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HABITUDES DANS LA RECHUTE :

Role des propriétés discriminatives des drogues d'abus dans l'automatisation des comportements

Membres du Jury

Dr Christelle BAUNEZ, DR2 CNRS (Marseille, France)	Président/Rapporteur
Pr Aldo BADIANI, PU (Rome, Italie)	Rapporteur
Dr Etienne COUTUREAU, CR1 CNRS (Talence, France)	Examinateur
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THESIS

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Subject: Sciences, Technology, Health

Specialisation: Neurosciences

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on 17th December 2012

by

María del Carmen GONZÁLEZ MARÍN

Born: 6th August 1982, Mexico City (Mexico)

HABITS IN RELAPSE:

Role of the discriminative stimulus properties of drugs of abuse in behavioral automatisms

Members of the Jury

Dr Christelle BAUNEZ, DR2 CNRS (Marseille, France)	President/Reviewer
Pr Aldo BADIANI, PU (Rome, Italy)	Reviewer
Dr Etienne COUTUREAU, CR1 CNRS (Talence, France)	Examiner
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RÉSUMÉ

L'addiction est une pathologie chronique caractérisée par de fréquentes rechutes même après des périodes prolongées d'abstinence. Chez le toxicomane, il a été démontré que la réexposition à la drogue contribue à précipiter le rétablissement d'une consommation compulsive. Chez l'animal de laboratoire, la réexposition à la drogue induit la réinstallation d'un comportement éteint de recherche de drogue même après une longue période d'abstinence. Pourtant, les mécanismes intimes par lesquels la réexposition à la drogue entraîne cette réinstallation restent mal connus. L'objectif de ce travail était de déterminer les processus comportementaux impliqués dans la réinstallation induite par la réexposition à la cocaïne, l'héroïne et la nicotine.

Question 1 :

Les propriétés de stimulus discriminatif de l'héroïne et de la nicotine sont-elles différentes de celles de la cocaïne ?

De précédents travaux réalisés au sein de notre équipe ont montré que les propriétés de stimulus discriminatif de la cocaïne jouent un rôle essentiel dans la réinstallation d'une réponse éteinte pour un renforçateur alimentaire (pastilles de nourriture). Afin d'évaluer si cette propriété est une caractéristique commune à toutes les drogues d'abus, nous avons comparé la cocaïne à d'autres drogues d'abus (héroïne et nicotine), et étendu l'étude à un autre type de renforçateur (solution de sucrose à 5%).

Notre objectif est de répondre à cette question en deux parties. Tout d'abord, la première partie de ce chapitre présente l'analyse de données obtenues au cours d'une expérience réalisée préalablement dans notre laboratoire. Cette expérience a eu pour but de comparer l'implication des propriétés de stimulus discriminatif de l'héroïne et de la cocaïne dans la réinstallation d'une réponse d'appui sur un levier pour une solution de sucrose après une procédure d'extinction. La deuxième partie de ce chapitre étudie l'implication des propriétés de stimulus discriminatif de la nicotine et de la cocaïne dans la réinstallation d'une réponse d'appui sur un levier pour une solution de sucrose.

Etude des propriétés de stimulus discriminatif de la cocaïne et de l'héroïne dans la réinstallation.

Les rats ont été exposés de manière identique à deux environnements différents (cages opérantes et cages individuelles) pendant 2 sessions journalières de 30 minutes chacune, tout au long de l'expérience. Durant la phase d'acquisition, les rats ont été entraînés à appuyer sur un des deux leviers (le levier actif) pour une solution de sucrose (5%), selon un programme de renforcement en FR-5, jusqu'à ce qu'ils aient atteint un niveau de 100 appuis par session.

Puis, au cours de la phase de conditionnement, les rats ont été répartis en différents groupes, "inside" et "outside". Les animaux des groupes "inside" ont reçu une injection périphérique non contingente de cocaïne (10 mg/kg; i.p.), d'héroïne (0.25 mg/kg; s.c) ou de NaCl (1 ml/kg; i.p.) avant d'être placés dans les cages opérantes (« inside » car les animaux sont sous l'effet de la drogue uniquement *à l'intérieur* des cages opérantes), alors que les animaux du groupe « outside » ont reçu soit de la cocaïne (10 mg/kg; i.p.), soit de l'héroïne (0.25 mg/kg; s.c.), soit du NaCl (1 ml/kg; i.p.) avant d'être placés dans les cages

individuelles, où ni sucrose ni levier n'étaient présents (nommé « outside » car les animaux sont sous l'effet de la drogue uniquement *à l'extérieur* des cages opérantes). Ainsi, les propriétés de stimulus discriminatif de la cocaïne et de l'héroïne pourront être associées aux réponses opérantes pour les groupes « inside », mais pas pour les groupes « outside ». Pour égaliser l'histoire pharmacologique et environnementale entre les groupes, les groupes « inside » ont également reçu des injections de NaCl dans les cages individuelles, et les groupes « outside » ont également reçu des injections de NaCl dans les cages opérantes.

