-hour blocks separately for both lighting periods, *i.e.* during the first ten hours of recording (light period; 5 blocks) and during the following twelve hours of recording (dark period; 6 blocks). The two last hours of recording were not analysed. However, there were discrepancies in the amount of data analysed according to the vigilance states and lighting period. Not only did rats spend very little time in REM sleep at the beginning of the recording sessions and during the dark period, but sleep onset latency was greatly enhanced following Ket administration (see article 3, page 103). A schematic representation of the blocks included in the different analyses is presented in **Figure 1**. Moreover, within some of the analysed blocks, some rats spent no time in REM sleep; therefore, data were imputed based on the mean value of the corresponding group (in the case of the first analysis) or session (in the case of the second analysis) for those time-points. It involved one imputation in two blocks during session 2, and two imputations in one block during session 3.

Although the analysis scripts provided data for each band of interest in each vigilance state of interest, we decided to only consider: ***i***) theta, alpha, beta, and gamma bands during Wake; ***ii***) delta and gamma bands during NREM sleep; ***iii***) theta and gamma bands during REM sleep, which are the most relevant bands according to the respective states (Adamantidis et al., 2019) (delta, for example, is practically absent during REM sleep).

To evaluate differences between NS and CMS-vulnerable animals, data from session 1 were analysed using ANOVAs with Group as between-subject factor and Block as within-subject factor. To evaluate the consequences of acute and chronic Ket regimens in CMS-vulnerable animals, data of sessions 1-3 in CMS-vulnerable animals were analysed with ANOVAs with Session and Block as within-subject factors. They were completed by Fisher's Least Significant Difference (LSD) post hoc tests when appropriate. The threshold for rejecting the null hypothesis was set at 0.05 throughout the study.

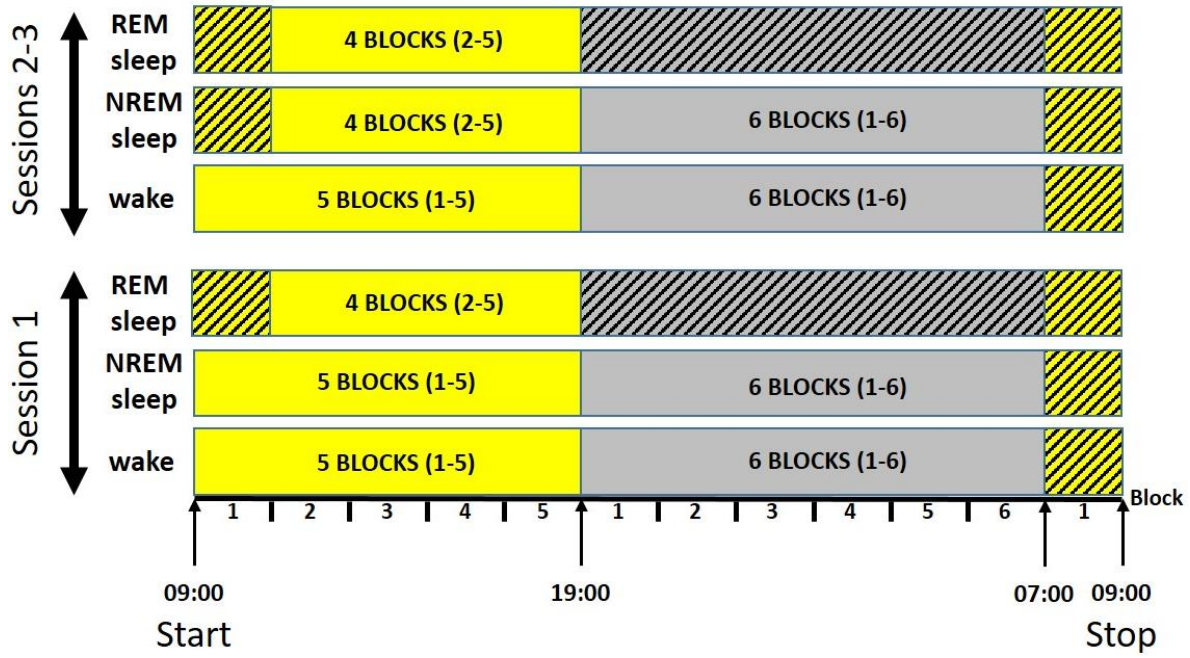

Figure 1. Representation of the number of 2-h blocks, within the light (yellow) and dark (grey) periods of each 24-hour recording session, considered for the different ANOVAs. The same colour code is used in subsequent figures. Dashed blocks represent blocks for which data were not taken into account for the analysis.

D) Results

Note: Only data concerning spectral power analyses are presented in the current section. Coherence results, which are not currently interpretable, have been removed (see final discussion paragraph for explanation).

1) Differences in relative spectral power between NS and CMS-vulnerable animals (session 1)

Full details of the ANOVAs' results are presented in **Supplementary Tables 1-3**. Data concerning NREM sleep delta and gamma power for which only a Block effect was present are available in supplementary information (**Supplementary Figures 1-2**). Data concerning HFO during Wake is also available in supplementary information (**Supplementary Figure 3**). Only the data for which there was a Group effect or a Group x Block interaction are described below.

PRL

During both light and dark periods, ANOVAs revealed a significant Group effect, no Block effect nor interaction for Wake beta (light, $p = 0.031$; dark, $p = 0.025$) and for NREM sleep gamma (light, $p = 0.031$; dark, $p = 0.06$). For both periods, beta relative power during Wake episodes was lower in CMS-vulnerable than in NS animals (**Figure 2A**) while gamma relative power during NREM sleep episodes was higher in CMS-vulnerable animals (**Figure 2E**).

ANOVAs revealed Group x Block interactions for theta, alpha and gamma during dark period Wake ($p = 0.025$, $p = 0.027$, $p = 0.005$, respectively). Post hoc on these interactions indicated a between-group difference for theta only. Theta relative power was lower in CMS-vulnerable animals than in NS animals but only during the last four blocks (at least $p < 0.05$, **Figure 2B**). Post hoc also indicated between-block variations for these bands but in opposite directions in the two groups. In NS, theta (**Figure 2B**) and alpha relative power (**Figure 2C**) increased between the beginning and the end of the dark period (theta: block 1 vs block 6; alpha: block 1 vs blocks 4-6, $p < 0.05$ for each comparison) whereas gamma relative power (**Figure 2D**) decreased (block 1 vs block 6, $p < 0.05$; block 2 vs blocks 4-6, $p < 0.05$ for each comparison). Conversely, in CMS-vulnerable animals, theta and alpha relative power decreased (theta: block 1-2 vs block 5; alpha: block 1 vs blocks 2-4, $p < 0.05$ for each comparison) across blocks (**Figures 2B and 2C**) whereas gamma relative power increased (block 1 vs blocks 3 and 6, $p < 0.05$ for each comparison; **Figure 2D**).

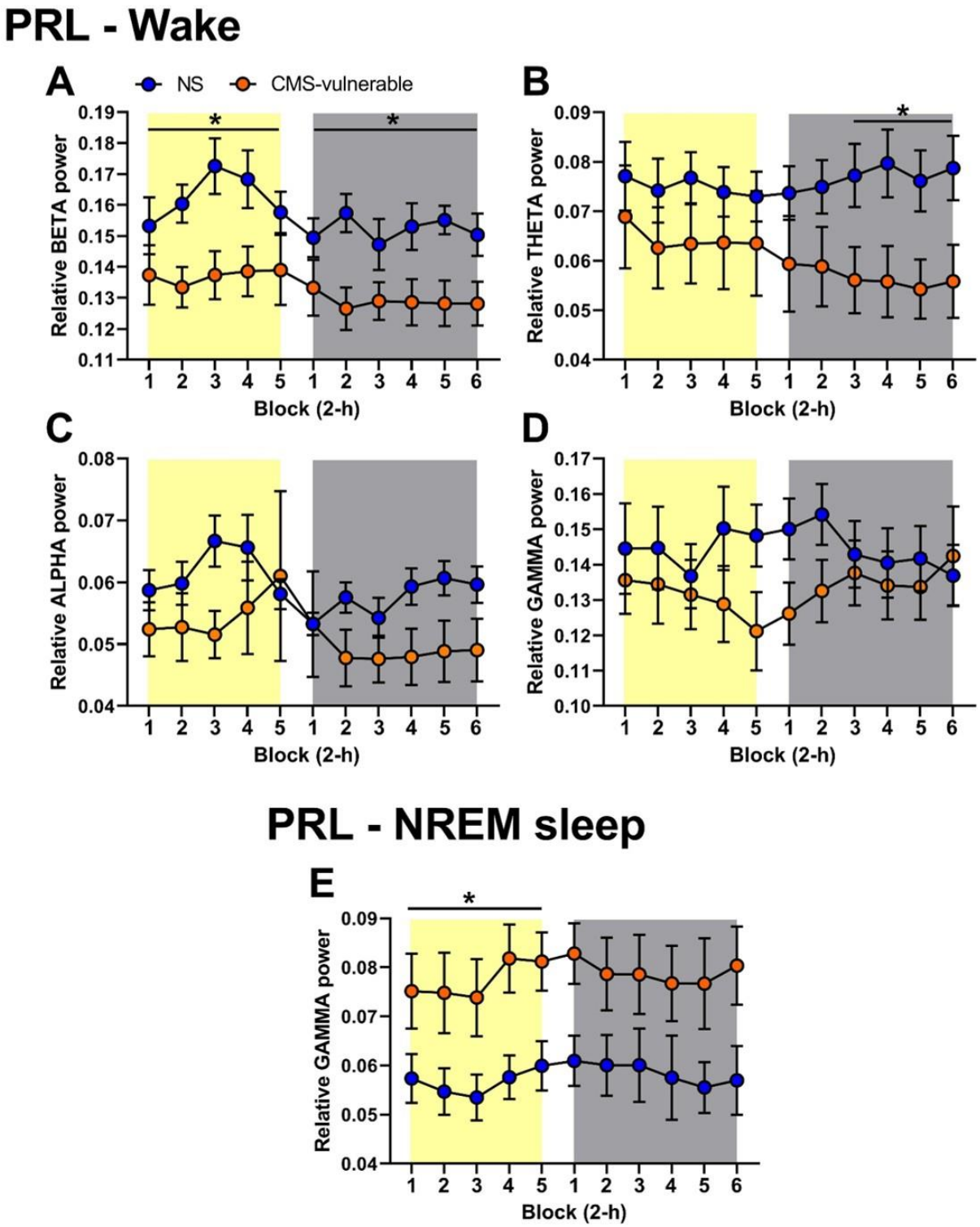

Figure 2. Significant relative power data in PRL during session 1: beta (A), theta (B), alpha (C) and gamma (D) during Wake episodes, and gamma (E) during NREM sleep episodes. *, difference between NS and CMS-vulnerable animals (at least $p < 0.05$). For reasons of figure legibility, statistics concerning differences between blocks within each group are not shown and are described only in the text. Yellow represents the light period, and Grey represents the dark period.

BLA

For data concerning REM sleep during the light period, ANOVAs revealed a significant Group effect with no Block effect nor interaction for theta and gamma (theta, $p = 0.02$; gamma, $p = 0.05$). Theta power was lower in CMS-vulnerable than in NS animals (**Figure 3A**) while gamma power was higher in CMS-vulnerable (**Figure 3B**).

For data concerning Wake during the dark period, there were Group x Block interactions for alpha and gamma ($p = 0.009$, $p = 0.042$, respectively). Subsequent post hoc tests indicated no difference between groups. There however were variations between blocks within each group for alpha but only in the CMS-vulnerable for gamma. As for the PRL, variations in alpha relative power were in opposite directions in the two groups (**Figure 3C**). In NS animals, alpha power increased across blocks (block 1 vs blocks 2 and 5-6, $p < 0.05$ for each comparison; block 2 vs block 3, $p < 0.05$; block 3 vs blocks 5-6, $p < 0.05$ for each comparison) whereas in CMS-vulnerable animals, it decreased (block 1 vs blocks 3-4, $p < 0.01$ for each comparison; block 1 vs block 6, $p < 0.05$). In CMS-vulnerable animals, gamma power increased across blocks (block 1 vs blocks 3-4, $p < 0.01$ for each comparison, block 1 vs block 6, $p < 0.05$, (**Figure 3D**).

In summary, CMS-vulnerable animals exhibited lower Wake beta power during the light and dark periods in the PRL, and lower theta power during REM sleep episodes in the BLA (though dark period REM sleep could not be analysed as explained in Materials and Methods). Interestingly, during the dark period, in both the PRL and the BLA, alpha and gamma during Wake varied across blocks but in opposite directions in the two groups: in CMS-vulnerable animals, alpha power decreased while gamma increased, whereas in NS animals, alpha power increased and PRL gamma power decreased.

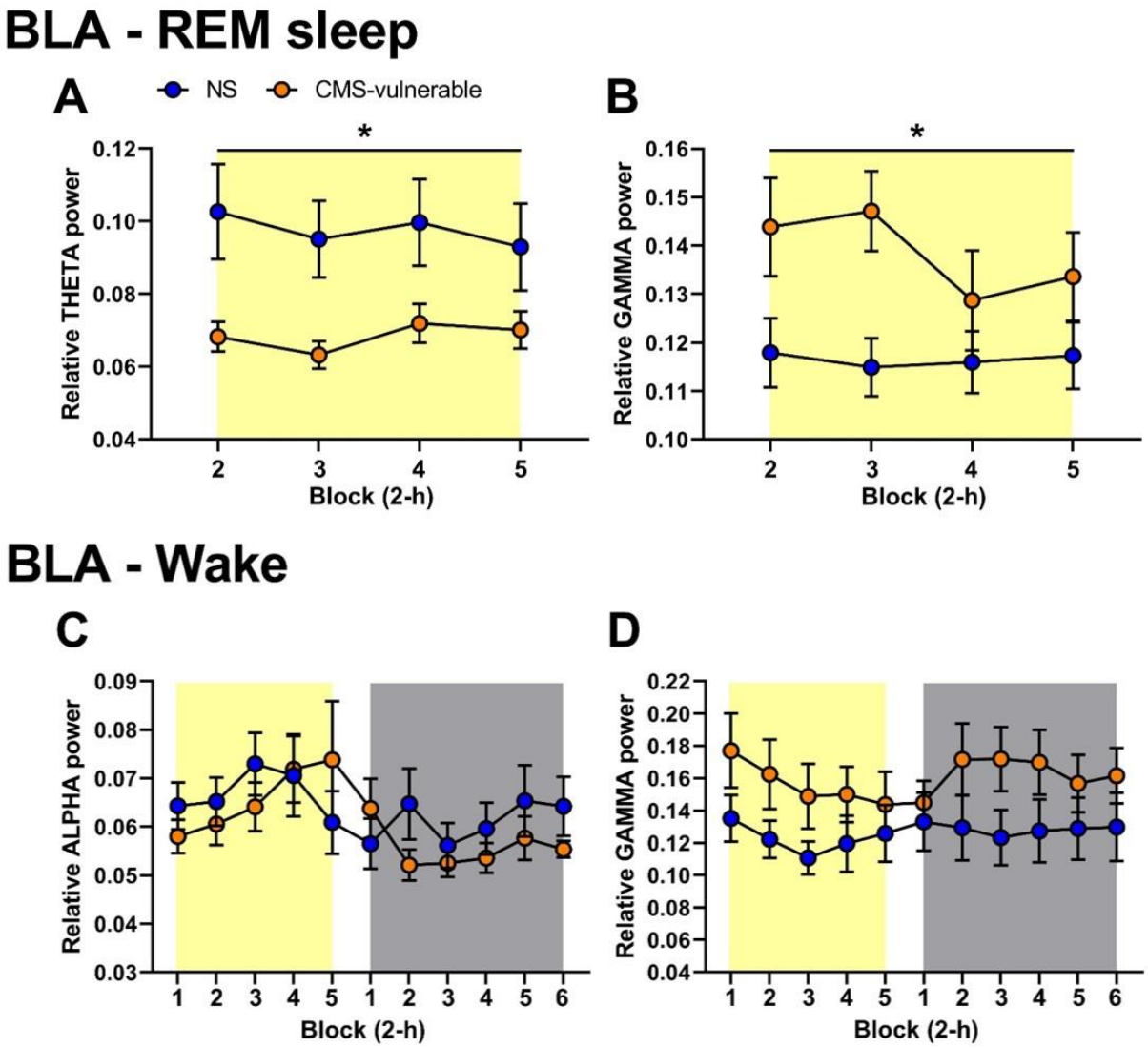

Figure 3. Significant relative power data in BLA during session 1: theta (A) and gamma (B) during REM sleep episodes, and alpha (C) and gamma (D) during Wake episodes. *, difference between NS and CMS-vulnerable animals (at least $p < 0.05$). For reasons of legibility, statistics concerning differences between blocks within each group are not shown and are described only in the text. Yellow represents the light period, and Grey represents the dark period.

2) Impact of acute and chronic Ket treatment on relative spectral power in CMS-vulnerable (sessions 1-3)

Full details of the results of ANOVAs results are presented in **Supplementary Tables 4-6**. Only the data for which there was a Session effect or a Session x Block interaction are described below.

PRL

A significant Session x Block interaction was present concerning Wake data of the light period for theta, beta and gamma ($p = 0.039$, $p = 0.030$, $p < 0.001$, respectively). Post hoc test on these interactions indicated differences between sessions for each band. For each band, they revealed

differences during block 1 between session 1 and the other two (theta, at least $p < 0.05$; beta, at least $p < 0.01$; gamma, at least $p < 0.0001$). At the beginning of the light period, theta and beta relative power were decreased after acute and chronic Ket treatments whereas gamma power was increased (**Figures 4A, 4B and 4C**). In addition, they indicated that whereas theta, beta and gamma power was similar between blocks during session 1, during sessions 2 and 3, theta and beta increased across blocks and gamma decreased (theta: block 1 vs blocks 2-3, $p < 0.02$; beta and gamma: block 1 vs blocks 2-5, at least $p < 0.02$).

Session x Block interactions were present regarding NREM sleep data of both the light and dark phase for delta ($p = 0.008$ and $p = 0.026$, respectively). Post hoc tests on these interactions indicated differences between session 1 and the other two sessions during block 2 of the light period, and during block 5 of the dark period (at least $p < 0.01$). At the beginning of the light period (block 2), acute and chronic Ket treatments increased relative delta power whereas they decreased it towards the end of the dark period (block 5) (**Figure 4D**). In addition, post hoc tests indicated a decrease in relative delta power throughout the light period for each session, whereas they revealed an increase between the beginning and the end of the dark period but only for sessions 1 and 2.

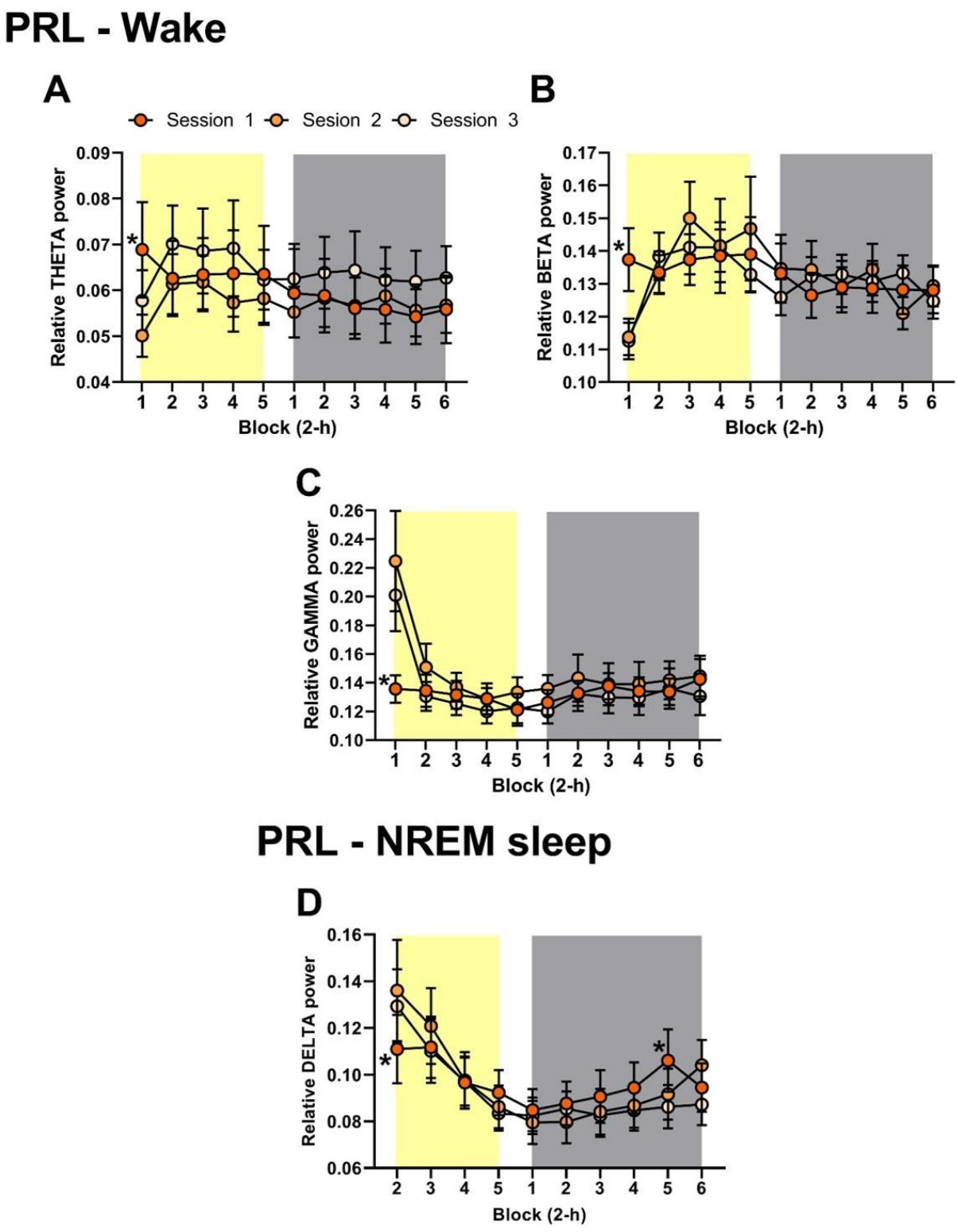

Figure 4. Significant relative power data in PRL during sessions 1-3 in CMS-vulnerable animals: theta (A), beta (B) and gamma (C) during Wake episodes, and delta (D) during NREM sleep episodes. *, session 1 vs sessions 2-3 (at least $p < 0.05$). For reasons of figure legibility, statistics concerning differences between blocks within each session are not shown and are described only in the text. Data are represented as average ± SEM. Yellow represents the light period, and Grey represents the dark period.

BLA

A Session x Block interaction was present regarding Wake data of the dark period for alpha ($p = 0.045$). Post hoc test showed differences between session 3 and sessions 1-2 during block 1 (at least $p < 0.05$), where chronic Ket decreased alpha power. In addition, post hoc test indicated that while alpha power decreased during session 1 (block 1 vs blocks 2-4 and 6, at least $p < 0.05$) it did not vary during sessions 2 and 3 (**Figure 5A**).

A significant Session x Block interaction was also found during Wake for both light and dark periods for beta ($p = 0.038$; $p = 0.018$, respectively). Regarding the light period, post hoc tests indicated differences between session 1 and sessions 2-3 during block 1 (at least $p < 0.01$). Acute and chronic Ket treatments decreased beta power during Wake at the beginning of the light period (**Figure 5B**). During the dark period, post hoc tests revealed differences between sessions 1 and 3 and session 2 during block 5 (at least $p < 0.05$), where beta relative power was lower after acute Ket treatment (**Figure 5B**). Finally, post hoc test indicated differences between sessions 1-2 and session 3 during block 1, and between sessions 1 and 3 during block 6 (at least $p < 0.05$). At both the beginning and the end of the dark period, chronic Ket treatment decreased beta power during Wake (**Figure 5B**).

Concerning NREM sleep data for both the light and dark periods, a Session x Block interaction was present for delta ($p = 0.0007$; $p = 0.003$, respectively). During the light period, post hoc tests indicated differences between session 1 and sessions 2-3 during block 2. Acute and chronic Ket treatment increased delta power during block 2 of the light period (**Figure 5C**). In addition, whereas no variations between blocks were observed during the first session, a gradual decrease was observed across blocks throughout the light period after acute and chronic Ket treatments (at least $p < 0.05$). During the dark period, post hoc tests showed differences between session 1 and sessions 2-3 during blocks 2 and 5 (at least $p < 0.05$), between session 1 and session 3 during block 4 ($p < 0.01$) and between sessions 1 and 3 and session 2 during block 6 (at least $p < 0.01$). Delta relative power was reduced after acute and chronic Ket treatments, except during the last block in which it was increased following acute treatment only. In addition, whereas dark period delta power during the first session showed initial increase, then decrease (block 1 vs blocks 4-5; block 2-4 vs block 5; block 5 vs block 6; all at least $p < 0.05$), it only increased throughout the dark period after acute Ket treatment (blocks 1-3 vs blocks 5 and 6; block 4 vs block 5; block 5 vs block 6; at least $p < 0.05$) and at the end of this period following chronic Ket treatment (block 1 vs block 6, $p < 0.05$).