Après 10 jours de traitement à la drogue, les rats ont été soumis à une période d'extinction (14 jours), pendant laquelle les appuis sur le levier ne conduisaient plus à la distribution de sucrose, et les injections de drogues étaient remplacées par des injections de NaCl. Au cours de cette période, les rats ont continué à être exposés aux deux environnements. Les sessions d'extinction ont été poursuivies jusqu'à ce que le nombre d'appuis sur le levier atteigne un niveau inférieur à 10 appuis par session, niveau auquel le comportement de recherche de sucrose a été considéré comme éteint. La réinstallation d'un comportement de recherche de sucrose a alors été quantifié (toujours en conditions d'extinction) lorsqu'une administration non contingente de cocaïne (10 mg/kg; i.p.) et d'héroïne (0.25 mg/kg; s.c.) a été réalisée chez chacun des groupes « inside » et « outside » traité avec la cocaïne, l'héroïne ou du NaCl. Chaque test de réinstallation induite par la drogue a été réalisé sur deux jours : un jour NaCl et un jour drogue. Enfin, tous les groupes (cocaïne, héroïne et NaCl) pour chaque condition (« inside » et « outside ») ont été testés sans distribution de sucrose, et avec distribution non contingente de sucrose dans les cages opérantes, sur deux jours consécutifs.

Cette expérience a démontré que :

1. Les injections non contingentes de cocaïne réinstallent les appuis sur le levier qui délivrait au préalable du sucrose uniquement chez les animaux « inside ». C'est-à-dire uniquement chez les animaux qui réalisaient la réponse instrumentale pour obtenir du sucrose sous l'influence de la cocaïne.
2. Les injections non contingentes d'héroïne ne réinstallent pas les appuis sur le levier associé à la présentation de sucrose, ni chez les rats prétraités à la cocaïne, ni chez ceux prétraités à l'héroïne.
3. La distribution non-contingente de sucrose réinstalle les appuis sur le levier associé à la présentation de sucrose chez tous les animaux, indépendamment du traitement reçu (cocaïne, héroïne ou NaCl) et de l'environnement où la drogue a été expérimentée (« inside » ou « outside »), indiquant qu'un comportement de recherche de sucrose peut être induit efficacement chez tous les groupes lorsque le renforcateur lui-même est réexperimenté.

Ces résultats sont identiques à ceux obtenus par Ronald Keiflin au laboratoire (Keiflin et al. 2008a) - concernant la cocaïne - et les étendent à un autre type de renforçateur naturel (solution de sucrose à 5%). Ces résultats indiquent aussi que le rôle joué par les propriétés de stimulus discriminatif de ces drogues dans la réinstallation peut être essentiel pour certaines drogues d'abus, telles que la cocaïne, mais pas pour d'autres, telles que l'héroïne.

Etude des propriétés de stimulus discriminatif de la cocaïne et de la nicotine dans la réinstallation.

Nous avons montré que les propriétés de stimulus discriminatif de la cocaïne jouent un rôle essentiel dans la réinstallation. Cependant, ceci ne s'étend pas à toutes les drogues d'abus, puisque nos résultats montrent que les propriétés discriminatif de l'héroïne ne joue pas un tel rôle. Ceci nous amène à nous demander si la cocaïne est unique par rapport aux autres drogues d'abus, en ce qui concerne l'implication de ses propriétés de stimulus discriminatif dans la réinstallation. La seconde expérience de ma thèse a donc consisté à comparer cette fois le rôle des propriétés de stimulus discriminatif de la cocaïne avec celle de la nicotine dans la réinstallation d'un comportement d'appui dirigé vers du sucrose.

Le protocole expérimental était presque identique à celui décrit précédemment, la seule différence étant que la nicotine (0.4 mg/kg; s.c.) a été testée à la place de l'héroïne.

Cette expérience a démontré que :

1. Les injections non contingentes de cocaïne réinstallent les appuis sur le levier pour le sucrose chez les rats ayant préalablement appris à appuyer sur le levier en étant sous les effets de la cocaïne, mais pas chez les rats ayant appris sous les effets de la nicotine.
2. Les injections non contingentes de nicotine réinstallent les appuis sur le levier pour le sucrose chez les rats ayant préalablement appris à appuyer sur le levier en étant sous les effets de la nicotine, mais pas chez les rats ayant appris sous les effets de la cocaïne.
3. La distribution non contingente de sucrose réinstalle les appuis sur le levier pour le sucrose chez tous les groupes, indépendamment du traitement reçu (cocaïne, nicotine ou NaCl) et de l'environnement dans lequel la drogue a été expérimentée (« inside » ou « outside »).