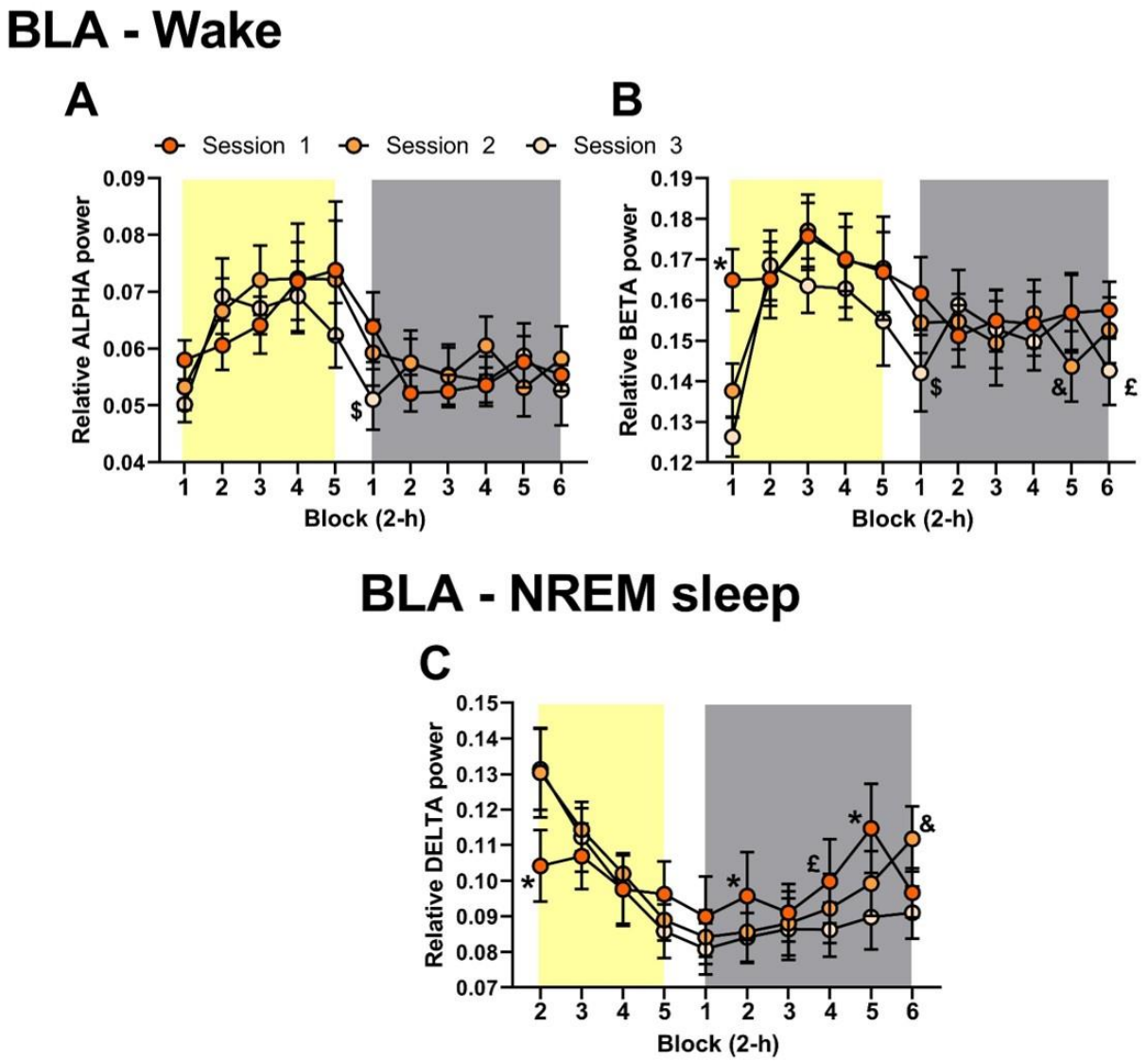

Figure 5. Significant relative power data in BLA during sessions 1-3 in CMS-vulnerable animals: alpha (A) and beta (B) during Wake episodes, and delta (C) during NREM sleep episodes. *, session 1 vs sessions 2-3 (at least $p < 0.05$); &, session 2 vs sessions 1-3 (at least $p < 0.05$); $, session 3 vs sessions 1-2 (at least $p < 0.05$); £, session 3 vs session 1 (at least $p < 0.05$). For reasons of figure legibility, statistics concerning differences between blocks within each session are not shown and are described only in the text. Data are represented as average ± SEM. Yellow represents the light period, and Grey represents the dark period.

dHPC

Concerning Wake data in the light period, there was a significant Session x Block interaction for all investigated bands (theta, $p = 0.013$; alpha, $p = 0.0003$; beta, $p < 0.0001$; gamma, $p < 0.001$). For theta, post hoc test indicated differences between session 1 and session 2 for blocks 2, 3 and 5 (at least $p < 0.05$). Theta power was increased in these blocks by acute Ket treatment (**Figure 6A**). Post hoc also indicated differences between sessions 1-2 and session 3 during block 1 ($p < 0.02$), where theta power was decreased by chronic treatment. For alpha, beta and gamma, post hoc test indicated

differences between session 1 and sessions 2-3 for blocks 1 (alpha, at least $p < 0.0001$; beta, at least $p < 0.001$; gamma, at least $p < 0.0001$) and 4 (for each band, at least $p < 0.05$). During the light period, both Ket treatments initially decreased Wake alpha (**Figure 6C** and beta (**Figure 6B**) relative power, and increased Wake alpha relative power during block 4. Conversely, they initially increased Wake gamma (**Figure 6D**) relative power in the light period (with a higher increase in the case of chronic, compared to acute treatment, $p < 0.05$), and decreased it during block 4. Post hoc test also revealed between-session differences during block 3. During this block, acute Ket treatment increased alpha power (at least $p < 0.05$ *vs* session 1) while it decreased gamma power (at least $p < 0.02$ *vs* sessions 1-3); chronic Ket treatment increased beta power (at least $p < 0.05$ *vs* sessions 1-2).

Regarding NREM sleep data in the light period, a significant Session x Block interaction was found for delta ($p = 0.0024$). Post hoc tests revealed differences between session 1 and session 2 during blocks 2, 3 and 5 (at least $p < 0.05$), between session 1 and session 3 during block 5 ($p < 0.001$) and between session 2 and session 3 during blocks 3 and 4 (at least $p < 0.05$). While acute Ket treatment initially increased, then finally decreased, delta power, chronic Ket treatment only decreased it at the end of the light period (**Figure 6E**).

In summary, both Ket regimens had immediate but transient effects on relative spectral power during Wake episodes. They decreased beta power in all three regions investigated, and increased gamma power in both the PRL and dHPC. Additionally, PRL theta and dHPC alpha power were also decreased. Furthermore, while in the light period during blocks 2 and/or 3, NREM sleep delta power was increased after both acute and chronic Ket treatments in the PRL and BLA, only acute treatment increased it in the dHPC. In this structure, delta power was reduced at the final block of the light period by both Ket treatments. During the dark period, acute and chronic Ket treatment decreased delta power in some, but not all blocks in the PRL and BLA; this effect was particularly evident at the penultimate block of the dark period (block 5).

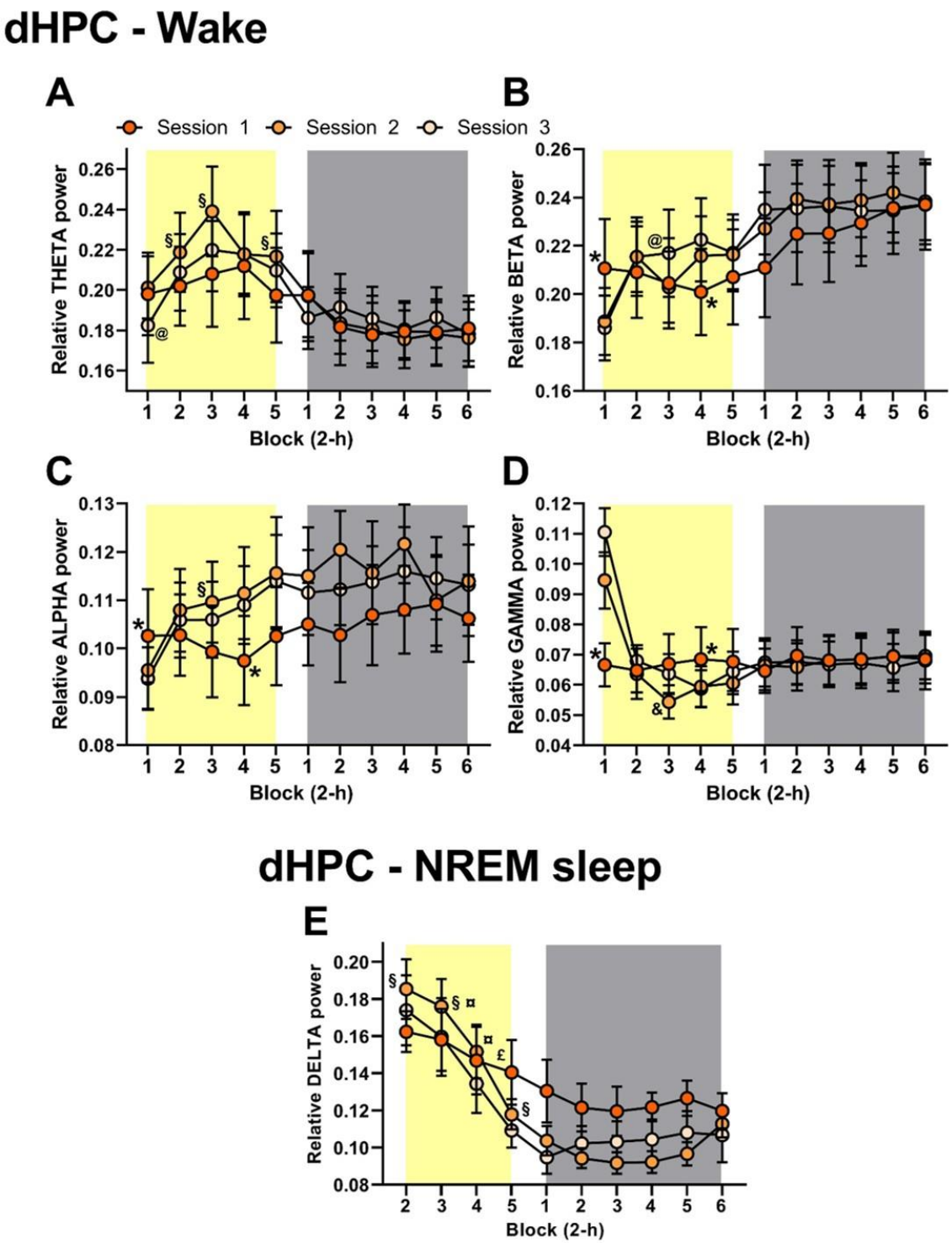

Figure 6. Significant relative power data in dHPC during sessions 1-3 in CMS-vulnerable animals: theta (A), beta (B), alpha (C) and gamma (D) during Wake episodes, and delta (E) during NREM sleep episodes. *, session 1 vs sessions 2-3 (at least $p < 0.05$); &, session 2 vs sessions 1-3 (at least $p < 0.05$); §, session 1 vs session 2 (at least $p < 0.05$); £, session 3 vs session 1 (at least $p < 0.05$); @, session 3 vs sessions 1-2 (at least $p < 0.05$); ¤, session 2 vs sessions 3 (at least $p < 0.05$). For reasons of figure legibility, statistics concerning differences between blocks within each session are not shown and are described only in the text. Data are represented as average ± SEM. Yellow represents the light period, and Grey represents the dark period.

E) Discussion

This study was designed to explore regional activity and inter-regional communication within a network composed of the PRL, BLA and dHPC through LFP recordings in CMS-vulnerable animals, and the effects of a Ket treatment depending on an acute and chronic regimen. Two analyses were performed: the first one comparing NS and CMS-vulnerable groups during the recording session 1; the second comparing the three recording sessions within CMS-vulnerable animals to evaluate the effects of both acute and chronic Ket treatment. We focused our investigations of electrophysiological data during both the light and dark periods, distinguishing states of Wake, NREM sleep and REM sleep. While coherence between region pairs was also computed for each investigated band, current results are not interpretable and have been removed from the article (see final discussion paragraph for explanation).

1) Differences in relative spectral power between NS and CMS-vulnerable animals

In the PRL, frequencies such as theta and beta showed lower relative power, while higher frequencies such as HFO (**Supplementary Figure 3**) showed higher spectral power. Gamma power was overall higher during NREM sleep, though no differences were found in REM sleep in the two investigated bands. In the BLA, a similar phenomenon was particularly evident during REM sleep, during which theta relative power was lower, while gamma was higher. The predominance of effects in the PRL supports the notion that medial prefrontal dysfunction is a core feature of chronic stress in vulnerable animals (Gerrits et al., 2003; Jing et al., 2021; Kumar et al., 2014), and is consistent with its central role in chronic stress induced mood pathology (Pizzagalli & Roberts, 2022). The BLA presented fewer alterations, whereas the dorsal hippocampus appeared largely preserved. Of note, another study using CMS found alterations in power in the mPFC or the ventral HPC (vHPC), but not the dHPC (Jacinto et al., 2013), suggesting dHPC neural dynamics to be less affected by CMS. As the relative, rather than absolute power was investigated in the present study, the observed changes reflect changes in the contribution of different frequency bands to the overall signal. The general spectral pattern of CMS-vulnerable animals, represented by overall reduced low-frequency and increased high-frequency relative power (See **Supplementary Figures 4-6** for power spectra of the PRL, the BLA and the dHPC, respectively ; the latter structure, which was preserved, serving as comparison), suggests modifications in the aperiodic components of power spectral density (PSD). Thus, one cannot directly compare PSD periodic differences in specific band power between the NS and CMS-vulnerable groups, which may be biased by aperiodic components. The aperiodic components include two parameters: the offset and the exponent, or 1/f slope. The offset corresponds to the power value at

the lowest frequency (Bush et al., 2024), so that lower offset means lower initial PSD power. The exponent determines how steep the 1/f slope is, *i.e.* how strongly power declines with increasing frequency. A smaller exponent therefore leads to a flatter 1/f slope. A decrease in both offset and exponent produces a flatter PSD with lower initial power, characterised by diminished low-frequency power and relatively enhanced high-frequency power, consistent with visual inspection of the data at the peak of such effects (**Supplementary Figures 4-6**). The exponent is determined by the excitation/inhibition (E/I) balance (Medel et al., 2023). Decreased inhibition, for example through impaired GABAergic interneuron function, leads to a flatter slope (Gao et al., 2017). Adequate E/I balance in the mPFC is critical for normal functioning ; of note, depression, like many psychiatric diseases, has been associated with impairments in such a balance (Ferguson & Gao, 2018). Chronic corticosterone administration has recently been shown to reduce the exponent of the PSD, *i.e.* impairing the E/I balance (Bergosh et al., 2024), further supporting similar changes induced by chronic stress. Additionally, increased power in higher frequencies (>30 Hz) has been proposed to reflect asynchronous firing of a local neural population (Guyon et al., 2021). Mechanistically, impaired E/I balance and asynchronous activity converge on impaired inhibitory function of GABAergic interneurons, notably the parvalbumin (PV) interneurons (Guyon et al., 2021). Cortical structures, and the mPFC particularly, contain interneurons such as those expressing parvalbumin (PV) which allow fine-tuning of neural activity (Ghosal et al., 2017). Indeed, phasic inhibitory firing of PV interneurons is critical in synchronising neural activity (Ferguson & Gao, 2018). PV function in rodent models of depression has been shown to be impaired. In a study using the learned-helplessness paradigm, vulnerable mice showed reduced excitation of PV interneurons, and artificial inhibition of PV interneuron activity was sufficient to induce vulnerability (Perova et al., 2015). Additionally, CMS has been shown to decrease GABAergic markers and inhibitory currents in the mPFC (Ghosal et al., 2020). Concerning the PSD offset, chronic corticosterone has also been shown to decrease this aperiodic parameter (Bergosh et al., 2024). PSD offset has been linked to firing rate of a local neural population (Gao, 2016), and CMS has also been shown to lead to decreased mPFC firing rate, a phenomenon rescued by classical antidepressants (Guo et al., 2014). This decreased firing rate could be related to prefrontal atrophy and synaptic losses, which are a hallmark of chronic stress and depression (Pizzagalli & Roberts, 2022). Altogether, these findings suggest impaired neural dynamics through changes in the aperiodic components of neural activity, predominantly affecting the PRL of CMS-vulnerable animals. Such alterations may be linked to impaired inhibitory regulation, reduced neuronal synchrony, and disrupted E/I balance. The resulting flattening of the PSD slope and enhancement of

high-frequency power may serve as electrophysiological markers of cortical dysregulation in vulnerable animals exposed to chronic stress and depression-like states.

While one cannot directly compare differences in band power between groups, regarding within-group variations of spectral power, NS animals showed a gradual increase in alpha and theta power in the PRL and a decrease in gamma power during wakefulness of the dark phase. As these reflect relative power changes, precise dynamics cannot be determined, as an increase in lower bands may drive decrease in higher bands, and vice-versa. These patterns however suggest a gradual transition from more active to quiet Wake. Indeed, prefrontal gamma during wakefulness increases with active behaviour (Furth et al., 2017). Prolonged time spent awake has been associated with decline of gamma power and increase of theta power in the frontal EEG during quiet Wake suggesting that it may represent a marker of sleep pressure (Grønli et al., 2016). Interestingly, alpha power also increased but in the somatosensory, rather than prefrontal cortex EEG in the same study. In contrast, CMS-vulnerable animals showed a decrease in PRL alpha and theta power and increase in gamma power, an inverse pattern relative to NS animals. According to the hypothesis above, this suggests a shift or dysregulated transition between wakefulness states, possibly suggesting more arousal, which has been linked to CMS-induced power enhancement in higher bands (Mirbolouk et al., 2023). While no late dark period changes were observed in sleep-wake parameters (**See article 3, page 103**) CMS-vulnerable did spend more time alseep during its early part. Furthermore, we have previously demonstrated delayed locomotor activity rhythms in CMS-vulnerable WKY rats, supporting more active Wake later on in the dark period (Loizeau et al., 2024). A similar phenomenon was observed in the BLA, in which alpha power increased in NS animals while it decreased in CMS-vulnerable, where gamma concurrently increased. In all structures and both groups, NREM delta power gradually decreased during the light phase, while it increased during the dark phase except in the dHPC. NREM sleep delta power is a marker of sleep pressure (Goldschmied et al., 2022), indicating dissipation during the light period and buildup during the dark period of sleep pressure in both groups. This similarity however contrasts with the pattern observed during dark period variations in Wake. Interestingly, interplay of both Wake gamma power and NREM sleep delta power may be indicative of dysregulated sleep homeostatic processes and is impaired in TRD. While this has not yet been investigated in the present data, a recent clinical study has shown that NREM sleep EEG delta power, which is correlated with Wake gamma power in healthy individuals, is no longer correlated in TRD patients (Kheirkhah et al., 2025).

2) Impact of acute and chronic Ket treatment on relative spectral power in CMS-vulnerable animals

Ket's effects on relative LFP power, which were evaluated within CMS-vulnerable animals, led to immediate changes after administration in Wake, *i.e,* in the first block for the light period. In all three structures, both Ket regimens reduced beta power, while gamma power was increased in the PRL and dHPC. Present results concerning beta are in line with the effects of Ket in rats (Bowman et al., 2022; Yu et al., 2020), including WKY rats (Manduca et al., 2020) and in non-human primates (Ma et al., 2018; Skoblenick et al., 2016). An integrated review concerning clinical data show mostly suppressed or no effect on beta power following Ket (McMillan & Muthukumaraswamy, 2020). Beta power inhibition has notably been linked to psychotomimetic features (Bowman et al., 2022). Some studies have however reported that baseline beta power, instead of being suppressed, can be enhanced by Ket (Páleníček et al., 2011; Qin et al., 2023), including in the WKY rat (Kantor et al., 2023), although stimuli evoked beta power is decreased (Qin et al., 2023), suggesting less sensitivity to external stimuli, which may be tied to Ket's anaesthetic and analgesic effects (Zanos et al., 2018). Indeed, frontal EEG beta power has been linked to sensory processing (Grønli et al., 2016). Concerning gamma power during Wake episodes, its enhancement is a hallmark of Ket's effects (Polis et al., 2019), in rodents (Kittelberger et al., 2012) including in the WKY rat (Manduca et al., 2020), in non-human primates (Ma et al., 2018) and in humans (McMillan & Muthukumaraswamy, 2020). Ket also decreased relative theta power. This was the case for both regimens in the PRL, while only chronic Ket decreased dHPC theta. However, chronic Ket led to particularly elevated dHPC gamma power at the same time point, so the effect may also be influenced by changes in the relative balance of powers. Results concerning Ket's effects on theta power are highly heterogeneous. It has been shown to decrease theta power in the mPFC of rats (Moran et al., 2015) or in the HPC (Kittelberger et al., 2012; Lazarewicz et al., 2010; Speers et al., 2023). However, no effect on PFC theta power was also reported (Bergosh et al., 2024), including in unstressed WKY rats (Manduca et al., 2020). Dose-dependent increases in frontoparietal theta EEG have also been reported with (R)- but not (S)-Ket (Pothorszki et al., 2024). Ket-induced decrease in theta power has been suggested to be due to a shift in the peak of theta oscillations towards higher frequency (Kittelberger et al., 2012), but has also been associated with reduced connectivity between the mPFC and the hippocampus (Moran et al., 2015), as prefrontal theta can be entrained by the hippocampus, though it also has local generation (O'Neill et al., 2013). Such heterogeneity of results may be linked to differences in how spectral power was calculated, and methods are regrettably rarely detailed.

These wake-related power changes were no longer evident after the initial block of recording: bands that were initially reduced, such as beta, returned to session 1 levels, while gamma, which was initially increased, decreased to session 1 levels. Ket's immediate modulation of oscillatory power during Wake appears strongly tied to its pharmacological properties, *i.e.* NMDA-R antagonism. As observed in the present study, or other studies (Mahdavi et al., 2020), it only lasts for up to 2 hours, which strongly aligns with its presence in the organism (Páleníček et al., 2011). Two opposing mechanisms have been suggested to explain such effects concerning gamma. Using a biophysical model, a study has shown this to be related to NMDA-R antagonism. By preventing NMDA-R controlled tonic inhibition of cortical networks, Ket may disinhibit both pyramidal neurons and phasic inhibitory interneurons, giving rise to highly synchronised gamma oscillations (Adam et al., 2024). However, an *in vivo* study also suggested that Ket-increased gamma reflects loss of synchronisation by preventing excitation of phasic inhibitory PV interneurons (Guyon et al., 2021), which, as stated above, can also enhance power in higher frequencies. Ket-induced decrease in theta power has been proposed to also involve dysregulation of critical inhibitory interneuron in cortical loops (Lazarewicz et al., 2010). Mechanistic explanations of Ket's modulation of beta power have not been proposed, though a similar loss of synchrony might explain reduced beta power, as beta oscillations also generate from cortical microcircuits involving inhibitory interneurons (Sherman et al., 2016). Ket's modulation of oscillatory dynamics appears dose-dependent (Adam et al., 2024; Manduca et al., 2020). Interestingly, a study showed that while at 9 mg/kg, Ket mainly affected gamma power, a treatment of 30 mg/kg increased power in all power bands (Páleníček et al., 2011). Ket's effects on spectral power are associated with reduced cerebral long-range communication and local hyperactivity (Ma et al., 2018).

Of note, while acute Ket did not initially reduce dHPC theta power, it increased it after the first block, an effect noticeable for up until 10 h post-administration. Interestingly, a study showed that (R)-, but not (S)-Ket, could increase frontoparietal EEG theta power for several hours post-administration (Pothorszki et al., 2024). This study proposed the involved mechanisms not to depend on NMDA-R antagonism, which indeed is likely: as explained above, passed ~2 h post-injection, Ket is mostly eliminated from the organism. Such a prolonged time scale suggests involvement of downstream signalling cascades, such as BDNF or mTOR activation which can last for several hours in the HPC (Caffino et al., 2024), through potential links with oscillatory power remain unclear. While a similar effect has not been explored in the alpha band, the fact that both are adjacent may hint to increase in the general 6-12 Hz range. Indeed, Pothorszki et al. observed increases at the upper edge of theta,

around 7-8 Hz (Pothorszki et al., 2024). The absence of this effect under chronic Ket likely reflects an adaptation to repeated exposure. Previous work has shown that 28 days of Ket treatment can induce compensatory changes in the hippocampus that produce effects opposite to those seen after acute administration (Luo et al., 2021). In contrast, the evaluation after a 7-day regimen as presently employed may reveal a point of balance where the acute effect is no longer detectable, yet potential longer-term compensatory changes observed after extended treatment do not appear. This interpretation aligns with the observation that theta power was decreased in the chronic condition: if decreased theta is driven by NMDA-R antagonism (Lazarewicz et al., 2010), in which (S)-Ket is more potent, while increased theta is driven by (R)-Ket modulated mechanisms, both influences could have cancelled each other out in the acute regimen, the drug being notably composed of equal amounts of each enantiomer (Zanos et al., 2018). With repeated treatment, adaptation to (R)-Ket induced mechanisms would prevent theta increases, allowing the (S)-Ket–mediated theta decrease to appear as significant. It is noteworthy that the dHPC was preserved from alterations compared to NS session, while the PRL and BLA were not. The fact that it was the only structure where this increase was revealed may suggest that neural dynamics impairments in CMS-vulnerable rats prevented this effect from appearing in the PRL and BLA. Another possibility is that this effect is generally region-dependent, which could be the case if these depend on signalling cascades (Caffino et al., 2024). While there were no other Wake-related differences in the light period, the BLA showed significant changes in the dark period, where acute Ket decreased beta power during the penultimate block, while chronic Ket decreased alpha power during the first block, and beta during the first and last blocks of Wake. Coincidentally, these blocks also showed increased total Wake time with chronic Ket in the sleep-wake study (**See article 3, page 103**). A direct parallel between increased Wake time and reduced beta power however seems insufficient to explain this, as Wake time was also higher during the first block in session 1, with no effect on BLA beta power. The change in alpha power could be related to change in that general frequency range, as both bands presented similar variations upon visual inspection of the data. While beta power in the BLA at rest has, to our knowledge, not been previously defined, beta in the BLA seems mainly increased during active engagement in tasks (Lee et al., 2025) or sensory processing and aversive behaviour (Chapuis et al., 2009), suggesting its decrease might reflect more calm Wake and/or less negative affect.

Concerning NREM sleep relative power changes, Ket led to transiently increased delta power in the PRL, BLA, and dHPC, although in the latter structure this effect was only significant with acute treatment. Ket treatment has previously been shown to enhance delta power during subsequent NREM

sleep in a dose dependent manner (Feinberg & Campbell, 1995). While Ket-induced anaesthesia also leads to enhanced delta waves (Dworak et al., 2011), one may suspect that the increased delta power presently evidenced during the NREM sleep could reflect increased sleep pressure. Indeed, Ket is known to suppress sleep, which was the case in the recorded CMS-vulnerable rats, and later led to sleep rebounds (**See article 3, page 103**). Furthermore, increased delta power post-Ket was proven to appear only when sleep was suppressed, and may also be enantiomer-specific, as (S)- but not (R)-Ket produces it (Koncz et al., 2023). Consistently with this hypothesis, after the initial increase, delta power in all structures decreased across NREM sleep during the light phase with both regimens, suggesting efficient sleep dissipation. It however did not in session 1, in line with the 'flatter' sleep-wake rhythm in these rats. This suggests that Ket could restore impairments in homeostatic sleep processes, which may involve its therapeutic mechanisms. Interestingly, however, dHPC delta power took longer to decrease, suggesting different modulation in this structure. This have been previously observed compared to motor/somatosensory areas (dos Santos Lima et al., 2019), although in this case dHPC NREM delta power was shown to have quicker drops prior to microarousal states. However, the absence of such a phenomenon with chronic Ket suggests adaptation, and overall parallels effects during Wake in increased theta/alpha power, suggesting similar mechanisms may be involved. During the dark phase, delta power was generally lower than in session 1 following both Ket regimens, likely as a consequence of better dissipation during the light phase, and gradually increased during the dark phase, indicative of rising sleep pressure with prolonged wakefulness.