Ces résultats indiquent que, lorsque l'on compare différentes drogues d'abus, les propriétés de stimulus discriminatif de la cocaïne et de la nicotine jouent un rôle significatif dans la réinstallation d'un comportement opérant éteint, non dirigé vers la drogue elle-même mais vers un renforçateur naturel (solution de sucrose à 5%). Ceci indique que les animaux utilisent les propriétés de stimulus discriminant des drogues pour guider leur comportement.

En conclusion, les résultats de ces deux expériences indiquent que le rôle des propriétés de stimulus discriminatif des drogues dans la rechute peut être crucial pour certaines drogues d'abus, telles que la cocaïne et la nicotine, mais par pour d'autres comme l'héroïne. Cette dissociation suggère que des processus neuropsychologiques différents pourraient sous-tendre le processus de rechute. Mieux comprendre comment les propriétés de stimulus discriminant des drogues d'abus mènent à la réinstallation aiderait à comprendre le processus de rechute, et pourrait fournir de nouvelles directions pour les thérapies de prévention de la rechute.

Question 2 :

Les propriétés de stimulus discriminatif de la cocaine et de la nicotine entrainent-t-elles la formation d'un comportement automatique ?

Réinstallation pour une récompense alimentaire (pastilles de nourriture) induite par la cocaïne: Comportement dirigé vers un but ou habitude ?

Dans les expériences précédentes, il a été montré que la réexposition à la cocaïne et à la nicotine réinstallait un comportement éteint de recherche de sucrose préalablement acquis sous l'influence de la cocaïne et de la nicotine, respectivement.

En utilisant une procédure de dévaluation, des données préliminaires obtenues dans notre laboratoire ont montré que la réexposition à la cocaïne réinstallait un comportement éteint de recherche de sucrose préalablement acquis sous l'influence de la cocaïne, même lorsque la récompense alimentaire (pastilles de nourriture) a été rendue aversive. Cette propriété semble être propre à la cocaïne, étant donné que la réinstallation du comportement de recherche de nourriture induite par la nourriture elle-même reste sensible à la valeur de la récompense. Ainsi, le but de l'expérience suivante était de répliquer les données préliminaires préalablement obtenues dans notre laboratoire, c'est-à-dire en utilisant des pastilles de nourriture (au lieu d'une solution de sucrose comme dans les précédentes expériences de cette thèse) et de la cocaïne.

Les rats ont été exposés de manière identique à deux environnements différents (cages opérantes et cages individuelles) pendant 2 sessions journalières de 30 minutes chacune, tout au long de l'expérience. Durant la phase d'acquisition, les rats ont été entraînés à appuyer sur un des deux leviers (le levier actif) pour un maximum de 70 pastilles de nourriture, selon un programme de renforcement en FR-5, jusqu'à ce qu'un maximum de pastilles ait été obtenu.

Puis, au cours de la phase de conditionnement, les rats ont été répartis en différents groupes. Les animaux du groupe « inside » ont reçu une injection périphérique non contingente de cocaïne (10 mg/kg; i.p.) avant d'être placés dans les cages opérantes, alors que les animaux du groupe « outside » ont reçu de la cocaïne (10 mg/kg; i.p.) avant d'être placés dans les cages individuelles, où ni sucrose ni levier n'étaient présents. Tout comme dans les expériences précédentes, cette méthode a entraîné une association (groupe « inside ») ou une indépendance (groupe « outside ») entre les propriétés de stimulus discriminant de la cocaïne et les réponses opérantes. Pour égaliser l'exposition aux injections, les animaux du groupe « inside » ont également reçu des injections de NaCl dans les cages individuelles, et ceux du groupe « outside » ont également reçu des injections de NaCl dans les cages opérantes.

Après 10 jours de traitement à la cocaïne, les rats ont été soumis à une période d'extinction pendant laquelle les appuis sur le levier ne conduisaient plus à la distribution de pastilles, et les injections de cocaïne étaient remplacées par des injections de NaCl. Au cours de cette période, les rats ont continué à être exposés aux deux environnements. Les sessions d'extinction ont été poursuivies jusqu'à ce que le nombre d'appuis sur le levier ait atteint un niveau inférieur à 10 appuis par session. Après l'extinction du comportement opérant, chacun des deux groupes (« inside » et « outside ») a été divisé en deux groupes : 1) dévalué et 2) non dévalué. La récompense alimentaire a été dévaluée en appariant les

pastilles obtenues au cours de l'apprentissage instrumental (100 pastilles dans un bol placé dans chaque cage individuelle pendant 10 minutes) avec des injections de LiCl induisant des malaises gastriques (0.3 M; 5 ml/kg; i.p.) immédiatement après les 10 minutes d'exposition aux pastilles de nourriture. Un jour sur deux, les animaux pour lesquels la récompense alimentaire n'a pas été dévaluée ont reçu des présentations non appariées des mêmes pastilles avec des injections de LiCl. Un total de quatre appariements nourriture- LiCl ou Ø-LiCl ont été réalisés, séparés de 48h.

Cette procédure garantit que tous les rats ont reçu la même exposition aux pastilles de nourriture et aux malaises gastriques induits par le LiCl, mais que seul le groupe dévalué a pu associer la consommation de pastilles de nourriture avec les malaises gastriques. La réinstallation du comportement instrumental de recherche de nourriture en conditions d'extinction a été testée par des injections non contingentes de cocaïne (10 mg/kg; i.p.) et de nicotine (0.4 mg/kg; s.c.) chez tous les groupes (« inside » et « outside ») dans les deux conditions (dévalué et non dévalué). Chaque test de réinstallation induite par la drogue a été réalisé sur deux jours : un jour NaCl et un jour drogue. Enfin, les deux groupes (« inside » and « outside ») dans les deux conditions (dévalué et non dévalué) ont été testés sans distribution de pastilles, et avec distribution non contingente de pastilles, sur deux jours consécutifs.

Cette expérience a démontré que :

1. Les propriétés de stimulus discriminatif de la cocaïne jouent un rôle significatif dans la réinstallation d'un comportement opérant éteint, non dirigé vers la drogue elle-même, mais vers un renforçateur naturel (pastilles de nourriture). Ceci indique que les animaux utilisent les propriétés de stimulus discriminatif de la cocaïne pour guider leur comportement (réplication des données préliminaires de notre laboratoire).
2. La réinstallation par la cocaïne n'est pas dirigée vers un but, c'est-à-dire qu'elle ne dépend pas de la représentation du renforçateur, puisque la cocaïne a induit la réinstallation malgré la dévaluation de la récompense. Par conséquent, la cocaïne contrôle l'expression de comportements automatiques, de type habituel.
3. Les injections non contingentes de nicotine ne réinstallent pas les appuis sur le levier chez les rats prétraités à la cocaïne.
4. Quand le renforçateur (pastilles de nourriture) est présenté de manière non contingente, un comportement de recherche de nourriture de type habituel (insensible à la dévaluation) est réinstallé, puisque les deux groupes (dévalué et non dévalué) réinstallent les appuis sur le levier, indépendamment de la dévaluation préalable de la nourriture et de l'environnement dans lequel les effets de la cocaïne ont été expérimentés (« inside » ou « outside », soit à l'intérieur ou à l'extérieur des cages opérantes).

Réinstallation pour une récompense alimentaire (pastilles de nourriture) induite par la nicotine: Comportement dirigé vers un but ou habitude ?

Nous avons montré, en utilisant une procédure de dévaluation, que la réexposition à la cocaïne réinstallait un comportement éteint de recherche de nourriture préalablement acquis sous l'influence de la cocaïne, même lorsque la récompense alimentaire (pastilles de nourriture) a été rendue aversive. Observerons-nous la meme chose avec la nicotine ? Ainsi, le but de cette expérience a été de savoir si la réexposition à la nicotine réinstallait un

comportement éteint de recherche de nourriture préalablement acquis sous l'influence de la nicotine, même lorsque la récompense (dans ce cas des pastilles de nourriture) a été rendue aversive par une procédure de dévaluation.

Le protocole expérimental était identique à celui décrit précédemment, la seule différence étant que la nicotine (0.4 mg/kg; s.c.) a été testée à la place de la cocaïne.

Cette expérience a démontré que :

1. Les propriétés de stimulus discriminatif de la nicotine jouent un rôle significatif dans la réinstallation d'un comportement opérant éteint, non dirigé vers la nicotine elle-même, mais vers un renforçateur naturel (pastilles de nourriture). Ceci indique que les animaux utilisent les propriétés de stimulus discriminatif de la nicotine pour guider leur comportement (réplication des données précedentes).
2. La réinstallation par la nicotine n'est pas dirigée vers un but, c'est-à-dire qu'elle ne dépend pas de la représentation du renforçateur, puisque la nicotine a induit la réinstallation malgré la dévaluation de la récompense. Par conséquent, la nicotine contrôle l'expression de comportements automatiques, de type habituels.
3. Les injections non contingentes de cocaïne réinstallent les appuis sur le levier chez les rats prétraités à la nicotine.
4. Quand le renforçateur (pastilles de nourriture) est présenté de manière non contingente, un comportement de recherche de nourriture dirigé vers un but (sensible à la dévaluation) est réinstallé, puisque seuls les groupes non dévalués ont réinstallé les appuis sur le levier, indépendamment de l'environnement dans lequel les effets de la nicotine ont été expérimentés (« inside » ou « outside », soit à l'intérieur ou à l'extérieur des cages opérantes). Ceci indique alors que la réinstallation peut être efficacement induite dans les deux groupes (« inside » out « outside »).