Overall, few differences were noted between the acute and chronic Ket treatments on spectral power. Both treatments had similar transient modulation of Wake oscillatory power during the first block of the light phase, and initially increased NREM sleep delta power, suggesting increased sleep pressure after sleep suppression. However, acute, but not chronic Ket seemingly led to prolonged dHPC-specific enhancement of theta and alpha Wake power, NREM sleep delta power, and decrease of gamma Wake power during the light phase, which may be tied to (R)-Ket-induced cascades. These PSD effects also hint to aperiodic alterations, possibly reflecting increased offset. Of note, Ket has been shown to enhance excitability in the CA1 region for several hours post washout (Jang & MacIver, 2021), which would be consistent with increased firing rate, *i.e.,* increased offset. We hypothesise the absence of this phenomenon in chronic Ket may be due to adaptation mechanisms. In the BLA, only chronic Ket reduced Wake beta power during the beginning and final part of the dark phase, possibly indicating more calm Wake and/or less negative affect.

3) Coherence methodology

Concerning coherence, some values obtained after bootstrap correction were negative. This result is not possible, as the value of magnitude squared coherence for any given frequency is always a real number between 0 and 1 (Malekpour et al., 2018). In other words, "real" coherence cannot fall below the value representing no coherence at all. This is seemingly the result of overcorrection due to the bootstrapping method used relying on 95th percentile. By producing spurious dependencies, percentile-based corrections using extreme bounds such as 95 % may exceed the true bias to be removed (Pukenas, 2015), leading to negative coherence values. Consequently, the present corrected dataset cannot be interpreted. Using other valid, but less conservative methods, such substracting mean or median of the bootstrap, or estimating error through confidence intervals (Dubey & Ray, 2016; Pukenas, 2015), rather than 95th percentile subtraction, may therefore yield exploitable results.

F) Conclusion

Altogether, we observed that CMS-vulnerable animals exhibited neural activity impairments suggesting alterations of aperiodic components of the LFP PSD, predominantly in the PRL. These impairments affected the BLA to a lesser extent, while sparing the dHPC. CMS-vulnerable animals also showed an altered dark-phase variation in alpha and gamma band power, displaying a pattern opposite to that of NS animals. Ket administration, regardless of regimen, transiently modulated cortical oscillations during wakefulness by suppressing theta and beta power and enhancing gamma power, likely via NMDA-R–mediated disruption of interneuron–pyramidal loops within cortical microcircuits. It increased delta power during subsequent NREM sleep, seemingly as a consequence of initial sleep suppression. Finally, we observed regimen-specific effects, such as in the dHPC, where acute treatment produced marked increases in low-frequency power such as in the theta and delta bands, and decreases in high-frequency power such as in the gamma band, possibly reflecting mechanisms driven by (R)-Ket. Their absence with chronic treatment suggests adaptation following repeated administration. In the BLA, chronic treatment decreased beta power at the beginning and the end of the dark period, which might reflect more calm Wake or less negative affect. Results concerning LFP coherence are not currently interpretable.

G) Limits

The present study has several limitations. Direct Wake behaviour was not monitored. Therefore, electrophysiological data could have been recorded during different behaviours for different rats, *e.g.* drinking, eating, moving or resting in the home cage. Such variability may have masked

results. The study was conducted exclusively in male, CMS-vulnerable rats and examined electrophysiological effects using only a single Ket dose. Most of all, at present, selected bootstrap method for bias correction prevented analysis of coherence results, as some coherence results were below 0 following correction.

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H) Supplementary information

Wake	Light period	Dark period
PRL		
Theta	Group: $F_{1,15} = 0.91$, p = 0.35 Block: $F_{4,60} = 1.79$, p = 0.14 G x B: $F_{4,60} = 0.45$, p = 0.77	Group: $F_{1,15} = 4.28$, p = 0.06 Block: $F_{5,75} = 0.53$, p = 0.75 **G x B: $F_{5,75} = 2.75$, p = 0.025**
Alpha	Group: $F_{1,15} = 0.85$, p = 0.37 Block: $F_{4,60} = 0.66$, p = 0.62 G x B: $F_{4,60} = 1.43$, p = 0.23	Group: $F_{1,15} = 2.05$, p = 0.17 Block: $F_{5,75} = 1.01$, p = 0.42 **G x B: $F_{5,75} = 2.69$, p = 0.027**
Beta	**Group: $F_{1,15} = 5.63$, p = 0.031** Block: $F_{4,60} = 1.70$, p = 0.16 G x B: $F_{4,60} = 1.53$, p = 0.21	**Group: $F_{1,15} = 6.19$, p = 0.025** Block: $F_{5,75} = 1.70$, p = 0.16 G x B: $F_{5,75} = 1.53$, p = 0.21
Gamma	Group: $F_{1,15} = 1.06$, p = 0.32 Block: $F_{4,60} = 0.91$, p = 0.46 G x B: $F_{4,60} = 2.28$, p = 0.07	Group: $F_{1,15} = 0.62$, p = 0.44 Block: $F_{5,75} = 0.62$, p = 0.68 **G x B: $F_{5,75} = 3.68$, p = 0.005**
BLA		
Theta	Group: $F_{1,15} = 0.41$, p = 0.53 Block: $F_{4,60} = 2.02$, p = 0.10 G x B: $F_{4,60} = 1.21$, p = 0.32	Group: $F_{1,15} = 1.05$, p = 0.32 Block: $F_{5,75} = 0.20$, p = 0.96 G x B: $F_{5,75} = 1.72$, p = 0.14
Alpha	Group: $F_{1,15} = 0.02$, p = 0.88 Block: $F_{4,60} = 1,55$, p = 0.20 G x B: $F_{4,60} = 1.70$, p = 0.16	Group: $F_{1,15} = 0.73$, p = 0.40 Block: $F_{5,75} = 1.97$, p = 0.09 **G x B: $F_{5,75} = 3.32$, p = 0.009**
Beta	Group: $F_{1,15} = 0.74$, p = 0.40 Block: $F_{4,60} = 0.98$, p = 0.42 G x B: $F_{4,60} = 0.94$, p = 0.45	Group: $F_{1,15} = 0.05$, p = 0.82 Block: $F_{5,75} = 1.33$, p = 0.26 G x B: $F_{5,75} = 0.92$, p = 0.54
Gamma	Group: $F_{1,15} = 2.08$, p = 0.17 **Block: $F_{4,60} = 3.03$, p = 0.024** G x B: $F_{4,60} = 0.69$, p = 0.60	Group: $F_{1,15} = 1.78$, p = 0.20 Block: $F_{5,75} = 0.97$, p = 0.44 **G x B: $F_{5,75} = 2.44$, p = 0.042**
dHPC		
Theta	Group: $F_{1,15} = 0.61$, p = 0.45 **Block: $F_{4,60} = 4.13$, p = 0.005** G x B: $F_{4,60} = 0.72$, p = 0.58	Group: $F_{1,15} = 0.12$, p = 0.73 Block: $F_{5,75} = 1.52$, p = 0.19 G x B: $F_{5,75} = 1.19$, p = 0.32
Alpha	Group: $F_{1,15} = 0.08$, p = 0.78 Block: $F_{4,60} = 0.21$, p = 0.93 **G x B: $F_{4,60} = 2.54$, p = 0.049**	Group: $F_{1,15} = 0.45$, p = 0.51 Block: $F_{5,75} = 1.86$, p = 0.11 G x B: $F_{5,75} = 0.96$, p = 0.45
Beta	Group: $F_{1,15} = 0.11$, p = 0.75 **Block: $F_{4,60} = 2.59$, p = 0.045** G x B: $F_{4,60} = 0.94$, p = 0.45	Group: $F_{1,15} = 0.19$, p = 0.67 **Block: $F_{5,75} = 2.58$, p = 0.033** G x B: $F_{5,75} = 1.96$, p = 0.09
Gamma	Group: $F_{1,15} = 0.45$, p = 0.51 Block: $F_{4,60} = 0.74$, p = 0.57 G x B: $F_{4,60} = 0.87$, p = 0.48	Group: $F_{1,15} = 0.27$, p = 0.61 Block: $F_{5,75} = 0.35$, p = 0.88 G x B: $F_{5,75} = 0.59$, p = 0.71

Table 1. Details of the analyses of power within each region of interest (PRL, BLA, dHPC) during Wake episodes of both the light and dark periods during session 1 (NS vs CMS-vulnerable animals).

NREM sleep	Light period	Dark period
PRL		
Delta	Group: $F_{1,15}$ = 2.13, p = 0.16 **Block: $F_{4,60}$ = 14.72, p = 1.10-7** G x B: $F_{4,60}$ = 2.12, p = 0.08	Group: $F_{1,15}$ = 2.05, p = 0.17 **Block: $F_{5,75}$ = 4.28, p = 0.002** G x B: $F_{5,75}$ = 0.68, p = 0.64
Gamma	**Group: $F_{1,15}$ = 5.67, p = 0.031** **Block: $F_{4,60}$ = 5.40, p = 9.10-4** G x B: $F_{4,60}$ = 0.79, p = 0.54	Group: $F_{1,15}$ = 4.27, p = 0.06 Block: $F_{5,75}$ = 1.25, p = 0.29 G x B: $F_{5,75}$ = 0.31, p = 0.91
BLA		
Delta	Group: $F_{1,15}$ = 2.91, p = 0.11 **Block: $F_{4,60}$ = 7.79, p = 4.10-5** G x B: $F_{4,60}$ = 1.88, p = 0.12	Group: $F_{1,15}$ = 2.95, p = 0.11 **Block: $F_{5,75}$ = 5.32, p = 3.10-4** G x B: $F_{5,75}$ = 2.21, p = 0.062
Gamma	Group: $F_{1,15}$ = 0.23, p = 0.63 **Block: $F_{4,60}$ = 4.75, p = 0.002** G x B: $F_{4,60}$ = 0.71, p = 0.58	Group: $F_{1,15}$ = 0.45, p = 0.51 **Block: $F_{5,75}$ = 3.09, p = 0.014** G x B: $F_{5,75}$ = 0.77, p = 0.57
dHPC		
Delta	Group: $F_{1,15}$ = 0.02, p = 0.90 **Block: $F_{4,60}$ = 9.89, p = 3.10-6** G x B: $F_{4,60}$ = 1.64, p = 0.17	Group: $F_{1,15}$ = 0.07, p = 0.79 Block: $F_{5,75}$ = 0.37, p = 0.87 G x B: $F_{5,75}$ = 0.74, p = 0.60
Gamma	Group: $F_{1,15}$ = 0.29, p = 0.59 Block: $F_{4,60}$ = 2.19, p = 0.08 G x B: $F_{4,60}$ = 0.63, p = 0.64	Group: $F_{1,15}$ = 0.26, p = 0.61 Block: $F_{5,75}$ = 1.45, p = 0.21 G x B: $F_{5,75}$ = 1.74, p = 0.14

Table 2. Details of the analyses of power within each region of interest (PRL, BLA, dHPC) during NREM sleep episodes of both the light and dark periods during session 1 (NS vs CMS-vulnerable animals).

REM sleep	Light period
PRL	
Theta	Group: $F_{1,15} = 0.87$, p = 0.36 Block: $F_{3,45} = 0.41$, p = 0.75 G x B: $F_{3,45} = 0.48$, p = 0.70
Gamma	Group: $F_{1,15} = 2.52$, p = 0.13 Block: $F_{3,45} = 0.07$, p = 0.97 G x B: $F_{3,45} = 1.11$, p = 0.35
BLA	
Theta	**Group: $F_{1,15} = 6.22$, p = 0.02** Block: $F_{3,45} = 2.48$, p = 0.07 G x B: $F_{3,45} = 1.56$, p = 0.21
Gamma	**Group: $F_{1,15} = 4.53$, p = 0.05** Block: $F_{3,45} = 1.56$, p = 0.21 G x B: $F_{3,45} = 1.69$, p = 0.18
dHPC	
Theta	Group: $F_{1,15} = 0.16$, p = 0.69 Block: $F_{3,45} = 4.36$, p = 0.008 G x B: $F_{3,45} = 0.52$, p = 0.67
Gamma	Group: $F_{1,15} = 0.48$, p = 0.50 Block: $F_{3,45} = 1.65$, p = 0.19 G x B: $F_{3,45} = 1.17$, p = 0.33

Table 3. Details of the analyses of power within each region of interest (PRL, BLA, dHPC) during REM sleep episodes of both the light and dark periods during session 1 (NS vs CMS-vulnerable animals).

Wake	Light period	Dark period
PRL		
Theta	Session: $F_{2,16} = 0.62$, p = 0.55 **Block: $F_{4,32} = 3.27$, p = 0.023** **S x B: $F_{8,64} = 2.20$, p = 0.039**	Session: $F_{2,16} = 1.19$, p = 0.33 Block: $F_{5,40} = 2.19$, p = 0.07 S x B: $F_{10,80} = 0.53$, p = 0.86
Alpha	Session: $F_{2,16} = 0.12$, p = 0.89 **Block: $F_{4,32} = 3.73$, p = 0.013** S x B: $F_{8,64} = 0.88$, p = 0.53	Session: $F_{2,16} = 0.54$, p = 0.59 Block: $F_{5,40} = 1.26$, p = 0.30 S x B: $F_{10,80} = 0.92$, p = 0.51
Beta	Session: $F_{2,16} = 0.11$, p = 0.89 **Block: $F_{4,32} = 7.41$, p = 0.0002** **S x B: $F_{8,64} = 2.30$, p = 0.030**	Session: $F_{2,16} = 0.03$, p = 0.97 Block: $F_{5,40} = 1.23$, p = 0.31 S x B: $F_{10,80} = 1.46$, p = 0.17
Gamma	**Session: $F_{2,16} = 5.07$, p = 0.019** **Block: $F_{4,32} = 15.85$, p = 1.10-6** **S x B: $F_{8,64} = 5.96$, p = 1.10-6**	Session: $F_{2,16} = 2.65$, p = 0.10 Block: $F_{5,40} = 2.01$, p = 0.098 S x B: $F_{10,80} = 0.65$, p = 0.76
BLA		
Theta	Session: $F_{2,16} = 0.06$, p = 0.94 **Block: $F_{4,32} = 2.95$, p = 0.034** S x B: $F_{8,64} = 1.07$, p = 0.40	Session: $F_{2,16} = 0.69$, p = 0.51 Block: $F_{5,40} = 0.12$, p = 0.98 S x B: $F_{10,80} = 1.29$, p = 0.25
Alpha	Session: $F_{2,16} = 0.22$, p = 0.81 **Block: $F_{4,32} = 6.07$, p = 9.10-4** S x B: $F_{8,64} = 0.79$, p = 0.61	Session: $F_{2,16} = 0.30$, p = 0.74 Block: $F_{5,40} = 0.99$, p = 0.43 **S x B: $F_{10,80} = 1.99$, p = 0.045**
Beta	Session: $F_{2,16} = 1.38$, p = 0.28 **Block: $F_{4,32} = 15.84$, p = 1.10-6** **S x B: $F_{8,64} = 2.21$, p = 0.038**	Session: $F_{2,16} = 0.48$, p = 0.63 Block: $F_{5,40} = 0.41$, p = 0.84 **S x B: $F_{10,80} = 2.33$, p = 0.018**
Gamma	Session: $F_{2,16} = 0.59$, p = 0.56 **Block: $F_{4,32} = 29.64$, p = 1.10-6** S x B: $F_{8,64} = 1.82$, p = 0.088	Session: $F_{2,16} = 0.15$, p = 0.86 Block: $F_{5,40} = 1.64$, p = 0.17 S x B: $F_{10,80} = 1.09$, p = 0.38
dHPC		
Theta	Session: $F_{2,16} = 0.60$, p = 0.56 **Block: $F_{4,32} = 6.95$, p = 0.00038** **S x B: $F_{8,64} = 2.67$, p = 0.013**	Session: $F_{2,16} = 0.03$, p = 0.97 **Block: $F_{5,40} = 2.63$, p = 0.038** S x B: $F_{10,80} = 1.86$, p = 0.063
Alpha	Session: $F_{2,16} = 0.67$, p = 0.52 **Block: $F_{4,32} = 3.37$, p = 0.002** **S x B: $F_{8,64} = 4.34$, p = 0.008**	Session: $F_{2,16} = 1.54$, p = 0.24 Block: $F_{5,40} = 1.35$, p = 0.26 S x B: $F_{10,80} = 1.17$, p = 0.32
Beta	Session: $F_{2,16} = 0.11$, p = 0.89 **Block: $F_{4,32} = 8.94$, p = 6.10-5** **S x B: $F_{8,64} = 5.42$, p = 3.10-5**	Session: $F_{2,16} = 0.96$, p = 0.40 Block: $F_{5,40} = 4.04$, p = 0.005 S x B: $F_{10,80} = 1.64$, p = 0.11
Gamma	Session: $F_{2,16} = 0.59$, p = 0.57 **Block: $F_{4,32} = 44.21$, p = 1.10-6** **S x B: $F_{8,64} = 17.07$, p = 1.10-6**	Session: $F_{2,16} = 0.01$, p = 0.98 Block: $F_{5,40} = 0.62$, p = 0.68 S x B: $F_{10,80} = 0.59$, p = 0.81

Table 4. Details of the analyses of power within each region of interest (PRL, BLA, dHPC) during Wake episodes of both the light and dark periods during sessions 1-3 in CMS-vulnerable animals (within subject design).

NREM sleep	Light period	Dark period
PRL		
Delta	Session: $F_{2,16}$ = 0.52, p = 0.60 **Block: $F_{3,24}$ = 12.66, p = 4.10-5** **S x B: $F_{6,48}$ = 3.33, p = 0.008**	Session: $F_{2,16}$ = 1.98, p = 0.17 **Block: $F_{5,40}$ = 6.06, p = 0.0003** **S x B: $F_{10,80}$ = 2.19, p = 0.026**
Gamma	Session: $F_{2,16}$ = 0.68, p = 0.52 **Block: $F_{3,24}$ = 5.43, p = 0.005** S x B: $F_{6,48}$ = 1.86, p = 0.11	Session: $F_{2,16}$ = 0.83, p = 0.45 Block: $F_{5,40}$ = 1.40, p = 0.25 S x B: $F_{10,80}$ = 0.89, p = 0.55
BLA		
Delta	Session: $F_{2,16}$ = 0.85, p = 0.45 **Block: $F_{3,24}$ = 21.01, p = 1.10-6** **S x B: $F_{6,48}$ = 4.77, p = 0.0007**	Session: $F_{2,16}$ = 1.26, p = 0.31 **Block: $F_{5,40}$ = 8.81, p = 1.10-5** **S x B: $F_{10,80}$ = 3.00, p = 0.003**
Gamma	Session: $F_{2,16}$ = 0.07, p = 0.93 **Block: $F_{3,24}$ = 11.84, p = 0.00006** S x B: $F_{6,48}$ = 0.94, p = 0.47	Session: $F_{2,16}$ = 0.15, p = 0.86 **Block: $F_{5,40}$ = 3.08, p = 0.019** S x B: $F_{10,80}$ = 1.51, p = 0.15
dHPC		
Delta	Session: $F_{2,16}$ = 0.95, p = 0.41 **Block: $F_{3,24}$ = 32.39, p = 1.10-6** **S x B: $F_{6,48}$ = 4.01, p = 0.0024**	**Session: $F_{2,16}$ = 4.60, p = 0.026** Block: $F_{5,40}$ = 0.78, p = 0.57 S x B: $F_{10,80}$ = 0.85, p = 0.58
Gamma	Session: $F_{2,16}$ = 0.34, p = 0.72 **Block: $F_{3,24}$ = 6.85, p = 0.002** S x B: $F_{6,48}$ = 1.77, p = 0.1	Session: $F_{2,16}$ = 0.22, p = 0.80 Block: $F_{5,40}$ = 0.95, p = 0.46 S x B: $F_{10,80}$ = 1.61, p = 0.12

Table 5. Details of the analyses of power within each region of interest (PRL, BLA, dHPC) during NREM sleep episodes of both the light and dark periods during sessions 1-3 in CMS-vulnerable animals (within subject design).

REM sleep	Light period
PRL	
Theta	Session: $F_{2,16} = 0.82$, p = 0.64 **Block: $F_{3,24} = 0.20$, p = 0.023** S x B: $F_{6,48} = 1.28$, p = 0.59
Gamma	Session: $F_{2,16} = 0.03$, p = 0.17 Block: $F_{3,24} = 1.72$, p = 0.19 S x B: $F_{6,48} = 0.60$, p = 0.4473
BLA	
Theta	Session: $F_{2,16} = 2.17$, p = 0.15 Block: $F_{3,24} = 1.10$, p = 0.37 S x B: $F_{6,48} = 1.70$, p = 0.14
Gamma	Session: $F_{2,16} = 0.02$, p = 0.97 **Block: $F_{3,24} = 5.53$, p = 0.005** S x B: $F_{6,48} = 0.79$, p = 0.58
dHPC	
Theta	Session: $F_{2,16} = 0.46$, p = 0.64 **Block: $F_{3,24} = 3.77$, p = 0.02** S x B: $F_{6,48} = 0.77$, p = 0.59
Gamma	Session: $F_{2,16} = 0.34$, p = 0.71 Block: $F_{3,24} = 0.15$, p = 0.93 S x B: $F_{6,48} = 2.08$, p = 0.07

Table 6. Details of the analyses of power within each region of interest (PRL, BLA, dHPC) during REM sleep episodes of both the light and dark periods during sessions 1-3 in CMS-vulnerable animals (within subject design).

Delta power during the NREM episodes in sessions 1

In the PRL, the BLA and the dHPC, the ANOVAs performed on delta during the NREM episodes revealed a significant Block effect without Group effect nor interaction during both the light period (PRL, p p < 0.001 ; BLA, p = 4.10-5; dHPC, p = 3.10-6) and the dark period, except in the dHPC (PRL, p = 3.10-4; BLA, p = 3.10-4; dHPC, p = 0.87). Globally, the delta power during the NREM episodes decreased between the beginning and end of the light period in the three regions while it increased throughout the dark phase in the PRL and BLA (**Supplementary Figure 1**).

Post hoc tests indicated significant differences between blocks as follows:

PRL:

Light period: blocks 1-3 vs blocks 4-5, at least p < 0.001.

Dark period: block 1 vs blocks 4-6, at least p < 0.05 ; blocks 2 and 3 vs block 5, at least p < 0.05.

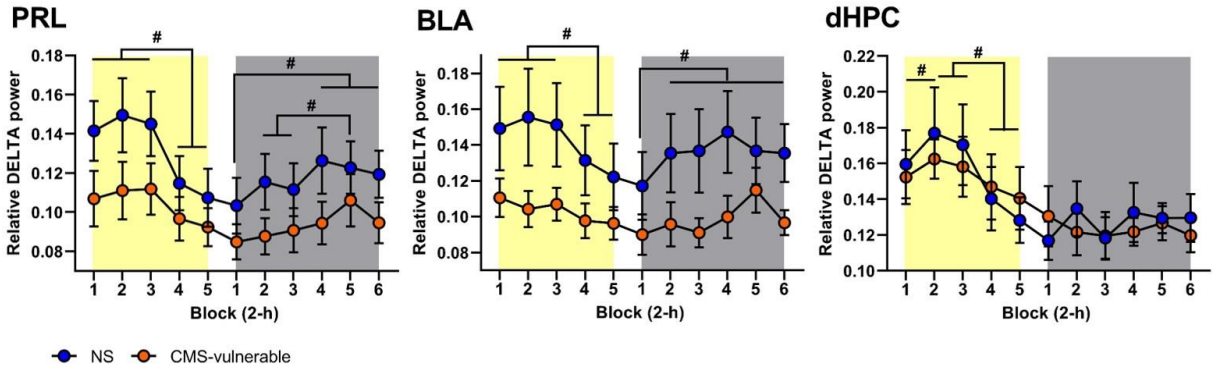
BLA:

Light period: blocks 1-3 vs blocks 4-5, at least p < 0.01.

Dark period: block 1 vs blocks 2-6, at least p < 0.05 ; block 2 vs block 5, at least p < 0.05, block 3 vs blocks 4-5, p < 0.05 for each comparison; block 5 vs block 6, p < 0.05.

dHPC:

Light period: block 1 vs blocks 2 and 5, at least p < 0.05, block 2 vs blocks 4-5, at least p < 0.001, block 3 vs blocks 4-5, at least p < 0.01).



Supplementary Figure 1. Significant delta power data during NREM sleep episodes of session 1. #p < 0.05 (between-block differences).

Gamma power during the NREM episodes of session 1

ANOVAs performed on gamma during the NREM sleep episodes revealed a significant Block effect without interaction during the light period in the PRL ($p = 9.10\text{-}4$) and the BLA ($p = 0.002$) and during the dark period only in the BLA ($p = 0.014$). Globally, in BLA gamma power during NREM sleep episodes increased across the light period and decreased throughout the dark phase whereas in PRL it increases throughout the light period only (**Supplementary Figure 2**).

Post hoc tests indicated significant differences between blocks as follows:

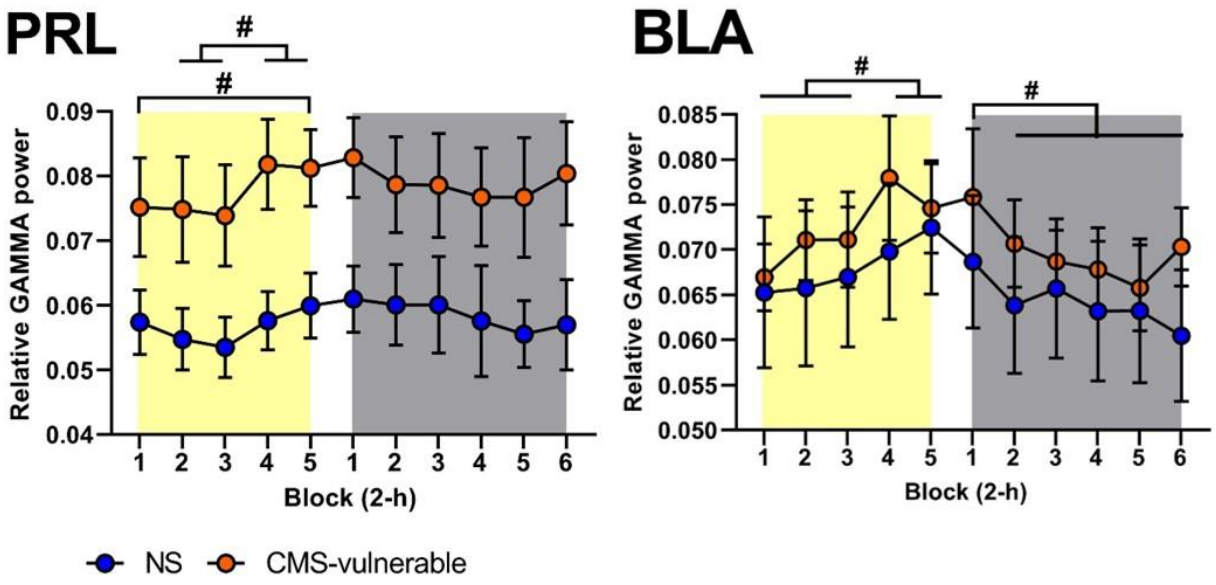
PRL:

Light period: bloc 1 vs block 5 $p < 0.05$; blocks 2-3 vs blocks 4-5 (at least $p < 0.01$).