Réinstallation pour une récompense alimentaire (pastilles de nourriture) induite par le contexte: Comportement dirigé vers un but ou habitude ?

Nous avons montré, en utilisant une procédure de dévaluation, que la réexposition à la cocaïne et à la nicotine réinstallait un comportement éteint de recherche de nourriture préalablement acquis sous l'influence de la cocaïne et de la nicotine respectivement, même lorsque la récompense alimentaire (pastilles de nourriture) a été rendue aversive. Afin de déterminer si cette propriété est propre aux drogues d'abus, une analyse a été effectuée à partir de données obtenues au cours d'une troisième expérience réalisée au sein de notre laboratoire, dans laquelle le stimulus « drogue » a été remplacé par un stimulus discriminant contextuel (DC+) (texture du sol de la cage + odeur). Le but de cette expérience était de savoir si la réexposition au DC+ réinstallait un comportement éteint de recherche de nourriture, préalablement acquis en présence de ce DC+, même lorsque la récompense (des pastilles de nourriture dans ce cas) a été rendue aversive par une procédure de dévaluation.

Le protocole expérimental était presque identique à celui décrit pour l'expérience précédente, la seule différence étant qu'un DC+ tactile et olfactif a été testé à la place de la nicotine.

Les rats ont été exposés de manière identique à deux environnements différents (cages opérantes et cages individuelles) pendant 2 sessions journalières de 30 minutes chacune, tout au long de l'expérience. Durant la phase d'acquisition, les rats ont été entraînés à

appuyer sur un des deux leviers (le levier actif) pour un maximum de 70 pastilles de nourriture, selon un programme de renforcement en FR-5, jusqu'à ce qu'un maximum de pastilles ait été obtenu.

Puis, au cours de la phase de conditionnement, les rats ont été répartis en différents groupes : les groupes « DC+ » et « Ø ». Pour le groupe « DC+ », les cages opérantes ont été adaptées avec un stimulus tactile et olfactif (un sol semi-rugueux avec de la sciure parfumée à l'anis éparpillée dessus), alors que, pour le groupe « Ø », les animaux ont expérimenté le même stimulus tactile et olfactif dans les cages individuelles, ceci garantissant que tous les groupes ont reçu une exposition identique au stimulus tactile et olfactif, soit dans les cages opérantes (groupe « DC+ »), soit dans les cages individuelles (groupe « Ø »). Tout comme dans les expériences précédentes, cette méthode a entraîné une association (groupe « DC+ ») ou une indépendance (groupe « Ø ») entre le stimulus tactile et olfactif et les réponses opérantes pour les pastilles de nourriture.

Après 10 jours d'entraînement au contexte discriminant, les rats ont été soumis à une période d'extinction pendant laquelle les appuis sur le levier ne conduisaient plus à la distribution de pastilles, et le stimulus tactile et olfactif était enlevé des cages individuelles et des cages opérantes. Au cours de cette période, les rats ont continué à être exposés aux deux environnements. Les sessions d'extinction ont continué jusqu'à ce que le nombre d'appuis sur le levier ait atteint un niveau inférieur à 10 appuis par session. Après l'extinction du comportement opérant, chacun des deux groupes (« DC+ » et « Ø ») a été divisé en deux groupes : 1) dévalué et 2) non dévalué. La récompense alimentaire a été dévaluée en appariant les pastilles obtenues au cours de l'apprentissage instrumental (100 pastilles dans un bol placé dans chaque cage individuelle pendant 10 minutes) avec des injections de LiCl induisant des malaises gastriques (0.3 M; 5 ml/kg; i.p.) immédiatement après les 10 minutes d'exposition aux pastilles de nourriture. Un jour sur deux, les animaux pour lesquels la récompense alimentaire n'a pas été dévaluée ont reçu des présentations non appariées des mêmes pastilles avec des injections de LiCl. Un total de trois appariements nourriture-LiCl ou Ø-LiCl ont été réalisés, séparés de 48h. Cette procédure garantit que tous les rats ont reçu la même exposition aux pastilles de nourriture et aux malaises gastriques induits par le LiCl, mais que seul le groupe dévalué a pu associer la consommation de pastilles de nourriture avec les malaises gastriques. La réinstallation du comportement instrumental de recherche de nourriture en conditions d'extinction a été testée par la réexposition au DC+ chez tous les groupes (« DC+ » et « Ø ») et dans les deux conditions (dévalué et non dévalué). Ce test de réinstallation induite par le contexte a été réalisé sur deux jours : un jour contrôle et un jour test. Enfin, les deux groupes (« DC+ » et « Ø ») dans les deux conditions (dévalué et non dévalué) ont été testés sans distribution de pastilles, et avec distribution non contingente de pastilles, sur deux jours consécutifs.

Cette expérience a démontré que :

1. Un stimulus discriminatif tactile et olfactif peut indure la réinstallation d'un comportement opérant éteint, dirigé vers un renforçateur naturel (pastilles de nourriture), indiquant que les animaux utilisent les indices contextuels discriminatifs pour guider leur comportement.
2. La réinstallation par un stimulus discriminatif tactile et olfactif est dirigée vers un but, c'est-à-dire qu'elle dépend de la représentation du renforçateur, puisque le stimulus

discriminatif tactile et olfactif n'a induit la réinstallation que chez le groupe non dévalué.

3. Quand le renforçateur (pastilles de nourriture) est présenté de manière non contingente, un comportement de recherche de nourriture dirigé vers un but (sensible à la dévaluation) est réinstallé, puisque seuls les groupes non dévalués ont réinstallé les appuis sur le levier, indépendamment de l'environnement dans lequel ils ont expérimenté le stimulus tactile et olfactif. Ceci indique alors que la réinstallation peut être efficacement induite dans les deux groupes (« DC+ » ou « Ø »).

En conclusion, les résultats indiquent que la réexposition à la cocaïne et à la nicotine réinstalle des comportements habituels et liés à la drogue, indépendamment de la valeur actuelle de la récompense, alors que la réexposition à un stimulus discriminatif tactile et olfactif réinstalle des comportements dirigés vers un but, dépendants de la valeur actuelle de la récompense. Ces habitudes induites par la cocaïne et la nicotine pourraient expliquer la perte de contrôle induite par la réexposition à la cocaïne et à la nicotine chez des toxicomanes, et la rapide reprise du comportement compulsif de prise de drogue, malgré la connaissance des conséquences néfastes qui en découlent.

Question 3 :

Un prétraitement à différentes drogues d'abus entraîne-t-il l'accélération de la formation d'un comportement habituel ?

L'exposition répétée à certaines drogues d'abus, telles que la cocaïne et l'amphétamine semble conduire à une progression plus rapide d'un comportement dirigé vers un but vers des réponses de type habitudes, caractérisées par une insensibilité aux modifications de la valeur de la récompense.

A ce jour, l'effet de la drogue sur l'accélération de l'automatisation comportementale en conditionnement opérant a été démontré uniquement après une sensibilisation à l'amphétamine.

Afin de pouvoir réellement attribuer à l'automatisation comportementale un rôle clé dans la rechute et donc dans l'addiction, il convient tout d'abord de vérifier si ces deux psychostimulants, à savoir l'amphétamine et la cocaïne, agissent de la même manière sur un comportement opérant, à travers les neuroadaptations qu'ils induisent au sein du système nerveux. Malgré leurs modes d'actions sensiblement différents au niveau du système nerveux, la cocaïne et l'amphétamine sont des drogues d'abus qui entraînent une augmentation de la neurotransmission dopaminergique : elles pourraient donc avoir un mécanisme commun dans le processus d'automatisation comportementale.

Nos travaux ont eu pour but d'étudier et de comparer l'effet d'un prétraitement à la cocaïne et à l'amphétamine sur la capacité des animaux à produire des actions dirigées vers un but, ceci afin de déterminer si la sensibilisation produite par ces drogues accélère ou non la transformation d'un comportement dirigé vers un but en un comportement automatisé.

Dans une première expérience, nous avons étudié l'effet d'un prétraitement à la cocaïne sur les performances de rats lors d'un conditionnement opérant pour obtenir une pastille alimentaire. Des animaux ont été prétraités avec différentes doses de cocaïne avant d'être soumis à un entraînement d'une durée limitée, puis, suite à la dévaluation du renforçateur à

l'aide de chlorure de lithium, nous avons comparé leurs performances instrumentales à celles d'animaux contrôles, prétraités avec du NaCl. Dans une seconde expérience, des animaux ont été prétraités à l'amphétamine ou à la cocaïne avant d'être soumis à un conditionnement opérant associant un appui sur le levier à l'obtention de nourriture. Afin de savoir si ces drogues accélèrent ou non le processus d'automatisation comportementale, nous avons étudié l'effet d'un prétraitement aux drogues sur la réponse instrumentale à différents moments de l'apprentissage instrumental : après une période courte d'entraînement puis après une période prolongée d'entraînement. Dans le but de pouvoir répliquer la dévaluation instrumentale pour étudier et comparer les performances des animaux à ces différents moments de l'apprentissage, la dévaluation a été réalisée par satiété spécifique, permettant ainsi la répétition de ce type de dévaluation.