BLA:

Light period: blocks 1-3 vs blocks 4-5 (at least $p < 0.05$).

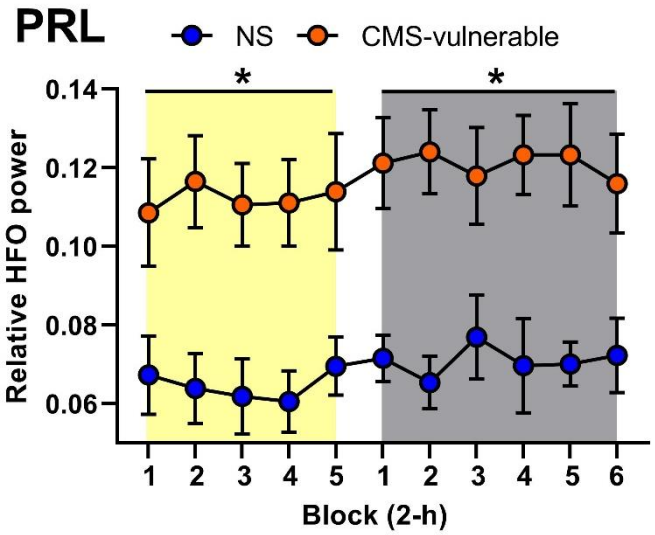
Dark period: block 1 vs blocks 2-6 (at least $p < 0.05$).



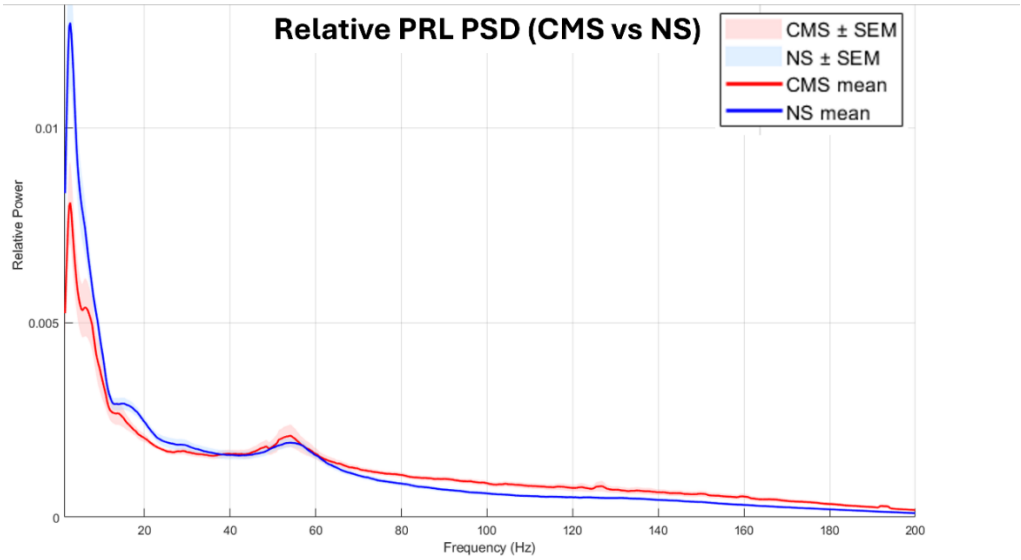
Supplementary Figure 2. Significant gamma power data during NREM sleep episodes of session 1. #$p < 0.05$ (between-block differences).

HFO power during Wake episodes of session 1

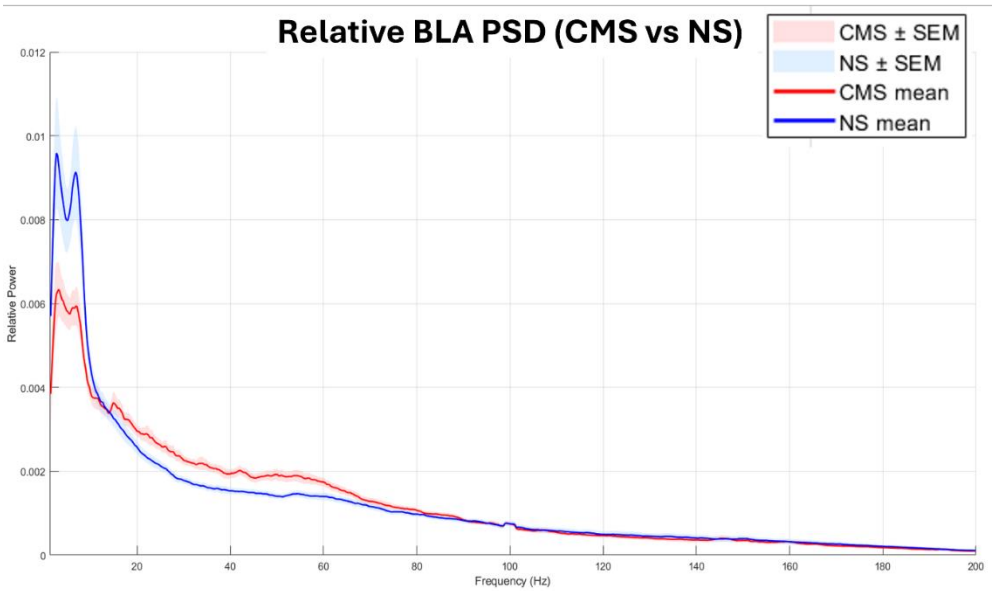
In the PRL, the ANOVAs performed on High frequency oscillations (HFO; 130-180 Hz) during Wake episodes revealed a significant Group effect without Block effect nor interaction during both the light and dark periods (light period: p = 0.006; dark period: p = 0.002). The Block factor was not significant in either phase (light period: p = 0.464; dark period: p = 0.978), and no Group × Block interaction was detected (light period: p = 0.558; dark period: p = 0.401). Globally, the measure remained stable across blocks in both periods, CMS-vulnerable animals consistently displayed elevated HFO power compared to controls across the entire light and dark periods. No HFO differences were observed within the BLA (light : Group: $F_{1,15} = 0.01$, p = 0.92 ; Block: $F_{5,75} = 2.38$, p = 0.06 ; G x B: F5,75 = 1.24, p = 0.30 ; dark : Group: $F_{1,15} = 0.02$, p = 0.89 ; Block: F5,75 = 0.43, p = 0.83; G x B: F5,75 = 0.39, p = 0.85) nor in the dHPC. (light : Group: F1,15 = 0.82, p = 0.38 ; Block: F4,60 = 1.13, p = 0.35 ; G x B: F4,60 = 1.06, p = 0.38 ; dark : Group: $F_{1,15} = 0.26$, p = 0.62 ; Block: $F_{5,75}$ = 1.85, p = 0.11 ; G x B: $F_{5,75} = 0.69$, p = 0.63).



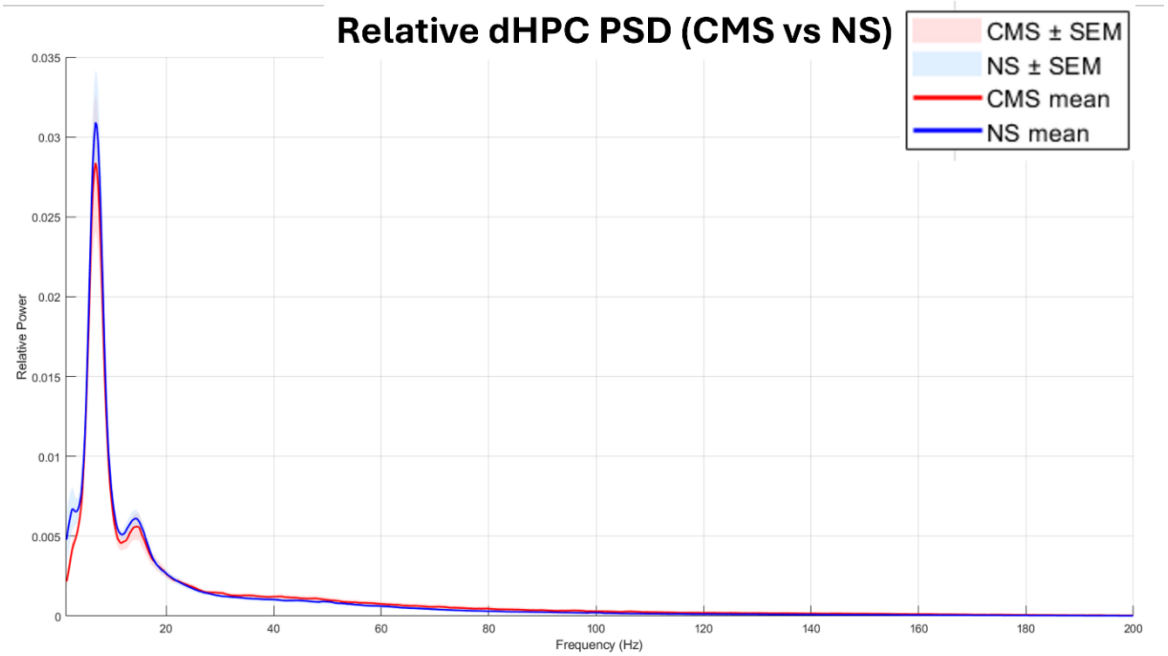
Supplementary Figure 3. High frequency oscillations (HFO; 130-180 Hz) power during Wake episodes of Session 1. *p < 0.05 at least.



Supplementary Figure 4. Relative power spectral density of CMS-vulnerable and NS animals in the PRL (during Wake at block 6 of the dark period *i.e.* peak of differences during session 1), illustrating changes in the aperiodic component of the spectrum. Notice the lower relative power of CMS-vulnerable animals up to 40 Hz, after which it is higher than in NS animals.



Supplementary Figure 5. Relative power spectral density of CMS-vulnerable and NS animals in the BLA (during REM at block 3 of the light period *i.e.* peak of differences during session 1), illustrating changes in the aperiodic component of the spectrum. Notice the lower relative power of CMS-vulnerable animals up to 15 Hz, after which it is higher than in NS animals up to 90 Hz.



Supplementary Figure 6. Relative power spectral density of CMS-vulnerable and NS animals in the dHPC (during Wake at block 1 of the light period, first recorded block arbitrarily selected), illustrating absence of change in the aperiodic component of the spectrum in contrast to the PRL and BLA.

Transcriptomic signatures of chronic stress and ketamine treatment according to vulnerability and sex in the Wistar-Kyoto rat model of depression

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A) Abstract

Major depressive disorder involves dysregulation of stress signalling, monoaminergic, glutamatergic, and neuroplasticity-related pathways across corticolimbic structures such as the prefrontal cortex (PFC), amygdala (AMY), and hippocampus (HPC). Chronic mild stress (CMS) is a validated preclinical model for inducing depressive-like phenotypes, yet individual vulnerability and sex differences remain poorly explored at the transcriptional level. Ketamine (Ket), a rapid-acting antidepressant effective in treatment-resistant depression, may restore synaptic plasticity through brain-derived neurotrophic factor (BDNF)-dependent mechanisms, though its precise molecular actions are unclear. We examined mRNA expression of seven genes related to the above-mentioned pathways (*Bdnf*, *Fkbp5*, *Gr*, *Gria1*, *Grin2b*, *Maoa*, *Psd95*) in the PFC, AMY and dorsal HPC (dHPC) of male and female Wistar-Kyoto rats. Animals underwent four weeks of CMS followed by behavioural phenotyping to classify vulnerable and non-vulnerable subgroups using the elevated plus maze and forced swim test, then received acute Ket (10 mg/kg) or saline treatment before tissue collection for real-time quantitative polymerase chain reaction analysis. CMS-vulnerable animals showed downregulated *Bdnf* expression in the PFC and AMY, while *Fkbp5* was upregulated in the dHPC across all CMS-exposed animals. Ket induced region-specific effects, upregulating *Bdnf* in the PFC, downregulating Mao_a in the PFC and dHPC, but paradoxically downregulating *Bdnf* and *Gr* while upregulating *Fkbp5* in the dHPC, likely through a confounding stressful effect of Ket. Sex differences were minimal, impacting PFC *Gr* expression, which was downregulated in females, and AMY *Grin2b* which was upregulated by Ket in females only in the AMY. Correlation matrix analyses suggested reduced inter-regional gene co-expression in CMS-vulnerable animals, which Ket may

partially normalise. These findings suggest that stress vulnerability involves region-specific transcriptional alterations, particularly BDNF pathway downregulation in the PFC. Ket's therapeutic effects may involve enhanced PFC synaptic plasticity, though acute stress responses observed in the dHPC may act as a confounding factor.

B) Introduction

Major depressive disorder is a leading cause of disability worldwide, affecting more than 322 million people and imposing a growing societal and economic burden (Chen et al., 2025; Friedrich, 2017; Rong et al., 2025). Depressed patients show dysfunction within corticolimbic circuits involved in emotional processing and regulation, notably including the prefrontal cortex (PFC) (Belleau et al., 2019; Pizzagalli & Roberts, 2022; Zheng et al., 2021), amygdala (AMY) (Drevets, 2003; Hamilton et al., 2008; Hossein et al., 2023), and hippocampus (HPC) (Nolan et al., 2020; Santos et al., 2018; Tartt et al., 2022). Abnormal stress-related neuroendocrine response and regulation are also hallmarks of the disorder (Jiang et al., 2025; Li et al., 2024; Sahu et al., 2022). Depression is associated with impairments in stress regulation (Jiang et al., 2025), in synaptic plasticity (Holmes et al., 2019; Normann et al., 2007; Pittenger & Duman, 2008; Tartt et al., 2022), in glutamatergic transmission (Moriguchi et al., 2019; Sarawagi et al., 2021), and in monoaminergic regulation (Chiuccariello et al., 2014; Meyer et al., 2006). However, this disorder is clinically heterogeneous, with strong interindividual differences in vulnerability, symptom profiles, and treatment response (Buch & Liston, 2021; Spigset & Mårtensson, 1999). Sex differences are also evident, as the disorder has a female:male ratio ranging from 1.25:1 to 1.95:1 (Lim et al., 2018; Salk et al., 2017), along with differences in neuroendocrine stress response (Wang et al., 2024) and in brain structure volumes, including atrophied AMY in depressed women (Mohammadi et al., 2023). Classical antidepressants targeting monoaminergic transmission, such as selective serotonin reuptake inhibitors (SSRIs), present the inconveniences of delayed onset of action (Lee et al., 2012) and inefficacy in about 30 % of patients, thus considered treatment resistant depressed patients (TRD) (McIntyre et al., 2023). By contrast, rapid-acting agents such as the N-methyl-D-aspartate receptor (NMDA-R) antagonist ketamine (Ket) produce antidepressant effects within minutes and have demonstrated efficacy in TRD (Shin & Kim, 2020). According to preclinical studies, Ket is believed to rapidly restore impaired synaptic plasticity (Duman et al., 2016) through the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA-R)-dependent expression of brain-derived neurotrophic factor (BDNF) (Suzuki et al., 2023), which could subsequently restore impaired inter-regional communication (Shin & Kim, 2020).

However, exact mechanisms of Ket's antidepressant action remain unclear, warranting the need for additional studies (Reddy et al., 2024).

Chronic stress is widely recognised as a major aetiological factor in depression, notably in interaction with genetic factors (Krasner et al., 2025; Pittenger & Duman, 2008) such as polymorphisms in genes involved in stress signalling (Chen et al., 2024; Fan et al., 2021; Peng et al., 2018; Zhang et al., 2023). Chronic stress paradigms are extensively implemented to induce depressive-like phenotypes in rodent, with the chronic mild stress (CMS) protocol being one of the most validated model (Willner, 2017). However, the question of sex differences is rarely addressed in such studies (Campbell et al., 2024), alongside the question of interindividual vulnerability (Strekalova et al., 2022). Several pathways appear to be involved in CMS-induced depressive-like phenotypes, particularly within corticolimbic structures such as the PFC, the AMY and the HPC (McEwen et al., 2015; Pizzagalli & Roberts, 2022). These pathways concern stress regulation itself, which involves glucocorticoid receptor (GR)-mediated negative feedback loops (Ulrich-Lai & Herman, 2009). Expression of GR, encoded by *Gr* (also known as *Nr3c1*), has been shown to be impacted in CMS models, although with heterogenous results, prompting further investigation. It has been shown to be downregulated or upregulated at the gene (Xing et al., 2015) and protein (Guidotti et al., 2013; Wei et al., 2016) levels. Additionally, GR may be involved in Ket's therapeutic effects, as Ket rescues stress-induced GR downregulation (Fraga et al., 2021). GR signalling is also modulated by its chaperone protein FKBP5, encoded by the Fkbp5 gene, which is often implicated in stress-related impairments (Zannas et al., 2016) and upregulated by CMS (Guidotti et al., 2013). CMS has marked deleterious effects on synaptic plasticity, notably through downregulation of BDNF at both the transcriptomic (*Bdnf*) (Abbasi Mehmandost Sofla et al., 2025; Luoni et al., 2015; Osacka et al., 2021) and protein (Willner, 2017) levels. These mechanisms are thought to play a causal role in depression (Krasner et al., 2025), and may be differently affected depending on vulnerability (Talaee et al., 2024). BDNF is also upregulated by Ket (Suzuki et al., 2023), though the substance's effects on *Bdnf* transcription are unclear. A downstream synaptic marker is postsynaptic density protein 95 (PSD95), encoded by *Psd95* (also known as *Dlg4*), involved in synapse maintenance, which is known to be downregulated, mainly in the PFC, at the gene (Lu et al., 2019; Zhuang et al., 2016) and protein level (Willner, 2017). However, differences regarding vulnerability remain unexplored. Other impaired pathways involve glutamatergic signalling (Dygalo et al., 2020). AMPA-R subunit GRIA1 (or GLUA1), encoded by *Gria1* (also known as *Glua1*) is crucial for synaptic plasticity and glutamatergic transmission as it allows AMPA-R trafficking to the membrane (Diering & Huganir, 2018). Not only does CMS

downregulate its gene (Duric et al., 2013) and protein expression (He et al., 2023), GRIA1 activation has also been shown to be involved in Ket's effects (Adaikkan et al., 2018). NMDA-R subunit GRIN2B (or GLUN2B), encoded by *Grin2b* (also known as *Glun2b*) may be involved in depression (Miller et al., 2014). It has also been proposed to be preferentially targeted by Ket as part of its therapeutic mechanisms through NMDA-R antagonism (Yavi et al., 2022; Zanos & Gould, 2018). In HPC, its expression, however shows inconsistent results with downregulated protein following CMS (Elhussiny et al., 2021), but upregulated protein in chronic restraint stress (Pacheco et al., 2017), and either no effect on gene regulation in these two studies, or downregulation (Dygalo et al., 2020) in another chronic restraint stress study, prompting the need for further investigation. Finally, there is evidence of monoamine regulation abnormalities, as upregulated monoamine oxidase A (MAO_A), encoded by *Mao*$_a$, has been observed following CMS or chronic social defeat at the gene (Geng et al., 2021; Harris et al., 2015; Lin et al., 2019; Stefanovic et al., 2016) and protein level (Geng et al., 2021; Stefanovic et al., 2016). Given that elevated cerebral MAOA is observed in depressed patients (Chiuccariello et al., 2014; Meyer et al., 2006), and MAO_A inhibition is a therapeutic approach, the effect of ketamine on MAO_A expression warrants investigation. Overall, transcriptomic effects of both CMS and Ket on the above-mentioned genes, *i.e.* mRNA expression, appear insufficiently studied, leaving a gap in our understanding of pathophysiological and therapeutic mechanisms. Of note, stress-induced transcriptional alterations are generally known to be highly region-specific (Chen et al., 2022; McEwen et al., 2015), highlighting the need to examine the above-mentioned corticolimbic regions to better understand underlying mechanisms. The additional factors of CMS-vulnerability and Sex may also lead to different transcriptional signatures regarding genes involved in the above-mentioned pathways.

The Wistar-Kyoto (WKY) rat is a strain which displays strong sensitivity to stress (Redei et al., 2023). When exposed to CMS, WKY rats develop a depression-like phenotype resistant to classical antidepressants such as SSRIs, but not to Ket, thus representing a putative model of TRD (Willner et al., 2019). Importantly, we have previously demonstrated sex-dependent behavioural differences in response to CMS as well as heterogeneity in individual vulnerability within the WKY-CMS model, where about 50 % of animals present behavioural impairments in the forced swim test (FST) and elevated plus maze (EPM) (Loizeau et al., 2024). Here, we aimed to characterise the transcriptional consequences of CMS and acute Ket treatment on a selection of genes belonging to the pathways presented above in the WKY rat. Using real-time quantitative polymerase chain reaction (RT-qPCR), we examined mRNA expression in the PFC, the AMY, and the dHPC with a specific focus on both

sexes and on differences between CMS-vulnerable and CMS-non-vulnerable animals to identify potential group-dependent transcriptomic differences. We hypothesise that CMS induces gene regulation patterns similar to its effects on protein expression, with more pronounced changes in CMS-vulnerable animals, and that Ket treatment can normalise these alterations.

C) Materials and methods

1) Animals

This study, approved by a local ethics committee (CREMEAS) and French authorities (APAFIS #43839), was conducted on 121 WKY rats (62 females, 59 males; Charles River, Sulzfeld, Germany) from two cohorts, each comprising both sexes. Upon arrival at the laboratory (4–5 weeks of age), animals were housed in groups of five of the same sex for one week to allow acclimation to the housing conditions. Following this period, for each sex, rats were randomly assigned to two experimental groups balanced in terms of body weight. Animals assigned to the CMS protocol (42 females, 41 males) were housed individually in the same room, while non-stressed Control rats (20 females, 18 males) were housed in pairs of the same sex in a different room.

All rats were maintained under a 12 h light/dark cycle (lights on at 07:00 A.M.), with controlled temperature (23 ± 1 °C). Food (Altromin, ref. 1324) and water were available *ad libitum*, except for CMS animals during food/water deprivation-based stressors.

2) Experimental Design

Rats assigned to the CMS group underwent the CMS protocol identically to our previous study (Loizeau et al., 2024) for four consecutive weeks. Briefly, animals were exposed daily to two varying physical, social or psychological stressors such as food deprivation, resident/intruder session or reduction of space in the cage. Animals were then phenotyped to assess vulnerability or non-vulnerability using the FST and the EPM. Application of the CMS protocol was then resumed for two additional weeks. Rats were then treated with either Ket or NaCl, according to their group assignment, and were sacrificed 2 hours later for collection of the PFC, AMY and dHPC samples. RT-qPCR was then conducted. To avoid confounding effects between chronic and acute stress responses, CMS protocol was stopped at least 24 hours before sample collection.

3) Behavioural phenotyping

Both control and CMS-rats were tested in the FST and the EPM such as described in our previous study (Loizeau et al., 2024). In each cohort, CMS rats of both sexes were then separately clustered into vulnerable and non-vulnerable populations, as described previously, using k-means clustering based on FST and EPM results (Loizeau et al., 2024). All groups (for each sex, Control, CMS-non-vulnerable and CMS-vulnerable) were then divided into two, balancing behavioural performance equally, with each subgroup destined for either NaCl or Ket treatment. The final experimental groups were constituted as follows:

Control males (NaCl, n = 10 ; Ket, n = 8) ; Control females (NaCl, n = 10 ; Ket, n= 10) ; CMS-non-vulnerable males (NaCl, n = 12 ; Ket, n = 12) ; CMS-non-vulnerable females (NaCl, n = 12 ; Ket, n= 10) ; CMS-vulnerable males (NaCl, n = 12 ; Ket, n = 12) ; CMS-vulnerable females (NaCl, n = 11 ; Ket, n= 6).

4) Drug administration and sample collection

At least 24 hours after cessation of the CMS protocol, rats received an intraperitoneal injection of either Ket (10 mg/kg) or 0.9% NaCl. Two hours after the injection, animals were placed in an anaesthetic induction chamber and exposed to 4% isoflurane for 4 minutes and decapitated. Brains were rapidly extracted, and the dHPC, PFC, and AMY were dissected on ice using a brain matrix and punch method as previously described (de Sa Nogueira et al., 2021). Tissue samples were snap-frozen at -80 °C until RNA extraction.

5) Real-time quantitative polymerase chain reaction procedure

Primers

Eight genes were assayed: *Rplp0* (housekeeping gene), *Bdnf*, *Fkbp5*, *Gr*, *Gria1*, *Grin2b*, Mao_a and *Psd95*. Primer sequences were designed using PrimerBLAST (Ye et al., 2012) and spanned exon-exon junctions where possible or taken from previous studies if suitable for our qPCR conditions (**Table 1**).

Name of *gene* (**protein**) GenBank accession no.	Forward sequence	Reverse sequence	Amplicon size (bp)	source
Rplp0/36b4 (**RPLP0**) NM_022402.2	CTGCCCGAGCCGGTGCCATC	TTCAATGGTACCTCTGGAG	131	Custom designed
Bdnf (**BDNF**) NM_012513.4	GGCCCAACGAAGAAAACCAT	CAGAAAGAGCAGAGGAGGCT	95	(Mingardi et al., 2023)
Fkbp5 (**FKPB5**) NM_001012174.1	AGTTATCTTCCCCGCTGCCC	GGAGACGGAGACGGAAGTCG	133	Custom designed
Gr/Nr3c1 (**GR**) NM_012576.2	AGCTCCCCCTGGTAGAGAC	GCGAAGATGCAGAAACCTTG	119	(Chen et al., 2021)
Gria1/Glua1 (**GRIA1/GLUA1**) NM_031608.1	ACTGAACGAGCAGGGGCTTT	GTCCTTGGAGTCACCTCCCC	91	Custom designed
Grin2b/Glun2b (**GRIN2B/GLUN2B**) NM_012574.1	GCATGATCGGTGAGGTGGTCA	CACAGTCCCATTGCTGCGAG	149	Custom designed
Mao$_a$ (**MAO$_A$**) NM_033653.1	TAGTCGTGATTGGAGGCGGC	CCCATTTGACATGCTCATTCCTCA	141	Custom designed
Psd95/Dlg4 (**PSD95**) NM_019621.2	CACCTCCCCAACCAGGCCAT	CCTCTGTTCCATTCACCTGCAAC	94	Custom designed

Table 1: List of primer sequences used in the study.

RNA Isolation, reverse transcription and qPCR

RNA was extracted following a precipitation-based protocol using the UptiZol™ reagent (Interchim). Briefly, tissue punches were lysed in UptiZol™, followed by chloroform–isoamyl (49:1) (Sigma-Aldrich, France) separation. Samples were centrifuged (12000 g, 15 minutes) and the aqueous phase containing RNA was pipetted out. Precipitation was then triggered with isopropanol (Propanol-2 ≥99.9 %, HiPerSolv CHROMANORM®, Avantor Sciences, USA) overnight at -20 °C. Samples were then centrifuged (12000 g, 15 minutes) and washed with 75 % ethanol twice (with centrifugation at 7500 g, 5 minutes after each wash). RNA pellets were air-dried and dissolved in nuclease-free water. RNA concentrations were measured using a NanoDrop spectrophotometer (Thermo Fisher, USA) and stored at -80°C. RT-qPCR was conducted as previously described (de Sa Nogueira et al., 2021). Briefly, cDNA synthesis was performed on 750 ng of total RNA using the iScript™ Reverse

Transcription Supermix for RT-qPCR (Bio-Rad). qPCR assays were conducted using real-time PCR detection systems (CFX Connect, CFX Opus 96, Bio-Rad, USA). Reactions were performed on diluted cDNA with SsoAdvanced Universal SYBR Green Supermix (Bio-Rad, USA) in triplicate in 96-well HardShell plates (Bio-Rad, USA). Thermal cycling conditions were as follows: an initial denaturation step at 95 °C for 30 s, followed by 40 cycles of denaturation at 95 °C for 5 seconds and annealing/extension at 60 °C for 45 seconds, during which fluorescence was recorded.

6) Data processing and statistics

Outliers within triplicates were identified and removed using Dixon's Q test. qPCR expression levels were normalised to the reference gene *Rplp0* (Cycle quantification values of Rplp0 in each structure is represented in **Supplementary Figure 1**). Relative quantification was calculated using the ΔΔCq method (de Sa Nogueira et al., 2021), with male NaCl-treated Control animals serving as the calibrator group to evaluate effects of sex, vulnerability to stress exposure, and treatment. Outlier values were identified and removed using generalised Extreme Studentised Deviate (gESD), but as subsequent matrix analyses cannot handle missing values, imputation based on mean was used (Nthoiwa & Sivasamy, 2024) to replace these without affecting the group's value. Normality and homogeneity of variance were assessed using Kolmogorov-Smirnov and Levene tests. Up to three RT-qPCR data groups for a given gene showed violations, but given adequate sample sizes (n = 10 on average), absence of non-parametric equivalents for three-way ANOVA, and considering ANOVA's robustness to moderate violations under these conditions (Blanca et al., 2017), data were analysed with ANOVA with Sex, Group (Control, CMS-vulnerable, and CMS-non-vulnerable) and Treatment as between-subjects factors. When appropriate, the ANOVAs were followed by post hoc comparisons using the Newman–Keuls multiple range test. The threshold for rejecting the null hypothesis was 0.05 throughout. To further investigate the results and identify potential transcriptomic signatures across groups, correlation matrices between gene expression levels in the different brain structures were generated using MATLAB scripts and Pearson's correlation coefficient. Data were pooled between sexes, as RT-qPCR results showed negligible effect of Sex (only significant for PFC *Gr* expression, and AMY Grin2B expression in interaction with Treatment). Data were analysed between NaCl groups (Control, Non-vulnerable, Vulnerable), and within groups between the NaCl- and Ket-treated animals. Matrix similarity between groups was assessed using a Bonferroni-corrected Mantel test at a significance threshold of $p < 0.05$.

D) Results

1) Post-CMS behavioural phenotyping

ANOVA performed on the time spent in the EPM revealed a main effect of Group ($F_{2,109}$ = 27.64, $p < 0.001$), but no effect of Sex and Treatment, nor interaction between factors. Post hoc analysis revealed a significant difference between the Control and CMS-vulnerable groups, as well as between CMS-non-vulnerable and CMS-vulnerable groups (NK: $p < 0.001$ for both), which was due to significantly less time spent in the open arms by vulnerable animals (**Figure 1A and 1C**).

ANOVA performed on the immobility time in the FST revealed a main effect of Sex ($F_{1,109}$ = 20.61, $p < 0.001$), a main effect of Group ($F_{2,109}$ = 18.32, $p < 0.001$), and a Sex × Group interaction ($F_{2,109}$=8.22, $p < 0.001$), but no effect of Treatment nor interaction between factors. In males, post hoc analyses revealed a significant difference between the Control and CMS-vulnerable groups, as well as between the Control and CMS-non-vulnerable groups (NK: $p < 0.001$ for both), due to significantly greater immobility in both CMS-exposed groups (**Figure 1B**). In females, post hoc analysis indicated a significant difference between the Control and CMS-vulnerable groups, as well as between the CMS-non-vulnerable and CMS-vulnerable groups (NK: $p < 0.001$ for both), driven by significantly higher immobility in vulnerable animals (**Figure 1D**).

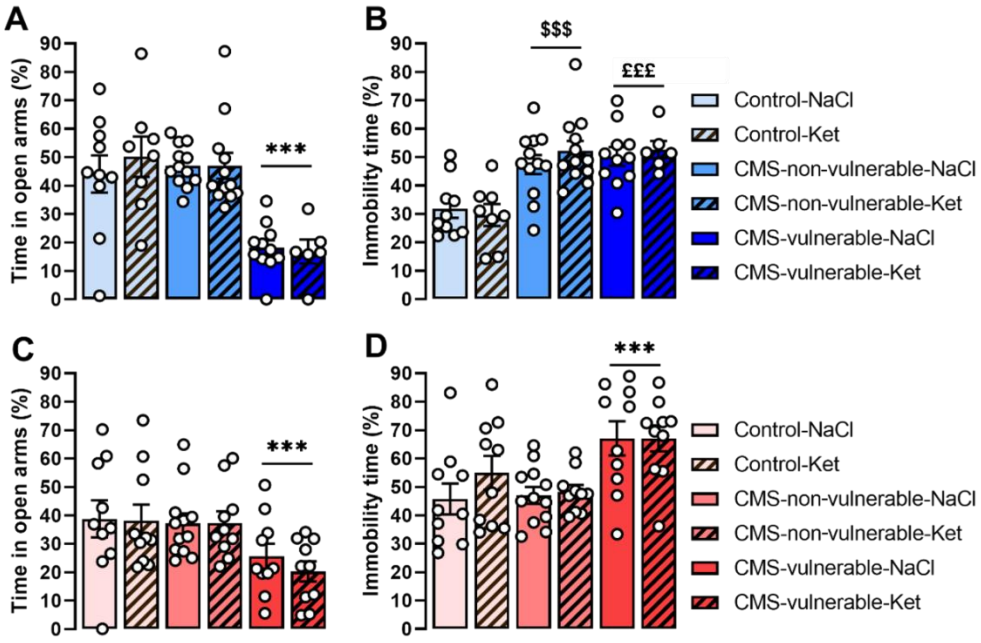

Figure 1. Behavioural performance in the EPM (A, C) and FST (B, D). Males are represented in blue on the top half (A, B), and females in red on the bottom half (C, D). Data are represented as mean ± SEM. *** $p < 0.001$ CMS-vulnerable vs. other groups ; $$$ $p < 0.001$ CMS-non-vulnerable vs. Control ; £££ $p < 0.001$ CMS-vulnerable vs. Control.

2) Relative gene expression in the dHPC

Full details of the ANOVAs' results are presented in **Supplementary Tables 1-3** for the dHPC, PFC and AMY, respectively. Only results for which significant effects were revealed are described below.

Results concerning relative gene expression in the dHPC are displayed in **Figure 2**. ANOVAs regarding this structure revealed significant effects of Group and/or Treatment but no effect of Sex or interactions between factors. *Fkbp5* was the only gene whose expression was modulated by the Group factor ($F_{2, 109} = 3.33$, $p < 0.05$), although the effect approached significance for *Grin2b* ($F_{2, 109} = 2.76$, $p = 0.067$), prompting post hoc analysis. *Fkbp5* expression was elevated in CMS-exposed animals compared to Control animals (**Figure 2B)**; with however, post hoc revealing significance only in CMS-non-vulnerable animals, and a trend in CMS-vulnerable rats (NK: $p < 0.05$ for non-vulnerable rats, $p = 0.06$ for vulnerable rats). Compared to Control animals, *Grin2b* also appeared upregulated in CMS-exposed animals (**Figure 2E)**, but post hoc only indicated a significant difference for CMS-vulnerable animals (NK: $p = 0.06$ for non-vulnerable rats, $p < 0.05$ for vulnerable rats). For genes whose expression was modulated by the Treatment factor, most were downregulated by Ket administration (*Bdnf*: $F_{1, 109} = 9.82$, $p < 0.01$, **Figure 2A** ; *Gr*: $F_{1, 109} = 7.90$, $p < 0.01$, **Figure 2C** ; Mao_a: $F_{1, 109} = 4.12$, $p < 0.05$, **Figure 2F**). *Fkbp5* was the only gene upregulated by Ket ($F_{1, 109} = 11.41$, $p < 0.01$, **Figure 2B**). Expression of *Gria1* and *Psd95* was not significantly affected by any factor (**Figure 2D and G**).

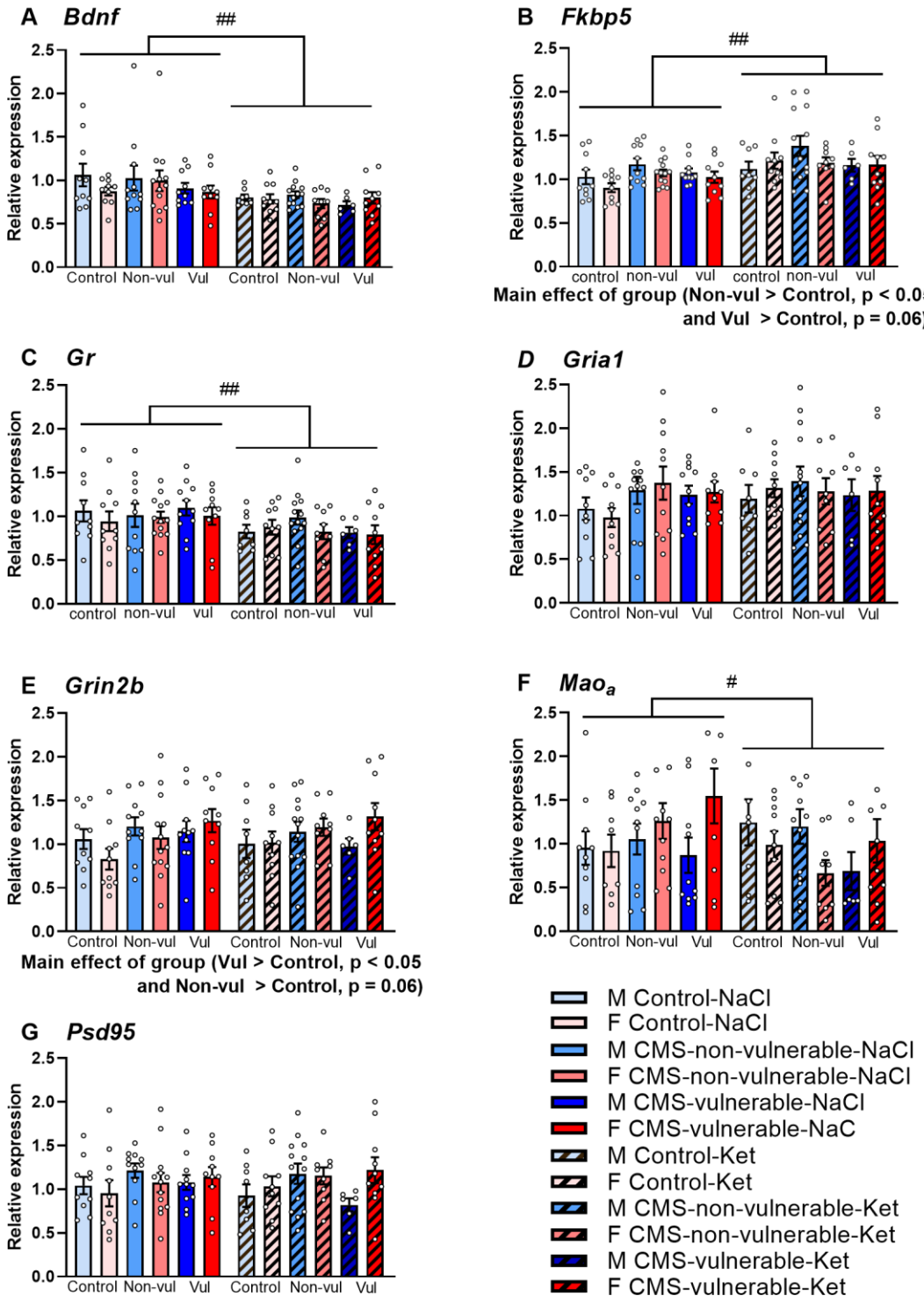

Figure 2. Relative expression of Bdnf (A), Fkbp5 (B), Gr (C), Gria1 (D), Grin2b (E), Mao$_a$ (F), and Psd95 (G), in the dHPC. M: Male, F: Female, Non-vul: CMS-non-vulnerable, Vul: CMS-vulnerable. Data are represented as mean ± SEM. # and ##, Ket vs. NaCl ($p < 0.05$ et $p < 0.01$).

3) Relative gene expression in the PFC

Results concerning relative gene expression in the PFC are displayed in **Figure 3**. ANOVAs regarding this structure revealed a significant effect of Sex for the expression of *Gr* only (Sex: $F_{1,109} = 15.55$, $p < 0.001$), with females, regardless of group and treatment, displaying lower levels of expression (**Figure 3C**). *Bdnf* was the only gene whose expression was significantly modulated by the Group factor ($F_{2,109} = 4.19$, $p < 0.05$). Post hoc analysis on the Group factor revealed a significant difference between Control and CMS-vulnerable animals only (NK: $p < 0.01$). These animals showed downregulated *Bdnf* expression relative to Control animals (**Figure 3A**). Regarding the Treatment factor, Ket upregulated *Bdnf* ($F_{1,109} = 26.87$, $p < 0.001$; **Figure 3A**) and downregulated Mao_a ($F_{1,109} = 4.07$, $p < 0.05$, **Figure 3F**), with no interaction between factors. Only ANOVAs performed on *Gria1* and *Grin2b* revealed significant interactions between factors. A significant Sex × Group interaction was observed for *Gria1* ($F_{2,109} = 3.80$, $p < 0.05$) and a Group × Treatment interaction for *Grin2b* ($F_{2,109} = 3.34$, $p < 0.05$). However, post hoc analyses did not reveal significant differences for either interaction (**Figure 3D and E**). *Psd95* was not affected by any factor (**Figure 3G**).

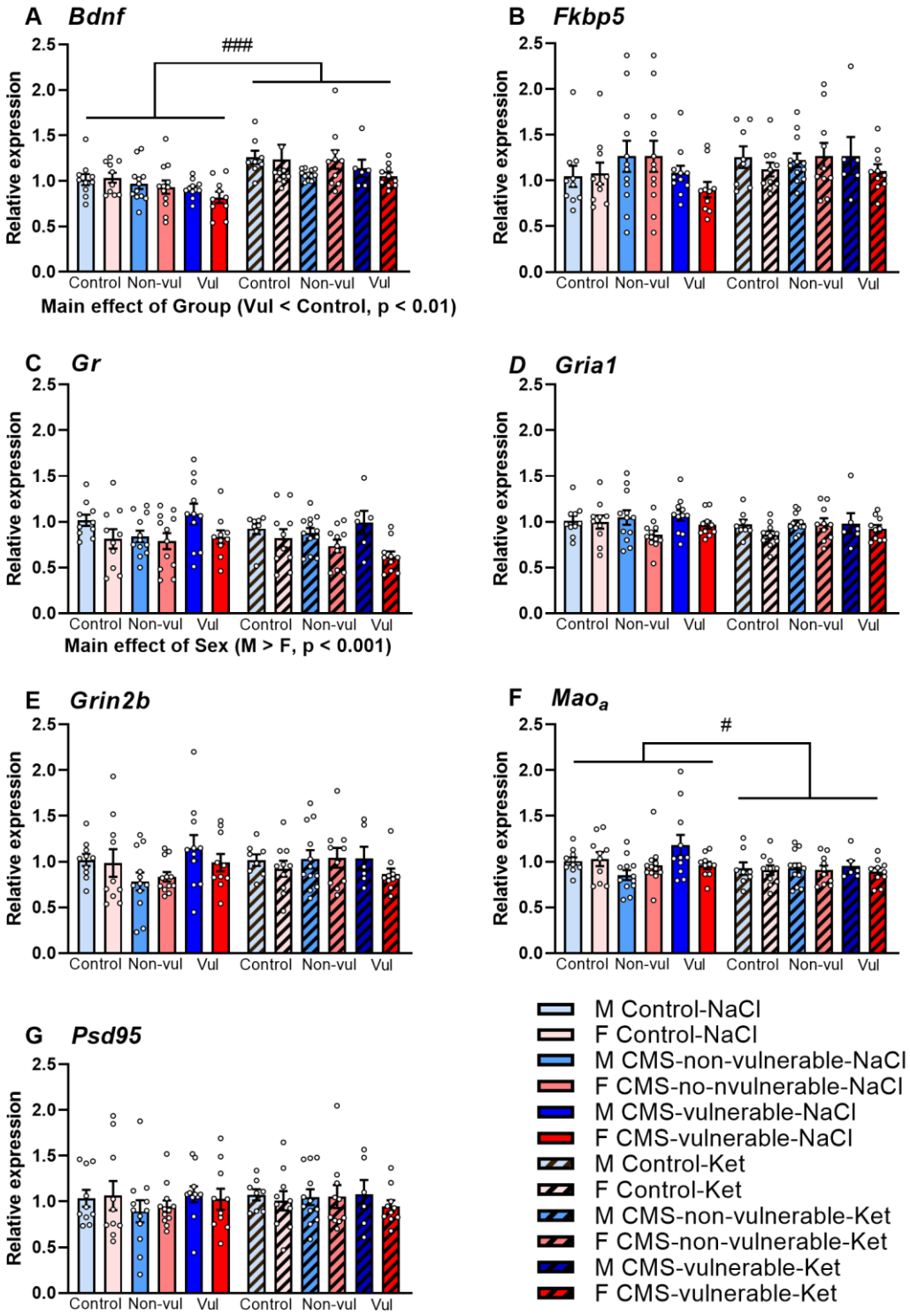

Figure 3. Relative expression of Bdnf (A), Fkbp5 (B), Gr (C), Gria1 (D), Grin2b (E), Mao$_a$ (F), and Psd95 (G), in the PFC. M: Male, F: Female, Non-vul: CMS-non-vulnerable, Vul: CMS-vulnerable. Data are represented as mean ± SEM. # and ###, Ket vs. NaCl ($p < 0.05$ and $p < 0.001$).

4) Relative gene expression in the AMY

Results concerning relative gene expression in the AMY are displayed in **Figure 4**. ANOVAs regarding this structure revealed that expression of *Bdnf* was significantly modulated by Group ($F_{2,109} = 6.20$, $p < 0.01$). Post hoc analysis indicated significant differences for both CMS-non-vulnerable and CMS-vulnerable animals, displaying downregulated *Bdnf* relative to Control animals (NK: $p < 0.01$ for both) (**Figure 4A**), regardless of Sex and Treatment (no interaction between factors). The expression of *Bdnf* was also significantly affected by Ket (Treatment: $F_{1,109} = 5.04$, $p < 0.05$) which downregulated its expression (**Figure 4A**). While the ANOVA on *Grin2b* presented a significant effect of Treatment ($F_{1,109} = 4.43$, $p < 0.05$), the Sex × Treatment interaction approached significance ($F_{1,109} = 3.14$, $p = 0.08$), with visual inspection of the data suggesting upregulation with Ket in females only. This was confirmed in post hoc analysis (NK: $p < 0.05$ for females, $p = 0.61$ for males, **Figure 4E**). A significant Sex × Group × Treatment interaction was observed for *Fkbp5* ($F_{2,109} = 6.09$, $p < 0.01$), but post hoc analyses did not indicate any significant pairwise differences (minimum $p = 0.20$; **Figure 4B**). No significant effects of Sex, Group, Treatment, or interactions were observed for *Gr*, *Gria1*, Mao_a, or *Psd95* (**Figure 4C, D, F, and G**).

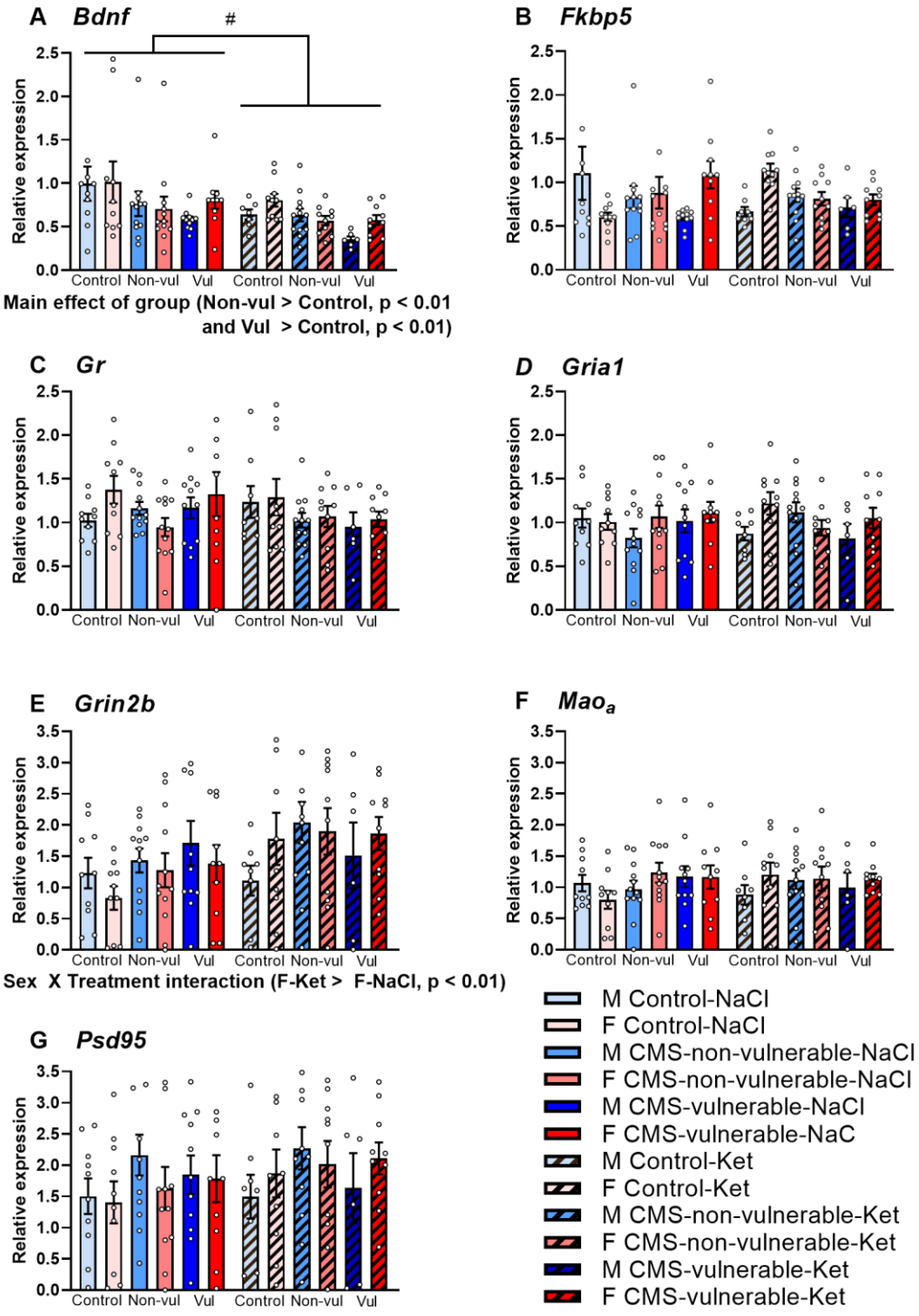

Figure 4. Relative expression of Bdnf (A), Fkbp5 (B), Gr (C), Gria1 (D), Grin2b (E), Mao$_a$ (F), and Psd95 (G), in the AMY. M: Male, F: Female, Non-vul: CMS-non-vulnerable, Vul: CMS-vulnerable. Data are represented as mean ± SEM. #, Ket vs. NaCl ($p < 0.05$).

5) Comparisons of gene expression correlation matrices

Correlation matrices generated in MATLAB were compared across NaCl-treated control, CMS-non-vulnerable, and CMS-vulnerable groups using the Mantel test to assess the similarity of interregional gene expression correlation patterns.

NaCl-treated groups

Comparison between NaCl-treated Control and CMS-non-vulnerable groups (Mantel r = 0.487, $p < 0.001$, **Figures 5A, 5B**) indicated a statistically significant similarity, as did the Control *vs.* CMS-vulnerable comparison (Mantel statistic = 0.438, $p < 0.001$, **Figures 5A, 5C**). Interestingly, while still significant, the non-vulnerable *vs.* vulnerable comparison (Mantel statistic = 0.240, $p = 0.002$, **Figures 5B, 5C**) revealed a weaker similarity. Visual inspection of the significant correlation matrix indicated 33 correlations in the Control group (**Figure 6A**), 51 in the CMS-non-vulnerable group (**Figure 6B**), and 25 in the CMS-vulnerable group (**Figure 6C**). Correlations were more numerous and broadly distributed in CMS-non-vulnerable animals, whereas they were fewer and more localised in CMS-vulnerable animals.