Ces expériences ont démontré qu'un prétraitement à la cocaïne (30mg/kg) favoriserait l'apparition de comportements automatiques (première expérience) bien qu'il faille souligner que l'effet obtyen u est très ténu. La deuxième expérience n'a pas permis de confirmer ces premiers résultats, et ne nous permet donc pas de vérifier l'hypothèse selon laquelle une exposition répétée à certaines drogues d'abus, telles que la cocaïne et l'amphétamine, conduirait à une progression plus rapide d'un comportement dirigé vers un but vers des réponses de type habitudes.

Conclusion

L'objectif de cette thèse était d'étudier les processus comportementaux impliqués dans la réinstallation induite par des drogues d'abus. Au vu des résultats obtenus, nous pouvons dire que la réinstallation induite par la cocaïne et la nicotine implique à la fois les propriétés de stimulus discriminatif de ces drogues, et les associations stimulus-réponse acquises sous les effets de la drogue. Afin d'attribuer un rôle clé aux automatismes comportementaux dans la rechute et dans l'addiction aux drogues, il est nécessaire de savoir si la formation des habitudes peut être généralisée aux autres drogues d'abus. Notre travail représente une première étape dans la comparaison de processus automatiques produits par les différentes drogues d'abus. Si la formation des habitudes est généralisée aux autres drogues d'abus, nous pourrons considérer que la reprise du comportement de recherche de drogue pourrait en partie être sous le contrôle de processus automatiques generés par les propriétés de stimulus discriminatif des drogues. Cela pourrait expliquer la forte probabilité de rechute durant l'abstinence, malgré la connaissance des conséquences néfastes qui en découlent.

ABSTRACT

Drug addiction can be considered as a chronic brain disease with recurrent relapse during abstinence periods which remains the major problem for the treatment of drug addiction. Using an animal model of drug relapse, it has been shown that a rodent can reinstate a drug-seeking behavior when re-exposed to the drug itself, drug associated cues or stress. In our research group, we assessed the relative contribution of the different properties of cocaine, heroin and nicotine (incentive, discriminative and reinforcing) in food-seeking reinstatement, and in order to dissociate the discriminative from the reinforcing properties, rats were trained to self administered a non-drug reward (food). We found that: 1) Cocaine and nicotine act as internal stimuli that acquires discriminative control over behavior, since cocaine and nicotine, but not heroin, can reinstate an extinguished food-seeking behavior when this behavior has been previously performed under the effects of cocaine and nicotine respectively. 2) Cocaine- and nicotine-induced reinstatement is independent of the current value of the outcome, which indicates that cocaine and nicotine control the activation of automatic, drug-related habitual behaviors. Then, in order to identify the way drugs of abuse lead to the formation of habits, we also examined the effects of cocaine sensitization at different stages of instrumental training for a food reward after outcome devaluation. We found that, globally, cocaine sensitization does not promote the development of habit-based behaviors. This series of experiments represent a first step in the comparison of automatic processes produced by cocaine and nicotine. If the activation of automatic, habit-based behaviors can be generalized to other drugs of abuse, we could consider that relapse to drug-seeking and drug-taking is partly under the control of automatic processes, which could explain the high probability of relapse, even after extended periods of abstinence and despite the knowledge of the adverse consequences.

Keywords: cocaine, heroin, nicotine, discriminative stimulus properties, habits, reinstatement, outcome devaluation.

PUBLICATIONS AND POSTER PRESENTATIONS

PUBLICATIONS

González-Marín, M.C., Vouillac, C., Keiflin, R. and Cador, M. The discriminative stimulus properties of cocaine and nicotine, but not heroin, play a critical role in reinstatement. *(submitted)*

González-Marín, M.C., Keiflin, R., Vouillac, C. and Cador, M. The discriminative stimulus properties of cocaine and nicotine reinstate an automatic, habit-based food-seeking behavior. *(in preparation)*

POSTER PRESENTATIONS

González-Marín, M.C. and Cador, M. (2012). The discriminative stimulus properties of nicotine reinstate an automatic, habit-based food-seeking behavior. Poster presented at the 2012 meeting of the Society for Neuroscience, New Orleans, USA, 13-17 October 2012. *[Aviesan-ITMO « Neurosciences, Sciences Cognitives, Neurologie, Psychiatrie » Travel Financial Support to assist to the Meeting of Society for Neuroscience (2012)]*

Cador, M., Vouillac, C., Jean-Charles, G., Keiflin, R. and **González-Marín, M.C.** (2012). The discriminative stimulus properties of cocaine and nicotine, but not heroin, play a key role in reinstatement in rats. Poster presented at the 2012 meeting of the Federation of European Neuroscience Societies, Barcelona, Spain, 14-18 July 2012.