NaCl vs. Ket comparisons

Correlation matrices between NaCl- and Ket-treated animals were compared within each group. The comparison for the Control groups (Mantel statistic r = 0.472, $p < 0.001$, **Figures 5A and 5D**), the CMS-non-vulnerable groups (Mantel statistic r = 0.374, $p < 0.001$, **Figures 5B and 5E**), and the CMS-vulnerable groups (Mantel statistic r = 0.337, $p < 0.001$, **Figures 5C and 5F**) each revealed significant similarity between NaCl and Ket matrices. Visual inspection of the significant correlation matrices showed 43 correlations in the Control-Ket group (**Figure 6D**), 35 in the CMS-non-vulnerable-Ket group (**Figure 6E**), and 34 in the CMS-vulnerable-Ket group animals (**Figure 6F**). The number of significant correlations was thus lower in CMS-non-vulnerable and CMS-vulnerable animals.

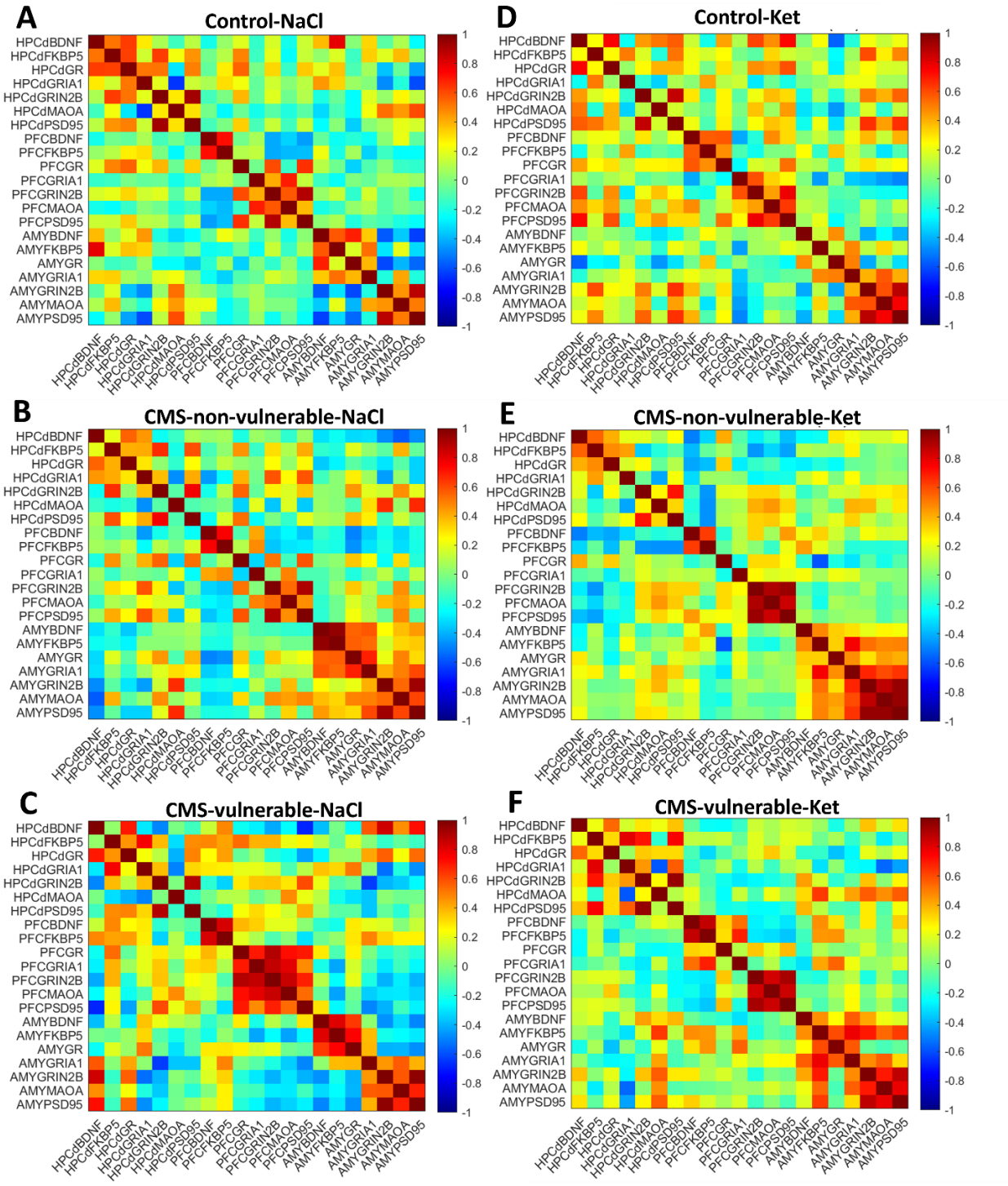

Figure 5. Inter-regional gene expression full correlation matrices. NaCl groups are represented on the left half with control-NaCl (A) CMS-non-vulnerable-NaCl (B) CMS-vulnerable-NaCl (C). Ket groups are represented on the right half with control-Ket (D) CMS-non-vulnerable-Ket (E) CMS-vulnerable-Ket (F). Matrices compare correlations of gene expression between regions. Heatmaps represent the strength of the correlation (blue: negative, red: positive).

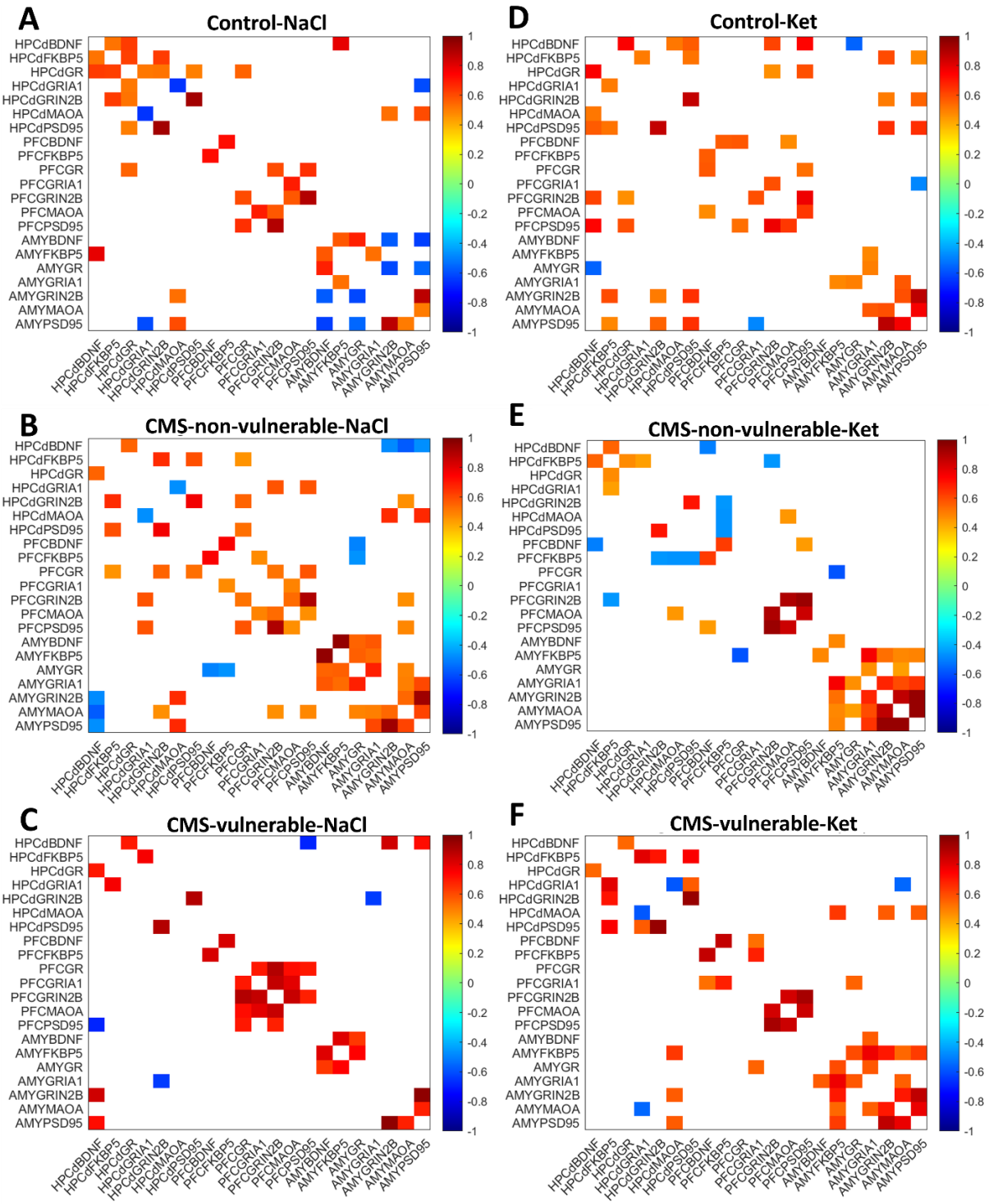

Figure 6. Inter-regional gene expression significant correlation matrices. NaCl groups are represented on the top half with control-NaCl (A) CMS-non-vulnerable-NaCl (B) CMS-vulnerable-NaCl (C). Ket groups are represented on the bottom half with control-Ket (D) CMS-non-vulnerable-Ket (E) CMS-vulnerable-Ket (F). Matrices indicate significant correlations of gene expression only between regions. Heatmap represents the strength of the correlation (blue: negative, red: positive).

E) Discussion

In this study, we investigated in WKY rats the consequences of CMS and acute Ket treatment on the expression of genes related to stress regulation, glutamatergic transmission and synaptic plasticity in three key structures involved in depression, the PFC, the AMY, and the dHPC; we particularly focussed on factors such as sex and vulnerability to stress exposure. We found that CMS exposure selectively downregulated *Bdnf* in the PFC of CMS-vulnerable animals, while downregulating *Bdnf* in the AMY and upregulating *Fkbp5* in the dHPC seemingly regardless of vulnerability. Conversely, Ket exerted region-specific and at times opposing effects, notably increasing *Bdnf* in the PFC but reducing it in the dHPC and AMY. Sex had minimal effects, affecting *Gr* expression which was lower in females in the PFC.

1) Behavioural profile

CMS-exposed animals labelled as 'vulnerable' presented reduced open-arm exploration of the EPM, reflecting heightened anxiety-like behaviour (Walf & Frye, 2007), and increased immobility in the FST, reflecting more passive stress-coping strategy (de Kloet & Molendijk, 2016). The other group, labelled 'non-vulnerable', showed control-like performance in both tests, except in males, where despite control-like behaviour in the EPM, it displayed increased immobility in the FST. This contrasts with our previous study, in which stressed animals of both sexes which presented control-like performance in the EPM also presented control-like performance in the FST (Loizeau et al., 2024). As suggested in a review discussing variability in CMS studies, this inter-cohort difference could be due to variation in a multitude of difficult-to-control environmental factors in the laboratory ; these range from seasonal effects to ambient noise (Markov & Novosadova, 2022). Environmental factors can also generate acute stress (Markov & Novosadova, 2022) and bias behaviour in the FST, which is closely linked to stress (de Kloet & Molendijk, 2016). Nevertheless, anxiety-like behaviour as evaluated in the EPM remains a good marker for vulnerability to CMS independent of FST behaviour (José Jaime et al., 2016), and anxiety is highly relevant to the study of depression (Vaccarino et al., 2008). For these reasons, and to avoid nomenclature complications, we decided to retain the name 'non-vulnerable' for these animals.

2) Gene expression results

ANOVAs on RT-qPCR results revealed distinct effects of Group, *i.e.* CMS exposure and/or vulnerability, and Treatment, *i.e.* Ket administration, on gene expression, with strong regional specificity. Sex modulated expression of one gene in the PFC, Group, of two in the dHPC and one in the PFC and AMY, and Ket modulated the expression of three genes in the dHPC, and two in the PFC and AMY. Changes were overall subtle in all brain structures within 0.5-fold change compared to the calibrator group.

GriA1 and *Psd95* expression remained unchanged across all conditions, suggesting a limited role for these synaptic markers in the transcriptional response to CMS, and to acute Ket at the 2-hour time point. *GriA1* has been observed to be downregulated following CMS in the dHPC (Duric et al., 2013), but another study found no changes (Elhussiny et al., 2021). The latter also found no effect of Ket on *GriA1* regulation at 1h post treatment (Elhussiny et al., 2021). Of note, GRIA1 protein has been shown to be upregulated at 30 or 40 minutes post-treatment, but not at 1h (Adaikkan et al., 2018) suggesting *GriA1* mRNA might be also transiently regulated before returning to baseline prior to evaluation in our study. *Psd95* has been shown to be downregulated following CMS in the HPC of mice (Lu et al., 2019) and in both the HPC and PFC of rats (Zhuang et al., 2016) although this last study employed longer CMS exposure (8 weeks), twice as long as the protocol in the present study, a length which might make some transcriptional effects more apparent. The effects of Ket on *Psd95* regulation are few and heterogenous. It has been shown to downregulate *Psd95* mRNA in the striatum, although at higher concentrations (25 and 50 mg/kg) (De Bartolomeis et al., 2013) at 1.5h post-administration; but 10 mg/kg S-Ket has also been shown to rescue downregulated *Psd95* mRNA in a prenatal stress model at a similar timepoint (Zhang et al., 2024).

Effect of Sex

Overall, sex seemed to have little to no impact: the only main effect of sex was on PFC *Gr* expression, with females showing lower expression than males. This aligns with the fact that GR protein expression is often greater in males in structures involved in negative feedback loops of the HPA axis, of which the PFC is part (McKlveen et al., 2015), an effect attributed to sex hormones (Heck & Handa, 2019).

Effect of Group

The observation of downregulated *Bdnf* mRNA expression in the PFC of CMS-vulnerable animals is coherent with previous findings investigating the impact of CMS in the rat PFC (Abbasi Mehmandost Sofla et al., 2025; Luoni et al., 2015; Osacka et al., 2021). It has also been shown to affect CMS-vulnerable only when vulnerability was considered (Talaee et al., 2024). *Bdnf* expression was downregulated in all CMS-exposed animals. While studies investigating the impact of CMS on AMY *Bdnf* mRNA levels are lacking, protein levels have been observed to be lowered, which align with our findings at the mRNA level (Luo et al., 2013; Zhang et al., 2014), although findings are heterogeneous, as increased BDNF protein expression has also been observed in rats following chronic stress (Zhang et al., 2018). While not directly investigated in the present study, chronic stress such as generated by the CMS paradigm leads to increased circulating concentration of the glucocorticoid hormone corticosterone (CORT) (Willner, 1997). In the brain, CORT can exert its effects by crossing the lipid membrane and binding to its receptor, GR, in the cytosol ; this induces GR signalling as the complex is translocated into the nucleus, where it can act as a transcription factor (Almawi & Melemedjian, 2002). GR signalling has been demonstrated to directly repress *Bdnf* mRNA expression (Chen et al., 2017), which likely explains our results in the PFC and AMY. Surprisingly, however, we did not observe *Bdnf* mRNA downregulation in the dHPC of CMS-exposed animals, while it is often the case (Jiang & Zhu, 2015; Stepanichev et al., 2014; Xing et al., 2015). Some studies however also report no changes with CMS (Allaman et al., 2008; Lucca et al., 2008) or even an upregulation of *Bdnf* (Osacka et al., 2021). These heterogeneous results may be due to subregion differences. Most of these studies investigated the whole HPC, and *Bdnf* mRNA expression is significantly higher in the ventral HPC (Gualtieri et al., 2017). The ventral HPC is more involved in emotional regulation (Nichols et al., 2023) compared to the dorsal part of the HPC. *Bdnf* downregulation in response to chronic stress might therefore be more evident in the ventral HPC, which may be one factor explaining the absence of effects here. Another possible mechanism may be related to *Fkbp5* expression in the dHPC, which was upregulated in CMS-non-vulnerable animals, and tended to be so in CMS-vulnerable animals. Such upregulation in the HPC is in line with previous studies using CMS (Guidotti et al., 2013; Xing et al., 2015) or chronic oral CORT treatment (Lee et al., 2010). These findings suggest an adaptive mechanism in response to chronic stress: FKBP5 lowers the affinity of GR for glucocorticoids such as CORT ; this hinders GR signalling (Zannas et al., 2016). FKBP5-mediated reduction in GR sensitivity may result in briefer GR signalling, as receptor activation may dissipate more quickly as glucocorticoid levels decrease (Zannas et al., 2016). In this hypothesis, CMS-induced GR signalling could have

returned to baseline during the 24 h delay between evaluation and stress cessation. Of note, a study using the WKY-CMS model failed to observe CMS-induced alterations in either *Gr* or *Fkbp5* expression in the HPC (Valdez et al., 2023). However, there were major differences in the employed CMS protocol's stressors and their application, which was ceased during the weekend [see (Henriquez et al., 2022) for details], compared to the present study, which might explain the different outcome concerning *Fkbp5* (Markov & Novosadova, 2022). *Grin2b* was also upregulated in the dHPC of CMS-vulnerable animals and tended to be upregulated in CMS-non-vulnerable animals. This contrasts with a study which observed its downregulation (Dygalo et al., 2020), although induced by chronic restraint. This discrepancy could be due to differences in the stress protocols, as different types of chronic stress paradigms have been shown to lead to distinct transcriptional responses (Yu et al., 2024). Of note, GRIN2B-dependent activity has been suggested to play a role in impaired synaptic plasticity (Miller et al., 2014), which is typically a consequence of CMS in the HPC (Dandi et al., 2023).

Together, these results show that stress vulnerability in WKY rats is related to BDNF impairments in prefrontal cortex circuits. CMS also seems to impair BDNF in amygdalar circuits, and lead to excessive GRIN2B hippocampal activity, while *Fkbp5* upregulation in the HPC may represent an attempt to buffer excessive glucocorticoid signalling.

Effect of Ket treatment in the dHPC

Most of the transcriptional changes observed in our study were driven by Ket administration, independent of stress exposure, with effects that were highly region- and gene-specific. The dHPC showed the strongest response, as Ket downregulated *Bdnf* and *Gr* expression while upregulating *Fkbp5*. These results appear counterintuitive: Ket has been shown to upregulate hippocampal *Bdnf* gene expression (Choi et al., 2017), and its therapeutic effects are believed to depend on a robust increase in BDNF protein expression (Suzuki et al., 2023). Studies evaluating its impact on *Gr* and *Fkbp5* gene expression are lacking, but Ket has been shown to restore downregulated GR at the protein level (Fraga et al., 2021). The response observed here rather suggests an acute stress response: GR signalling is known to downregulate both *Bdnf* (Chen et al., 2017) and *Gr* (Burnstein et al., 1991; Glantschnig et al., 2019), but upregulate *Fkbp5* (Spies et al., 2021). This paradoxical effect could be driven by Ket's effects. Indeed, a study showed that Ket increased both circulating CORT concentration and sgk1 expression, a downstream effector of GR signalling in the HPC. These effects were observed only in conscious, but not in anesthetised rats and evaluated 1 hour after administration (Wegman-Points et al., 2020) suggesting that internal state generated by Ket administration is a

stressful stimuli that activates the HPA axis. Considering that the WKY strain is particularly stress-sensitive, and that samples were collected two hours after Ket administration, Ket likely induced similar CORT elevations, activating GR signalling in the HPC, acting as a confounding factor. It is however important to note that Ket has been shown to increase hippocampal BDNF protein levels independently of *Bdnf* transcript expression (Autry et al., 2011). Therefore, the observed downregulated *Bdnf* levels may not prevent Ket-induced BDNF protein expression, which is a consistent observation in the HPC including in the WKY rat (Aleksandrova et al., 2019). In addition, hippocampal subregion specificity may be involved as explained above: much like its basal expression, *Bdnf* mRNA may be mainly upregulated by Ket in the ventral HPC, but less so in the dHPC, which may explain the discrepancies between our results and those of Choi et al. in which the whole HPC was studied (Choi et al., 2017). This also raises the question of the absence of dHPC *Bdnf* downregulation in CMS-exposed animals, but as hypothesised above, this may be tied to the CMS-induced upregulation of *Fkbp5*. Potentially more transient effects of GR signalling due to upregulated FKBP5 (Zannas et al., 2016) could allow acute stressors, in this case, Ket-induced, to exert effects in a shorter time window. These might therefore be noticeable during the 2-h time window between Ket administration and evaluation, but not after the 24-h delay after CMS cessation. Mao_a mRNA was seemingly downregulated by Ket. There is an apparent lack of studies investigating the impact of Ket on this parameter, but of note, the dHPC receives serotonin projections from the dorsal raphe whose stimulation has been shown to improve synaptic plasticity and reduce depressive and cognitive impairments in a mice model of Alzheimer disease (Chen et al., 2024). Reduced MAOa activity, suggested by Mao_a downregulation, may therefore enhance dorsal raphe signalling to the dHPC, potentially contributing to therapeutic effects.

Effect of Ket treatment in the AMY

In the AMY, Ket also downregulated *Bdnf*, which could be explained by the same mechanism. The fact that Ket had no impact on the expression of AMY *Gr* and *Fkbp5* suggests different effects of GR signalling in both structures. It is known that stress differentially affects gene expression depending on structure, possibly due to different GR distributions (McEwen et al., 2015; Meijer et al., 2018; Shirazi et al., 2015). Supporting this hypothesis, *Fkbp5* mRNA was upregulated in stressed animals in the dHPC, but not in the AMY or PFC. *Grin2b* was also upregulated by Ket in females only, which suggests involvement of sex hormones. There is evidence that *Grin2b* mRNA regulation by some substances is sexually dimorphic, such as with bisphenol A (Alavian-Ghavanini et al., 2018), although mechanistic underpinnings are not known. Interestingly, one proposed mechanism of Ket's therapeutic

effects is a preferential antagonism of GRIN2B containing NMDA-R (Miller et al., 2014). Therefore, we can speculate that a concurrent upregulation of *Grin2b* may represent a compensation to GRIN2B containing NMDA-R blockade.

Effect of Ket treatment in the PFC

In the PFC, Ket induced upregulation of *Bdnf* and concurrent downregulation of Mao_a expression. PFC *Bdnf* mRNA increases following Ket are a consistent observation (Kim et al., 2022; Yang et al., 2013), and aligns with the enhancement of BDNF protein expression in the PFC at the center of Ket's antidepressant action (Suzuki et al., 2023). There is an apparent lack of studies investigating the impact of Ket on Mao_a mRNA. However, Ket has been shown to increase prefrontal monoamine levels (Ago et al., 2019; Pham et al., 2017). For serotonin, this effect has been linked to increased firing of serotonergic neurons on the PFC (Pham & Gardier, 2019), but decreased MAOA expression could also contribute to the elevated extracellular monoamine levels if gene downregulation also translates to protein downregulation.

3) Correlation matrices

Concerning the correlation matrices, the absence of significant differences between the comparisons limits further analysis. However, it is interesting to note that for NaCl comparisons, Mantel statistic was highest in the Control *vs.* non-vulnerable comparison, and lowest in the non-vulnerable *vs.* vulnerable comparison, suggesting more common features and less divergence in the former than in the latter. The number of significant correlations was also greater in the non-vulnerable group, and they were more dispersed, suggesting vulnerability may be tied to lesser brain-wide co-expression, *i.e.* more independently functioning brain regions. Concerning the NaCl *vs.* Ket comparisons, the Mantel statistic was highest in the Control comparison, and lowest in the CMS-vulnerable comparisons. This interestingly parallels the results concerning PFC *Bdnf* regulation, which was upregulated by Ket in all groups, but downregulated in CMS-vulnerable animals only. Additionally, compared to the NaCl groups, the Ket groups showed lesser total number of significant correlations in both Control animals and non-vulnerable animals, but greater number in vulnerable animals. This suggests Ket could increase brain-wide co-expression in behaviourally impaired animals, but decrease it in Control animals or non-vulnerable animals.

F) Conclusion

Overall, we observed that CMS downregulated *Bdnf* expression in the PFC of vulnerable animals specifically, a coherent observation suggesting impaired synaptic plasticity. Of note, loss of

synaptic connections between these structures is closely associated with depressive phenotype. CMS also generally downregulated AMY *Bdnf*, and upregulated *Grin2b* and *Fkbp5* in the dHPC of all animals, the latter result possibly reflecting an adaptive mechanism. Ket upregulated PFC *Bdnf* and downregulated Mao_a both in the PFC and dHPC, suggesting enhanced synaptic plasticity and reduced monoamine degradation, consistent with improved monoaminergic neurotransmission. It had unexpected effects on *Bdnf* expression in both dHPC and AMY, likely stemming from confounding CORT secretion which activated region specific GR signalling effects. Taken together, these findings highlight the critical importance of timing to evaluate the transcriptomic effects of both CMS and Ket. Further studies exploring how such mRNA changes impact protein expression, such as using Western blot would be helpful to better understand the mechanisms between chronic stress and Ket's regulation of BDNF, FKBP5, GR, GRIA1, GRIN2B and PSD95 notably in the WKY-CMS model.

The results of this study indicate that stress exposure and vulnerability, and Ket administration produce highly selective, region-dependent and at times opposing changes in the expression of genes implicated in the pathophysiological mechanisms of depression. However, Ket may act as a confounding factor by generating acute stress in animals.

G) Limits

The present study has several limitations that should be acknowledged. First, the unexpected stress response observed following acute ketamine administration may have masked potential transcriptional effects, notably concerning *Bdnf*. Second, only a single ketamine dose and one post-administration time point were examined. Future studies would benefit from assessing multiple doses as well as additional time points, including both earlier intervals (e.g., 30 minutes post-administration) and longer-term effects, to better characterize the temporal dynamics of ketamine-induced transcriptional changes.

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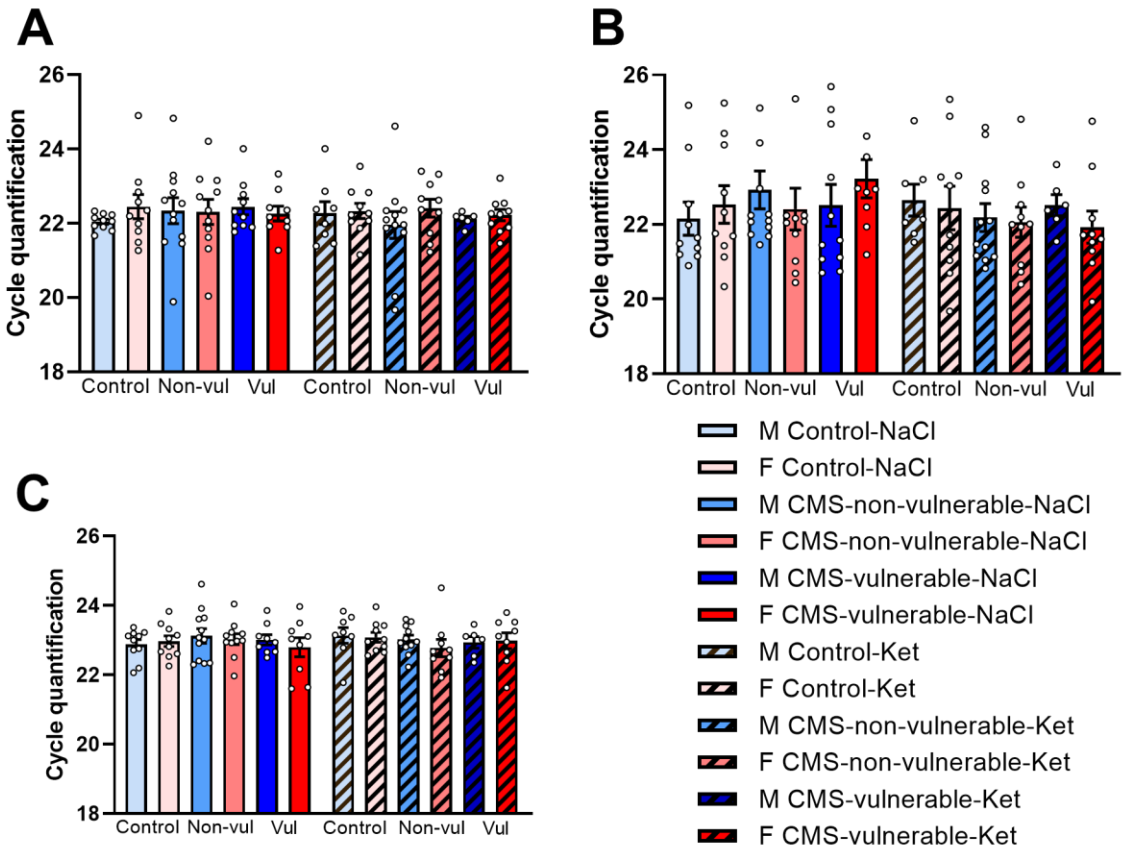
H) Supplementary information



Supplementary Figure 1. Cycle quantification numbers for Rplp0 (housekeeping gene) in the dHPC (A), the PFC (B) and the AMY (C). M: Male, F: Female, Non-vul: CMS-non-vulnerable, Vul: CMS-vulnerable Data are represented as mean ± SEM.

Gene	Statistics	Post hoc
Bdnf	Sex: $F_{(1, 109)} = 1.70$, p = 0.195 Group: $F^{(2, 109)} = 0.19$, p = 0.982 **Treatment: $F_{(1, 109)} = 9.82$, p = 0.002** Sex × Group: $F_{(2, 109)} = 0.03$, p = 0.968 Sex × Treatment: $F_{(1, 109)} = 1.32$, p = 0.252 Group × Treatment: $F_{(2, 109)} = 0.11$, p = 0.899 Triple interaction: $F_{(2, 109)} = 0.92$, p = 0.403	N/A
Fkbp5	Sex: $F_{(1, 109)} = 1.93$, p = 0.167 **Group: $F_{(2, 109)} = 3.33$, p = 0.039** **Treatment: $F_{(1, 109)} = 11.41$, p = 0.001** Sex × Group: $F_{(2, 109)} = 0.86$, p = 0.427 Sex × Treatment: $F_{(1, 109)} = 0.39$, p = 0.534 Group × Treatment: $F_{(2, 109)} = 0.32$, p = 0.729 Triple interaction: $F_{(2, 109)} = 1.02$, p = 0.364	Con vs Non-vul **p=0.032** ; Con vs Vul p=0.485 ; Non-vul vs Vul p=0.066
Gr	Sex: $F_{(1, 109)} = 1.05$, p = 0.307 Group: $F_{(2, 109)} = 0.09$, p = 0.914 **Treatment: $F_{(1, 109)} = 7.90$, p = 0.006** Sex × Group: $F_{(2, 109)} = 0.10$, p = 0.905 Sex × Treatment: $F_{(1, 109)} = 1.11$, p = 0.742 Group × Treatment: $F_{(2, 109)} = 0.52$, p = 0.595 Triple interaction: $F_{(2, 109)} = 0.70$, p = 0.496	N/A
Gria1	Sex: $F_{(1, 109)} = 0.04$, p = 0.845 Group: $F_{(2, 109)} = 0.68$, p = 0.191 Treatment: $F_{(1, 109)} = 0.76$, p = 0.384 Sex × Group: $F_{(2, 109)} = 0.24$, p = 0.788 Sex × Treatment: $F_{(1, 109)} = 0.15$, p = 0.696 Group × Treatment: $F_{(2, 109)} = 0.68$, p = 0.507 Triple interaction: $F_{(2, 109)} = 0.19$, p = 0.823	N/A
Grin2b	Sex: $F_{(1, 109)} = 0.14$, p = 0.705 Group: $F_{(2, 109)} = 2.76$, p = 0.067 Treatment: $F_{(1, 109)} = 0.02$, p = 0.898 Sex × Group: $F_{(2, 109)} = 1.77$, p = 0.174 Sex × Treatment: $F_{(1, 109)} = 2.05$, p = 0.155 Group × Treatment: $F_{(2, 109)} = 0.26$, p = 0.775 Triple interaction: $F_{(2, 109)} = 0.01$, p = 0.985	Con vs Vul **p=0.040** ; Con vs Non-vul p=0.058 ; Non-vul vs Vul p=0.582
Maoa	Sex: $F_{(1, 109)} = 0.09$, p = 0.767 Group: $F_{(2, 109)} = 0.34$, p = 0.715 **Treatment: $F_{(1, 109)} = 4.12$, p = 0.045** Sex × Group: $F_{(2, 109)} = 0.16$, p = 0.855 Sex × Treatment: $F_{(1, 109)} = 0.44$, p = 0.510 Group × Treatment: $F_{(2, 109)} = 0.64$, p = 0.529 Triple interaction: $F_{(2, 109)} = 0.78$, p = 0.459	N/A
Psd95	Sex: $F_{(1, 109)} = 0.59$, p = 0.444 Group: $F_{(2, 109)} = 2.17$, p = 0.119 Treatment: $F_{(1, 109)} = 0.21$, p = 0.650 Sex × Group: $F_{(2, 109)} = 1.81$, p = 0.168 Sex × Treatment: $F_{(1, 109)} = 2.44$, p = 0.121 Group × Treatment: $F_{(2, 109)} = 0.24$, p = 0.785 Triple interaction: $F_{(2, 109)} = 0.21$, p = 0.811	N/A

Supplementary Table 1. Details of the analyses of relative gene expression within the dHPC.

Gene	Statistics	Post hoc
Bdnf	Sex: $F_{(1, 109)} = 1.02$, p = 0.870 **Group: $F_{(2, 109)} = 4.19$, p = 0.018** **Treatment: $F_{(1, 109)} = 26.87$, p < 0.0001** Sex × Group: $F_{(2, 109)} = 1.52$, p = 0.223 Sex × Treatment: $F_{(1, 109)} = 0.83$, p = 0.363 Group × Treatment: $F_{(2, 109)} = 0.64$, p = 0.938 Triple interaction: $F_{(2, 109)} = 0.94$, p = 0.394	Control vs Non-vul: p = 0.110 Control vs Vul: **p = 0.006** Non-vul vs Vul: p = 0.127
Fkbp5	Sex: $F_{(1, 109)} = 0.50$, p = 0.483 Group: $F_{(2, 109)} = 1.65$, p = 0.197 Treatment: $F_{(1, 109)} = 3.26$, p = 0.074 Sex × Group: $F_{(2, 109)} = 1.20$, p = 0.306 Sex × Treatment: $F_{(1, 109)} = 0.03$, p = 0.863 Group × Treatment: $F_{(2, 109)} = 0.62$, p = 0.539 Triple interaction: $F_{(2, 109)} = 0.26$, p = 0.774	N/A
Gr	**Sex: $F_{(1, 109)} = 15.55$, p = 0.0001** Group: $F_{(2, 109)} = 1.44$, p = 0.241 Treatment: $F_{(1, 109)} = 1.95$, p = 0.165 Sex × Group: $F_{(2, 109)} = 1.27$, p = 0.285 Sex × Treatment: $F_{(1, 109)} = 1.15$, p = 0.701 Group × Treatment: $F_{(2, 109)} = 0.57$, p = 0.567 Triple interaction: $F_{(2, 109)} = 0.47$, p = 0.625	N/A
Gria1	Sex: $F_{(1, 109)} = 0.07$, p = 0.793 Group: $F_{(2, 109)} = 0.22$, p = 0.805 Treatment: $F_{(1, 109)} = 1.29$, p = 0.258 **Sex × Group: $F_{(2, 109)} = 3.80$, p = 0.025** Sex × Treatment: $F_{(1, 109)} = 1.05$, p = 0.307 Group × Treatment: $F_{(2, 109)} = 1.63$, p = 0.200 Triple interaction: $F_{(2, 109)} = 0.61$, p = 0.544	Sex × Group post hoc: min p = 0.237
Grin2b	Sex: $F_{(1, 109)} = 1.67$, p = 0.199 Group: $F_{(2, 109)} = 0.91$, p = 0.407 Treatment: $F_{(1, 109)} = 0.12$, p = 0.727 Sex × Group: $F_{(2, 109)} = 0.55$, p = 0.580 Sex × Treatment: $F_{(1, 109)} = 0.12$, p = 0.734 **Group × Treatment: $F_{(2, 109)} = 3.34$, p = 0.039** Triple interaction: $F_{(2, 109)} = 0.01$, p = 0.993	Group × Treatment post hoc: min p = 0.177
Maoa	Sex: $F_{(1, 109)} = 0.70$, p = 0.403 Group: $F_{(2, 109)} = 1.89$, p = 0.156 **Treatment: $F_{(1, 109)} = 4.07$, p = 0.046** Sex × Group: $F_{(2, 109)} = 1.94$, p = 0.149 Sex × Treatment: $F_{(1, 109)} = 0.01$, p = 0.923 Group × Treatment: $F_{(2, 109)} = 1.64$, p = 0.198 Triple interaction: $F_{(2, 109)} = 1.40$, p = 0.250	N/A
Psd95	Sex: $F_{(1, 109)} = 0.38$, p = 0.540 Group: $F_{(2, 109)} = 0.39$, p = 0.674 Treatment: $F_{(1, 109)} = 0.14$, p = 0.712 Sex × Group: $F_{(2, 109)} = 0.14$, p = 0.868 Sex × Treatment: $F_{(1, 109)} = 0.33$, p = 0.567 Group × Treatment: $F_{(2, 109)} = 0.80$, p = 0.450 Triple interaction: $F_{(2, 109)} = 0.04$, p = 0.958	N/A

Supplementary Table 2. Details of the analyses of relative gene expression within the PFC.

Gene	Statistics	Post hoc
Bdnf	Sex: $F_{(1, 109)} = 0.50$, p = 0.480 **Group: $F_{(2, 109)} = 6.20$, p = 0.003** Treatment: $F_{(1, 109)} = 5.04$**, p = 0.027** Sex × Group: $F_{(2, 109)} = 1.29$, p = 0.280 Sex × Treatment: $F_{(1, 109)} = 0.01$, p = 0.942 Group × Treatment: $F_{(2, 109)} = 0.99$, p = 0.906 Triple interaction: $F_{(2, 109)} = 0.09$, p = 0.918	Control vs Non-vul: **p = 0.009** Control vs Vul: **p = 0.003** Non-vul vs Vul: p = 0.471
Fkbp5	Sex: $F_{(1, 109)} = 1.67$, p = 0.199 Group: $F_{(2, 109)} = 0.26$, p = 0.774 Treatment: $F_{(1, 109)} = 0.03$, p = 0.859 Sex × Group: $F_{(2, 109)} = 1.49$, p = 0.229 Sex × Treatment: $F_{(1, 109)} = 1.51$, p = 0.222 Group × Treatment: $F_{(2, 109)} = 0.17$, p = 0.840 **Triple interaction: $F_{(2, 109)} = 6.09$, p = 0.003**	Triple interaction post hoc: min p = 0.200
Gr	Sex: $F_{(1, 109)} = 1.59$, p = 0.209 Group: $F_{(2, 109)} = 1.55$, p = 0.217 Treatment: $F_{(1, 109)} = 1.71$, p = 0.194 Sex × Group: $F_{(2, 109)} = 0.95$, p = 0.389 Sex × Treatment: $F_{(1, 109)} = 0.18$, p = 0.675 Group × Treatment: $F_{(2, 109)} = 2.64$, p = 0.076 Triple interaction: $F_{(2, 109)} = 2.29$, p = 0.106	N/A
Gria1	Sex: $F_{(1, 109)} = 1.15$, p = 0.285 Group: $F_{(2, 109)} = 1.17$, p = 0.840 Treatment: $F_{(1, 109)} = 0.03$, p = 0.872 Sex × Group: $F_{(2, 109)} = 1.61$, p = 0.205 Sex × Treatment: $F_{(1, 109)} = 1.79$, p = 0.183 Group × Treatment: $F_{(2, 109)} = 0.79$, p = 0.454 Triple interaction: $F_{(2, 109)} = 0.60$, p = 0.550	N/A
Grin2b	Sex: $F_{(1, 109)} = 0.03$, p = 0.852 Group: $F_{(2, 109)} = 2.20$, p = 0.113 **Treatment: $F_{(1, 109)} = 4.43$, p = 0.037** Sex × Group: $F_{(2, 109)} = 0.07$, p = 0.928 Sex × Treatment: $F_{(1, 109)} = 3.14$, p = 0.079 Group × Treatment: $F_{(2, 109)} = 0.65$, p = 0.526 Triple interaction: $F_{(2, 109)} = 0.45$, p = 0.641	N/A
Maoa	Sex: $F_{(1, 109)} = 0.13$, p = 0.720 Group: $F_{(2, 109)} = 0.88$, p = 0.416 Treatment: $F_{(1, 109)} = 0.02$, p = 0.882 Sex × Group: $F_{(2, 109)} = 0.04$, p = 0.958 Sex × Treatment: $F_{(1, 109)} = 3.68$, p = 0.058 Group × Treatment: $F_{(2, 109)} = 0.37$, p = 0.688 Triple interaction: $F_{(2, 109)} = 0.42$, p = 0.660	N/A
Psd95	Sex: $F_{(1, 109)} = 0.09$, p = 0.757 Group: $F_{(2, 109)} = 1.78$, p = 0.172 Treatment: $F_{(1, 109)} = 0.70$, p = 0.406 Sex × Group: $F_{(2, 109)} = 0.27$, p = 0.766 Sex × Treatment: $F_{(1, 109)} = 1.10$, p = 0.296 Group × Treatment: $F_{(2, 109)} = 0.10$, p = 0.905 Triple interaction: $F_{(2, 109)} = 0.02$, p = 0.979	N/A

Supplementary Table 3. Details of the analyses of relative gene expression within the AMY.

DISCUSSION GÉNÉRALE

Cette section présente une synthèse du contexte, des objectifs et des principaux résultats de la thèse. Après avoir discuté les limites des présents travaux, je propose un modèle mécanistique intégratif s'appuyant sur la littérature pour expliquer les différents résultats obtenus, incluant des éléments de discussion spéculatifs non abordés dans les articles. Enfin, je conclus sur les perspectives ouvertes par ces travaux.

I) Récapitulatif des travaux

Cette thèse, initiée dans le cadre d'un projet translationnel (ERA-NET NEURON) visant à optimiser l'utilisation thérapeutique de la Ket dans la TRD, a eu deux objectifs principaux : (1) mieux décrire l'impact du CMS chez le rat WKY, et (2) évaluer les effets d'un traitement de Ket, selon une modalité aiguë ou chronique, i.e. une administration quotidienne pendant une semaine. Cinq études complémentaires ont été conduites. La première a été centrée sur la réponse comportementale d'animaux soumis au CMS, notamment *via* l'exploration de différences liées au sexe ou à la vulnérabilité au protocole. La deuxième a été consacrée à l'étude des mécanismes de plasticité synaptique dans le dHPC chez des animaux mâles CMS-vulnérables, et des effets dans cette population d'une administration aiguë ou chronique de Ket, *via* des enregistrements *ex vivo*. Les troisième et quatrième études ont permis d'analyser le rythme veille-sommeil et l'activité cérébrale *via* des analyses spectrales dans le PRL, la BLA et le dHPC chez des animaux mâles CMS-vulnérables. Ces analyses ont impliqué trois sessions d'enregistrement *in vivo* : la session 1, avant traitement de Ket, permettant de comparer les animaux CMS-vulnérables à des témoins non stressés ; la session 2, suite à un traitement aigu, et la session 3, suite à un traitement chronique chez les animaux CMS-vulnérables, permettant étudier les effets de la Ket selon ces modalités en comparaison à la session 1. Enfin, la cinquième étude a évalué, *via* une approche transcriptomique par RT-qPCR, l'expression de gènes impliqués dans des voies liées à la réponse au CMS ou à la Ket, dans le mPFC, l'AMY et le dHPC, en considérant la vulnérabilité au CMS, le sexe et l'effet d'un traitement aigu à la Ket.

Dans la première étude (**cf. p. 83**), environ la moitié (M : 46% F : 38%) des animaux ont présenté une anxiété accrue telle qu'évaluée dans l'EPM, ainsi qu'une stratégie d'adaptation passive en situation stressante sans échappatoire dans le FST, constituant la population classée comme « CMS-vulnérable ». De façon surprenante, une augmentation de la préférence au saccharose a été constatée dans le SPT avec le temps chez tous les groupes, sans effet du CMS sur celle-ci. Tous les mâles exposés au CMS ont eu une moindre prise pondérale, et eu une réaction d'hyperlocomotion face à un

environnement nouveau. Lors de l'évaluation de l'activité locomotrice spontanée, un décalage du rythme d'activité nycthéméral, caractérisé par un retard de phase, a été observé seulement chez les mâles CMS-vulnérables. Dans le SPT, une moindre consommation de saccharose a été constatée uniquement chez les femelles CMS, sans que cela n'affecte la préférence.

Dans la seconde étude (**cf. p. 84**), chez des mâles CMS-vulnérables, la LTP n'a été observée qu'après un délai, tandis que la LTD n'a pu être déclenchée. Si un traitement aigu de Ket a pu rétablir la LTP, mais pas la LTD, un traitement chronique a pu rétablir la LTD mais a conduit à une perte de la LTP. Une interprétation possible concernant l'absence de LTD chez les animaux CMS-vulnérables, non mentionnée dans l'article, est que ce mécanisme pourrait être en état de saturation (O'Connor et al., 2005). En effet, étant donné la sensibilité accrue au stress chez le rat WKY (Paré, 1989), le stress chronique, généralement décrit comme facilitant le mécanisme de LTD, aurait pu conduire à une saturation de la LTD chez les animaux CMS-vulnérables. Dans ce cas, la Ket aurait pu dans un premier temps rétablir une potentialisation, entraînant la possibilité de déprimer la synapse dans un second temps.

La troisième étude (**cf. p. 103**) a permis d'observer, chez des mâles CMS-vulnérables, plusieurs perturbations rappelant celles observées dans la dépression. L'analyse des hypnogrammes des mâles CMS-vulnérables a révélé une plus grande latence à l'endormissement en sommeil NREM, et une tendance à une latence plus faible d'entrée en sommeil REM. Le rythme veille-sommeil a été globalement altéré, caractérisé par un profil aplani et une plus grande fragmentation en période nocturne. Les deux modalités de traitement de Ket ont transitoirement empêché l'endormissement, avec une latence d'entrée en sommeil NREM accrue et ont provoqué l'augmentation de la latence d'entrée en sommeil REM, avant d'entraîner un rebond de sommeil. Néanmoins, seul le traitement chronique de Ket a pu rétablir un rythme veille-sommeil plus proche de celui des témoins en période nocturne, sans toutefois affecter la fragmentation.

Dans la quatrième étude (**cf. p. 137**), en raison de la grande variabilité d'échelle des valeurs de puissance spectrale, il n'a pas été possible de comparer les valeurs de puissance absolue entre les enregistrements, d'où l'emploi des valeurs de puissance relative. De fait, une discussion concernant les différences de puissance absolue dans les différentes bandes de fréquences entre les groupes serait spéculative. Néanmoins, des modifications de puissance absolue suivant le profil des variations de puissance relative pourraient être cohérentes avec les conséquences du CMS (voir Annexe). Les animaux CMS-vulnérables ont présenté des perturbations au sein du PRL et de la BLA dans les composantes apériodiques du spectre de puissance de l'activité cérébrale, suggérant un déséquilibre

de la balance excitation/inhibition (E/I) dans ces structures. De plus, pendant la phase nocturne, les données ont indiqué au sein du PRL en particulier, une diminution graduelle de la puissance relative dans la bande alpha et une augmentation dans la bande gamma, ce qui est inverse au profil observé chez les animaux témoins. En revanche, aucun changement n'a été observé dans le dHPC. La Ket a généré des effets transitoires dans les trois structures, notamment une diminution de la puissance bêta et une augmentation de la puissance gamma exceptée dans la BLA, suggérant une potentielle désynchronisation transitoire de l'activité au sein des structures. Une augmentation transitoire de la puissance dans la bande delta a également été constatée lors du sommeil NREM qui a suivi la période d'éveil prolongé. Après ces effets transitoires, la Ket a également induit d'autres effets, variant selon le mode d'administration. Parmi ceux-ci, le traitement aigu a entraîné dans le dHPC sur plusieurs heures lors de la période diurne, une augmentation des puissances dans la bande thêta pendant l'éveil et dans la bande delta lors du sommeil NREM, et une diminution de la puissance gamma, effet qui n'a pas été constaté avec le traitement chronique. Dans la BLA, lors de la période nocturne, le traitement aigu a induit une diminution de la puissance bêta en veille en fin de la période. Le traitement chronique a eu le même effet, en plus de diminuer la puissance bêta et alpha en début de la période. Enfin, les deux types de traitement ont également entraîné une moins grande puissance delta à divers moments de la période nocturne. En l'état, les données de cohérence corrigée ne permettent pas de conclure sur la connectivité fonctionnelle.

Enfin, la cinquième étude (**cf. p. 183**) a permis de constater chez les animaux CMS-vulnérables des deux sexes, une moindre transcription de *Bdnf* dans le mPFC. Les animaux exposés au CMS ont présenté une transcription accrue de *Fkbp5* et de *Grin2b* dans le dHPC, et une moindre transcription de *Bdnf* dans l'AMY. La Ket, administrée de façon aiguë, a augmenté la transcription de *Bdnf* dans le mPFC, tout en la diminuant dans l'AMY et dans le dHPC. Dans ce dernier, un amoindrissement de la transcription de *Gr* et un accroissement de celle de *Fkbp5* ont également été observés, suggérant une réaction de stress aigu généré suite au traitement de Ket. La Ket a aussi entrainé une moindre transcription de Mao_a dans le PFC et le dHPC. Concernant les différences liées au sexe, une moindre expression de *Gr* dans le mPFC, ainsi qu'une tendance à une transcription accrue de *Grin2b* par la Ket dans l'AMY, ont été constatées chez les femelles.

Dans l'ensemble, nous n'avons pas observé de nombreuses divergences entre mâles et femelles lorsque ce paramètre était pris en compte. La moindre prise de poids n'a affecté que les mâles, un effet fréquemment décrit dans la littérature (Hodes, 2018; Liang et al., 2008; Murison & Hansen, 2001; Sterrenburg et al., 2011; Xing et al., 2013). L'hyperlocomotion face à un environnement nouveau a été

constatée chez les mâles uniquement, tandis qu'une consommation affaiblie de saccharose l'a été uniquement chez les femelles. Dans l'étude transcriptomique, seule l'expression basale de *Gr* dans le mPFC et une tendance à une expression accrue de *Grin2b* dans l'AMY suite à la Ket a démontré des différences liées au sexe. L'absence d'interactions liées au sexe sur les effets du CMS dans l'étude transcriptomique suggère que la plasticité hippocampique ou les LFP pourraient être altérées de la même façon chez les femelles que chez les mâles. En revanche, puisque la phase du rythme d'activité locomotrice n'a pas été perturbée chez les femelles CMS-vulnérables, il est possible que les altérations du rythme veille-sommeil soient moins importantes, voire absentes, chez les femelles.

II) Limites des travaux

Si les travaux de cette thèse ont permis d'étudier divers aspects de l'impact du CMS chez le rat WKY, ainsi que les effets d'un traitement aigu ou chronique par la Ket, ils présentent plusieurs limites.

Le sexe et la non-vulnérabilité au stress n'ont été pris en compte que dans les études comportementales et transcriptomiques, les études électrophysiologiques *ex vivo* et *in vivo* n'ayant été réalisées que chez des mâles CMS-vulnérables. D'une part, ceci résulte du fait que les femelles de la première cohorte, au sein de laquelle la plupart des animaux ont été sélectionnés pour ces études, ont été transmises, suite au CMS, chez des collaborateurs dans le cadre du projet ERA-NET NEURON. D'autre part leur inclusion ultérieure aurait impliqué de multiplier les effectifs, ce qui, en raison de contraintes temporelles et logistiques relatives à la thèse, n'aurait pu être accompli à temps notamment en raison du volume de données à analyser. Hormis dans l'étude transcriptomique, l'effet de la Ket n'a pas été évalué chez les animaux témoins, limitant l'interprétation concernant de potentielles interactions entre traitement et état pathologique. Néanmoins, le projet dans lequel ces travaux s'inscrivaient avait pour but essentiel d'évaluer les effets de traitements de Ket dans le cadre d'un modèle de la pathologie, plutôt que les effets généraux de la substance dans une condition non pathologique, ces derniers ayant déjà été abondamment décrits dans la littérature.

De plus, les effets de la Ket n'ont pas pu être évalués sur le comportement. Bien que cette évaluation était prévue *via* le SPT, ce dernier n'a jamais permis de démontrer d'anhédonie, c'est-à-dire une baisse de la préférence au saccharose, suite au CMS. Comme discuté dans l'article de la première étude, ce test présente d'importants problèmes de reproductibilité (Markov, 2022), et de tels résultats ne sont pas inédits (Willner, 2005). Il n'aurait pas non plus été possible de répéter les tests servant à phénotyper les animaux, comme l'EPM et le FST, en raison d'une modification du

comportement qu'entraîne leur répétition, ce qui en aurait biaisé l'interprétation (Mezadri et al., 2011; Walf & Frye, 2007).

III) Hypothèse mécanistique expliquant les résultats des différentes études

Si les travaux de cette thèse ont été concentrés sur certains aspects des conséquences du CMS et des effets de la Ket, ils permettent, conjointement avec les données de la littérature, d'établir des liens entre les résultats des différentes études.

L'exposition au CMS a probablement entraîné une activation chronique de l'axe HPA, conduisant à une sécrétion, voire hypersécrétion de CORT (Willner, 1997), l'axe HPA étant particulièrement réactif aux mécanismes de rétrocontrôle inhibiteurs moins efficaces chez le WKY (Aleksandrova et al., 2019). La CORT, circulant dans l'organisme, a pu agir sur les structures cérébrales comme le mPFC, la BLA et le dHPC, structures exprimant de façon importante les GR (Su et al., 2025). En se liant aux GR, la CORT a pu déclencher une signalisation modulant l'expression de divers gènes (Almawi & Melemedjian, 2002). La trace de cette signalisation a notamment été constatée dans le dHPC par la transcription accrue de *Fkbp5*, une réponse de l'organisme suggérant une tentative de mitiger les effets de la CORT (Zannas et al., 2016). Au sein de l'HPC, la signalisation GR chronique a probablement perturbé l'expression et/ou l'activité de kinases fondamentales pour la LTP comme la CaMKII (Gerges et al., 2004; Park et al., 2015), ainsi que des voies de signalisation comme JAK2/STAT3 (Virijevic et al., 2025) et potentiellement des mécanismes comme l'autophagie protéique (Yang et al., 2025) nécessaires à la LTD (Kallergi et al., 2022; Nicolas et al., 2012). En conséquence, les mécanismes de LTP et de LTD y ont été perturbés et bloqués, respectivement. S'il n'est pas possible de conclure sur de tels effets chez les femelles, l'absence de différences liées au sexe concernant la transcription de *Fkbp5*, ainsi que la dérégulation CaMKII et JAK2/STAT3 décrite chez des femelles WKY exposées au CMS (Virijevic et al., 2025) suggère que les mêmes perturbations de la plasticité synaptique pourraient y être observées.

Dans le mPFC et l'AMY, la signalisation GR a probablement entraîné l'inhibition de la transcription de *Bdnf* constatée (Chen et al., 2017). Ceci reflète probablement la première étape d'une chaine mécanistique menant à une moindre production de la protéine BDNF (Chen et al., 2017), dont la dérégulation est une conséquence bien établie du stress chronique (Krasner et al., 2025). Il est donc probable que des altérations des plasticités synaptiques similaires à celles constatées dans le dHPC ont également eu lieu dans ces structures (Krasner et al., 2025). De telles pertes de plasticité extra-

hippocampiques ont notamment déjà été décrites chez le rat WKY en comparaison à d'autres souches et le CMS pourraient les aggraver (Aleksandrova et al., 2019). En plus de cet amoindrissement des capacités plastiques des synapses, le CMS a pu entraîner des pertes synaptiques concernant les projections depuis et vers d'autres structures, au sein de la microcircuiterie locale, voire des pertes cellulaires (Khan et al., 2020; Wang et al., 2023). En conséquence, l'activité inhibitrice impliquant des interneurones GABAergiques au sein des microcircuits locaux a probablement été amoindrie (Ghosal et al., 2017). En effet, le BDNF est fondamental à l'activité normale des interneurones GABAergiques (Guyon et al., 2021), dont la dysfonction a également été démontrée dans l'AMY dans un modèle de stress chronique par contention (Gilabert-Juan et al., 2011). Ces perturbations au sein des microcircuits ont pu déséquilibrer la balance E/I et la synchronie de l'activité neuronale locale (Ferguson & Gao, 2018), comme suggéré par le profil spectral de l'activité enregistrée au sein de ces structures. Ceci, conjointement aux potentielles pertes synaptiques entre les structures aurait pu réduire la connectivité fonctionnelle, voire structurelle, entre les différentes régions (Wang et al., 2023). De fait, ces structures auraient pu fonctionner plus indépendamment les unes des autres, ce qui aurait entraîné une moindre capacité de régulation émotionnelle (Mavrych et al., 2025). En conséquence, des perturbations comportementales, comme l'anxiété et la stratégie d'adaptation passive face au stress ont pu émerger, reflétant un état pathologique. Si cette hypothèse concerne des perturbations à l'âge adulte, il est intéressant de noter qu'il existe un dimorphisme sexuel dans le développement préfrontal d'interneurones GABAergiques à l'adolescence, qui est BDNF-dépendant. Des déficits de BDNF pouvant davantage affecter les interneurones PV chez les mâles, et SST chez les femelles (Du et al., 2018). Il est donc possible qu'un dérèglement des interneurones lié à des perturbations du BDNF pourrait présenter des subtilités selon le sexe.

Des niveaux circulants élevés de CORT ont pu augmenter la latence d'endormissement et la fragmentation du sommeil (Yao et al., 2023). La latence d'entrée en sommeil REM, en revanche, a été réduite, un phénomène impliquant potentiellement une moindre excitation de neurones REM-OFF *via* projections préfrontales, ou du raphé dorsal, (Monti, 2010; Wang et al., 2015). La signalisation GR aurait également pu perturber l'expression des gènes horloges régulant les rythmes circadiens, par exemple Per2 (Calabrese et al., 2016; Sarrazin et al., 2024), perturbant d'une part le rythme d'activité locomotrice, comme constaté dans l'étude comportementale, et d'autre part le rythme veille/sommeil. Si les résultats présents ne permettent pas de tirer de conclusions chez les femelles, l'absence du décalage de rythme constaté dans l'étude comportementale chez les femelles CMS-vulnérables suggère un potentiel impact différent du CMS sur les mécanismes circadiens, incluant le rythme

veille/sommeil. Il est également intéressant de noter que l'expression de *Gr* était constitutivement réduite dans le mPFC des femelles, et que les gènes horloges semblent particulièrement affectés par le stress chronique dans le mPFC (Sarrazin et al., 2024).

Néanmoins, chez environ la moitié des individus exposés au CMS, certains facteurs ont pu protéger les animaux d'effets délétères du stress chronique, ces animaux constituant donc la population dite non-vulnérable. Ces facteurs ont pu être génétiques, le rat WKY conservant une part de variabilité génétique (Will et al., 2003), mais également épigénétiques, comme *via* le soin maternel périnatal, facteur modulant la vulnérabilité au stress, notamment dans des modèles de CMS (Caldji et al., 2000; Henningsen et al., 2012; Weaver et al., 2004). Ces facteurs protecteurs ont pu d'une part limiter l'hyperactivation de l'axe HPA et d'autre part mitiger les effets de la signalisation GR dans les structures cérébrales (Brivio et al., 2021), notamment concernant l'expression de BDNF (Weaver et al., 2017). L'étude transcriptomique n'a effectivement pas permis de constater chez ces animaux une perturbation de l'expression *Bdnf* en ce qui concerne le PFC. Il est donc possible que les animaux non-vulnérables ne présenteraient pas, ou présenteraient en moindre mesure, les pertes synaptiques supposées chez les animaux CMS-vulnérables. Ainsi, les microcircuits locaux et l'équilibre de la balance E/I pourraient être plus préservés. Le réseau PRL-BLA-dHPC pourrait donc potentiellement être moins altéré, permettant une meilleure régulation émotionnelle malgré l'exposition au CMS. Ces phénomènes pourraient donc expliquer le comportement différent des animaux non-vulnérables dans les tests comportementaux, en particulier dans l'EPM. Cette hypothèse est cependant à nuancer puisqu'une baisse de l'expression de *Bdnf* a été constatée dans la BLA chez tous les animaux. Chez les animaux mâles non-vulnérables, des facteurs protecteurs vis-à-vis des effets du stress auraient également pu limiter des perturbations de gènes horloges, comme suggéré par l'absence de décalage du rythme d'activité locomotrice dans ce groupe. En conséquence, il est possible qu'ils ne présenteraient pas d'altérations marquées du rythme veille/sommeil. Néanmoins, des effets métaboliques du CMS y ont tout de même été constatés, tels qu'une moindre prise de poids. Chez les femelles, qui n'ont pas présenté ces altérations, les hormones sexuelles auraient pu prévenir un effet catabolisant lié à l'activation de l'axe HPA (Moench & Wellman, 2017; Rivest et al., 1989).

L'administration de Ket a eu plusieurs effets immédiats. Les résultats de l'étude transcriptomique dans le dHPC suggèrent qu'elle a déclenché une réponse aiguë de stress probablement médiée par ses effets psychomimétiques, sympathomimétiques et vestibulaires, une telle réponse étant constatée chez des rats conscients, mais pas anesthésiés (Wegman-Points et al., 2020). *Via* son action pharmacologique, *i.e.* un antagonisme des NMDA-Rs, la Ket a accru la puissance

oscillatoire dans la bande gamma, mais a pu réprimer la puissance dans les bandes thêta, alpha et bêta selon les régions, par une modulation de l'activité des interneurones inhibiteurs (Adam et al., 2024; Guyon et al., 2021). Une potentielle sécrétion de CORT aurait également pu contribuer à cet effet (Wang et al., 2014). L'antagonisme des NMDA-R a également pu prévenir l'action de neurones impliqués dans l'initiation du sommeil, par exemple au sein du noyau hypothalamique préoptique latéral (Miracca et al., 2022), région clé dans l'initiation du sommeil (Gallopin et al., 2000) ou dans les noyaux suprachiasmatiques (Kawai et al., 2015), entraînant l'inhibition transitoire du sommeil sur une durée correspondant à celle de la présence de la Ket dans l'organisme (Páleníček et al., 2011). Ces effets dépendant de l'antagonisme NMDA-R auraient notamment pu concerner en particulier les récepteurs riches en sous-unité GRIN2A, une étude ayant montré que l'antagonisme sélectif des NMDA-R riches en sous-unité GRIN2B ne perturbait pas le sommeil et n'élicitait pas d'oscillations gamma dans la période post-administration (Kocsis, 2012). Ils auraient également pu être médiés principalement par la (S)-Ket, cette dernière ayant une affinité plus importante pour les NMDA-R et un effet inhibiteur plus marqué sur le sommeil (Koncz et al., 2023).

En parallèle, dans différentes régions cérébrales, la Ket a probablement stimulé l'activité de kinases comme la CaMKII (Adaikkan et al., 2018; Yang et al., 2018), l'activation d'AMPA-R (Zaytseva et al., 2023) et la production de BDNF (Yang et al., 2013; Zhou et al., 2014) comme suggéré par la transcription accrue du gène *Bdnf* dans le mPFC et le rétablissement de la LTP dans le dHPC suite au traitement aigu. Probablement en raison d'une signalisation GR provoquée une sécrétion de CORT induite par la Ket (Wegman-Points et al., 2020), une transcription amoindrie de *Bdnf* a été constatée dans l'AMY et surtout dans le dHPC. Ainsi, l'effet transcriptomique de la Ket dans ces structures a potentiellement été masqué. Néanmoins, du BDNF aurait pu tout de même être produit, une étude *in vitro* ayant notamment montré une production hippocampique de BDNF par la Ket indépendamment de sa transcription (Autry et al., 2011). Il est également intéressant de constater que dans le dHPC, en plus du rétablissement de la LTP, la Ket en traitement aigu a également entraîné une modulation de la puissance spectrale durant les heures suivant son administration, un effet potentiellement médié par la (R)-Ket (Pothorszki et al., 2024). Étant donné cette convergence sur le plan temporel, et le fait que la (R)-Ket ait un effet plus marqué que la (S)- Ket sur l'augmentation de la signalisation BDNF et la synaptogenèse (Yang et al., 2015), il peut être supposé que la modulation de la puissance spectrale et les effets sur la plasticité pourraient être liés.

Les effets pro-plasticité de la Ket auraient pu contrecarrer les déficits synaptiques décrits précédemment. En effet, celle-ci aurait pu rétablir de potentielles pertes de densité synaptique induites

par le stress chronique dans le mPFC et l'hippocampe (Zhornitsky et al., 2023) restaurant un fonctionnement normal du réseau PRL-BLA-dHPC. Ainsi, une régulation émotionnelle normale aurait pu être rétablie, comme la Ket permet de le faire notamment dans l'EPM et le FST (Hou et al., 2022; Wen et al., 2019). L'inhibition de la transcription de *Mao*$_a$ pourrait conduire à une moindre expression de MAO$_A$, ce qui aurait également pu contribuer à l'augmentation de la concentration de 5-HT dans le PFC et le dHPC. Une telle augmentation, impliquant des projections du raphé dorsal, a notamment été constatée dans le PFC suite à un traitement de Ket (Pham et al., 2017). Une concentration de 5-HT accrue aurait également pu d'une part moduler l'activité d'interneurones GABAergiques dans le mPFC (Athilingam et al., 2017) et d'autre part contribuer à augmenter la latence d'entrée en sommeil REM, l'activité sérotoninergique inhibant cet état de vigilance (Cespuglio, 2018).

Un traitement aigu n'a pas semblé suffisant pour rétablir les mécanismes de LTD tels qu'évalués 2 heures après l'administration, leur rétablissement n'ayant été observé qu'après le traitement chronique. Le fait qu'un rythme veille/sommeil normal n'ait pas pu être rétabli suite au traitement aigu de Ket suggère également qu'une telle modalité de traitement ne suffit pas à rétablir les dysfonctions sous-jacentes. Si les perturbations circadiennes dépendent d'une dérégulation des gènes horloges par le stress chronique, un traitement de Ket répété pourrait être nécessaire pour leur réalignement. Néanmoins, la fragmentation du sommeil n'a pu être rétablie par aucune modalité de traitement. Il est cependant possible que cette fragmentation ait été présente en amont de l'application du protocole de CMS chez les animaux CMS-vulnérables. En effet, une étude impliquant un modèle de défaite sociale répétée a démontré qu'une fragmentation du sommeil pré-stress permettait de prédire la vulnérabilité au paradigme de stress (Radwan et al., 2021), ce qui aurait pu également être le cas des animaux CMS-vulnérables évalués. Ainsi, si la fragmentation résulte de mécanismes dysfonctionnels indépendants du stress chronique, il est possible que ceux-ci ne soient pas rétablis par l'administration de Ket.

En somme, la Ket pourrait rétablir les déficits comportementaux observés chez les animaux CMS-vulnérables en rétablissant d'une part la plasticité synaptique, *via* une modulation de facteurs neurotrophiques et de kinases et d'autre part en modulant des régulateurs de rythmicité circadienne. Les résultats de cette thèse suggèrent également qu'une modalité de traitement idéale pour rétablir l'ensemble des déficits pourrait être sub-chronique plutôt qu'aiguë. En effet, si un traitement aigu a permis de rétablir certains paramètres, il n'a pas semblé suffisant pour rétablir le rythme veille/sommeil en période nocturne, ou la LTD. Néanmoins, un traitement chronique a semblé saturer la LTP, un phénomène pouvant induire des déficits cognitifs. En effet, une saturation de la LTP hippocampique

perturbe les capacités d'apprentissage et la flexibilité cognitive (Barnes et al., 1994; Guntupalli et al., 2023). Des études évaluant des traitements répétés de Ket ont déjà décrit une augmentation de l'immobilité dans le FST (Weston et al., 2021). Une étude ayant évalué les effets d'un traitement par de la Ket durant 28 jours consécutifs a même constaté un amoindrissement de la densité synaptique, de l'expression de marqueurs synaptiques et de l'expression et la phosphorylation de kinases comme la CaMKII, ainsi qu'une LTP perturbée et des moins bonnes performances en tâche de mémoire spatiale (Luo et al., 2021). Cet effet pourrait représenter de potentiels mécanismes d'adaptation engagés face au traitement de Ket à long terme. Globalement, les résultats des présents travaux, conjointement avec ces données, suggèrent l'existence d'un point de bascule dans la modalité de traitement avant lequel la Ket a un effet thérapeutique incomplet, et après lequel elle peut entraîner un effet délétère.

Il est également intéressant de noter qu'outre ses effets thérapeutiques sur les conséquences physiologiques et comportementales du stress chronique, plusieurs études précliniques ont également décrit un effet préventif de la Ket, permettant d'empêcher les effets délétères du stress chronique dans divers paramètres (Brachman et al., 2016; Evers et al., 2022; Krzystyniak et al., 2019; Santos et al., 2025).

Bien que les mécanismes décrits ci-dessus ne puissent pas être directement extrapolés à l'humain, la Ket à usage thérapeutique est souvent accompagnée d'une augmentation de la concentration sérique de BDNF ; néanmoins, ces résultats sont hétérogènes et pourraient n'affecter que les patients répondant le plus efficacement au traitement (Pardossi et al., 2024). La Ket permet également de normaliser les dysfonctionnements de connectivité entre différentes structures cérébrales, effets qui semblent corrélés à ses mécanismes thérapeutiques (Evans et al., 2018). Par ailleurs, elle peut moduler le rythme circadien d'activité chez des patients résistants à au moins un traitement antérieur, en induisant une avance de phase (Duncan et al., 2017), suggérant que ses mécanismes thérapeutiques impliquent effectivement une régulation de gènes horloges. S'il n'a pas été évalué dans la dépression en général, un effet thérapeutique préventif de la Ket a également été décrit dans le cas de dépression postpartum (Evers et al., 2022). À l'inverse, des données issues d'usagers récréatifs de Ket suggèrent que des expositions fréquentes ou intensives à la substance ont des effets délétères. Ces derniers incluent une réduction de la concentration sérique de facteurs neurotrophiques, notamment le BDNF (Ke et al., 2014), une perte de substance grise et blanche et des déficits de connectivité entre diverses structures cérébrales, perturbations proportionnelles à la durée et à l'intensité de la consommation (Strous et al., 2022).

IV) Perspectives

Les résultats et la discussion des présents travaux soulèvent de nouvelles questions et ouvrent des perspectives de recherche.

Sur le plan pharmacologique, seule une dose de traitement de Ket (10 mg/kg) et un unique point temporel (2 heures post-administration) ont été étudiés. Explorer d'autres concentrations et d'autres délais permettrait de mieux caractériser la dynamique des effets du traitement au sein des différentes études.

Sur le plan comportemental, l'évaluation d'autres paramètres pertinents dans la modélisation de la dépression, tels que les biais cognitifs, les altérations de la prise de décision ou de la mémoire de travail ou à long terme, permettraient également d'enrichir la caractérisation du modèle WKY-CMS et potentiellement approfondir sa validité.

Sur le plan électrophysiologique, une analyse des composantes apériodiques des spectres de fréquences, notamment via des méthodes d'analyse telles que la toolbox FOOOF (Donoghue et al., 2020), permettrait non seulement de confirmer les différences suggérées par le profil spectral, mais également d'isoler ces paramètres pour analyser les composantes périodiques de manière indépendante. Concernant les données de cohérence, d'autres approches de correction comme par la moyenne du bootstrap permettraient potentiellement de révéler des différences interprétables concernant les animaux CMS-vulnérables et NS. Dans ce cas, des analyses comme la causalité de Granger permettraient de mieux caractériser la directionnalité des échanges d'information entre les structures présentant des cohérences oscillatoires. L'étude du rôle des interneurones GABAergiques dans les perturbations du PRL et de la BLA permettrait de soutenir ou non l'hypothèse de la perturbation des microcircuits neuronaux et de déséquilibre de la balance E/I dans ces structures. Il serait par exemple intéressant d'évaluer l'effet sur le profil spectral de leur inactivation transitoire par récepteurs artificiels activés par ligand de synthèse (Designer Receptor Exclusively Activated by Designer Drugs, DREADD) chez des animaux non stressés.

Sur le plan moléculaire, il serait intéressant de compléter les données transcriptomiques par une évaluation protéique, par exemple par immunohistochimie ou Western blot. Ceci permettrait de déterminer dans quelle mesure les modifications observées au niveau de la transcription se traduisent au niveau de l'expression protéique. L'analyse de l'expression de gènes horloges permettrait également de confirmer l'hypothèse de dérèglements chez les animaux CMS-vulnérables, et d'une potentielle restauration par la Ket chronique. De plus, étant donné l'implication d'une inflammation et neuroinflammation dans la dépression, une quantification de marqueurs tels que IL-6, TNF-alpha, ou

une évaluation de l'activation de la microglie, potentiellement impliqués dans les déficits synaptiques, serait intéressante.

L'inclusion de l'hippocampe ventral, davantage impliqué dans les processus émotionnels, dans l'étude transcriptomique permettrait de mieux caractériser les altérations hippocampiques et potentiellement confirmer l'hypothèse d'une modulation du BDNF plus importante dans cette sous-région. L'étude de l'activité électrophysiologique *in vivo* au sein de l'habenula latérale serait également intéressante étant donné les dysfonctions mises en causes dans la dépression et les mécanismes thérapeutiques de la Ket.

Annexe : Interprétation spéculative des données de puissance spectrale CMS-vulnérable *vs.* NS

Bien qu'aucune conclusion ne puisse être tirée concernant des différences dans la puissance spectrale absolue, les variations en puissance relative entre animaux CMS-vulnérables et témoins suggèrent un profil qui, s'il se confirmait en absolu, resterait biologiquement plausible.

Concernant le cortex prélimbique (PRL), la baisse de puissance dans la bande thêta chez les animaux CMS-vulnérable pourrait refléter des traits de vulnérabilité. En effet, une étude a examiné la puissance spectrale d'animaux WKY avant et après exposition au CMS ; cette étude a constaté, en amont de l'exposition, une moindre puissance thêta dans le PFC de femelles vulnérables, mais pas non-vulnérables au CMS, bien que chez les mâles l'effet n'était pas significatif (Thériault et al., 2021). Une réduction de la puissance thêta dans le mPFC a également été observée dans un modèle de traitement chronique au CORT (Bergosh et al., 2024). De plus, une telle diminution a été décrite dans le PRL de rats mâles exposés à un stress précoce, un facteur fortement impliqué dans la vulnérabilité au stress et le risque de dépression (Reincke & Hanganu-Opatz, 2017). Une diminution de la puissance thêta dans le PRL a été constatée lors de l'exposition à une situation anxiogène, dans les bras ouverts d'un EPM, tandis qu'une augmentation a été observée lors de la sortie des bras ouverts (Jacinto et al., 2016). Néanmoins, une étude a rapporté une augmentation de la puissance thêta dans le mPFC post-CMS, bien qu'évaluée sous anesthésie (Oliveira et al., 2013). Dans l'ensemble, ces résultats suggèrent qu'une moindre puissance thêta dans le PRL pourrait refléter un état d'anxiété. La puissance thêta préfrontale augmente également lors de la locomotion, la prise de décision ou l'engagement dans des tâches, impliquant par exemple la mémoire de travail (O'Neill et al., 2013). Bien que dans le cas présent les enregistrements ont été réalisés au repos, ces résultats pourraient suggérer que les rats CMS-vulnérable sont également moins actifs physiquement et/ou dans un état d'engament cognitivement. La puissance préfrontale alpha, affaiblie chez les animaux CMS, a également été associée à de meilleures performances d'apprentissage dans des tâches comportementales (Hernández-González et al., 2012) et avec un affect positif accru (Burgdorf & Moskal, 2023). Il est intéressant de noter que la puissance alpha dans la BLA est associée à l'encodage d'issues positives telles que l'évitement de chocs électriques (Schaich Borg et al., 2017), et est affaiblie dans un contexte de sevrage généré par l'absence d'amphétamine lors de l'exposition à une chambre dans laquelle un conditionnement à cette substance a été généré (Nukitram et al., 2021). De plus, les traitements anxiolytiques et antidépresseurs peuvent augmenter la puissance alpha dans la BLA (Antonoudiou et al., 2022). Une moindre puissance

alpha dans la BLA pourrait donc refléter un affect négatif. Les animaux CMS-vulnérable ont également présenté une moindre puissance prélimbique dans la bande bêta. Chez des rats mâles exposés à du stress précoce, facteur de vulnérabilité, un tel phénomène a également été constatée. (Reincke & Hanganu-Opatz, 2017). Des altérations dans la bande bêta ont aussi été associées à un état pathologique chez des souris vulnérables dans un modèle de défaite sociale (Hultman et al., 2016). L'augmentation de la puissance gamma dans la BLA, observée chez les animaux vulnérables, pourrait refléter un état d'appréhension et de vigilance accrue (Headley et al., 2021). Il est également intéressant de noter qu'une puissance gamma accrue dans la BLA est associée à des symptômes plus sévères chez les patients TRD (Scangos et al., 2021). L'augmentation de la puissance gamma dans la BLA lors du sommeil REM pourraient refléter une plus forte consolidation de souvenirs aversifs (Mavanji et al., 2003). En effet, la stimulation ou l'inhibition de la puissance gamma renforce ou affaiblie cette consolidation, respectivement (Kanta et al., 2019). L'augmentation de la puissance prélimbique en gamma durant le sommeil NREM durant la phase d'obscurité pourrait traduire un état de sommeil moins profond (Cano et al., 2008), ce qui serait cohérent avec la fragmentation accrue lors de cette période.

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Vincent LOIZEAU

Étude des effets de la kétamine dans un modèle de dépression résistante aux traitements : le rat Wistar-Kyoto soumis au stress léger chronique

Résumé

La dépression touche jusqu'à 13 % de la population, avec un tiers de patients résistants pour lesquels la kétamine constitue une option, bien que son mécanisme reste mal compris. Cette thèse utilise le rat Wistar-Kyoto soumis au stress léger chronique (CMS) comme modèle de dépression résistante, avec deux objectifs : caractériser l'impact du CMS sur divers paramètres comportementaux et physiologiques, et évaluer les effets d'un traitement aigu ou chronique de kétamine. Comportementalement, environ la moitié des animaux montrent une vulnérabilité au CMS (anxiété, coping passif face à un stress). Le CMS perturbe aussi la plasticité synaptique hippocampique, le rythme veille-sommeil, l'activité cérébrale dans le cortex préfrontal et l'amygdale basolatérale, ainsi que l'expression de gènes liés au stress et à la plasticité (ex. *Bdnf*). Ces travaux indiquent un rôle central du BDNF dans le cortex préfrontal et des effets thérapeutiques partiels de la kétamine dépendant du mode de traitement.

Mots clés : Stress chronique, Vulnérabilité, Dépression résistante aux traitements, Kétamine, Plasticité synaptique

Abstract

Depression affects up to 13% of the population, with one-third of patients being resistant, for whom ketamine is an option despite its poorly understood mechanism. This thesis uses chronic mild stress (CMS)-exposed Wistar-Kyoto rat as a model of treatment-resistant depression, with two goals: to characterise the impact of CMS on various behavioural and physiological parameters, and to assess the effects of acute or chronic ketamine treatment. Behaviourally, about half of the animals show vulnerability to CMS (anxiety, passive coping under stress). CMS also disrupts hippocampal synaptic plasticity, sleep–wake rhythms, and brain activity in the prefrontal cortex and basolateral amygdala, as well as the expression of stress- and plasticity-related genes (e.g., *Bdnf*). These findings highlight a central role of BDNF in the prefrontal cortex and partial therapeutic effects of ketamine depending on the treatment regimen.

Keywords: Chronic stress, Vulnerability, Treatment-resistant depression, Ketamine, Synaptic plasticity