González-Marín, M.C. and Cador, M. (2012). Nicotine discriminative stimulus properties reinstate an automatic, habit-based behavior. Poster presented at the 2012 Journée Scientifique de l'Ecole Doctorale des Sciences de la Vie et de la Santé (Université Bordeaux Segalen), Arcachon, France, 28th March 2012.

González-Marín, M.C., Vouillac, C., Jean-Charles, G., Keiflin, R. and Cador, M. (2011). The discriminative stimulus properties of cocaine and nicotine, but not heroin, play a key role in reinstatement in rats. Poster presented at the 2011 meeting of the European Behavioral Pharmacology Society, Amsterdam, the Netherlands, 26-29 August 2011. *(Bursary from the EBPS)*

González-Marín, M.C., Vouillac, C., Jean-Charles, G., Keiflin, R. and Cador, M. (2011). The discriminative stimulus properties of cocaine and nicotine, but not heroin, play a key role in reinstatement in rats. Poster presented at the 2011 Journée Scientifique de l'Ecole Doctorale des Sciences de la Vie et de la Santé (Université Bordeaux Segalen), Arcachon, France, 7th April 2011.

LIST OF FIGURES

LIST OF ABBREVIATIONS

MOR	μ-opioid receptor
A-O	Action-outcome
BLA	Basolateral amygdala
BNST	Bed nucleus of the stria terminalis
CeA	Central amygdala
CNS	Central nervous system
CPP	Conditioned place preference
CS	Conditioned stimulus
CRF	Corticotropin-releasing factor
THC	Delta-9-tetrahydrocannabinol
DC+	Discriminative contextual stimulus
DA	Dopamine
DAT	Dopamine transporter
DD	Drug discrimination
FI	Fixed-interval
FR	Fixed-ratio
FT	Fixed-time
GABA	Gamma-aminobutyric acid
HCl	Hydrochloride
ICSS	Intracranial self-stimulation
i.p.	Intraperitoneally
IV	Intravenous
LiCl	Lithium chloride
mPFC	Medial prefrontal cortex
6 MAM	Metabolite 6-monoacetylmorphine
MDMA	Methylenedioxymetamphetamine

nAChRs	Nicotinic acetylcholine receptors
NA	Noradrenaline
LTg	Noradrenaline cell groups of the lateral tegmental nuclei
NAc	Nucleus accumbens
PFC	Prefrontal cortex
RT	Random-time
5-HT	Serotonin
NaCl	Sodium chloride
SSD	Specific satiety devaluation
S-R	Stimulus-response
s.c.	Subcutaneously
DSM-IV	The diagnostic and statistical manual of mental disorders, 4th. edition
DSM-5	The diagnostic and statistical manual of mental disorders, 5th. edition
ICD-10	The international statistical classification of diseases and related health problems
US	Unconditioned stimulus
VI	Variable-interval
VP	Ventral pallidum
VTA	Ventral tegmental area
GHB	ϒ-hydroxybutyrate

Contents

Introduction

1. Definition of Drug Addiction

At some time in their life, most people try a potentially addictive drug (e.g. alcohol). However, few become addicts (Robinson and Berridge 2008).

The Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) (American Psychiatric Association, 1994) and the International Statistical Classification of Diseases and Related Health Problems (ICD-10) (World Health Organization, 1992) distinguish between three levels of substance use:

1) occasional, controlled or social use;
2) drug abuse or harmful use;
3) drug addiction.

Therefore, drug addiction is more than mere drug use. Drug addiction, also known as substance dependence (DSM-IV, 1994), is a chronically relapsing disorder characterized by:

- compulsion to seek and take the drug, which occupies an inordinate amount of an individual's time and thoughts and persists despite adverse consequences (Hasin et al. 2006);
- loss of control in limiting intake;
- emergence of a negative emotional state (e.g. dysphoria, anxiety, irritability) reflecting a motivational withdrawal syndrome when access to the drug is prevented (Koob and Le Moal 1997).

The DSM-IV also emphasizes compulsive use as a criterion for addiction. Therefore, individuals suffering from drug addiction fail to limit or stop their drug use, despite knowledge of the adverse consequences on their health and social life (see Figure 1). The fifth edition of the DSM (DSM-5), planned for release in 2013, is likely to have this terminology revisited. Under consideration is a transition from the abuse/dependence terminology. At the moment, abuse is seen as an early, or less hazardous, form of the disease, compared to the dependence criteria. However, the American Psychiatric Association's definition of *dependence*, does not mean that physiological dependence is present but rather means that a disease state is present, one that most would likely refer to as an addicted state.

Drug addiction has also been conceptualized as a disorder that involves elements of impulsivity and compulsivity with varying contributions of positive and negative reinforcement (Koob and Le Moal 2008a), which generate an addiction cycle comprising three stages:

1) preoccupation/anticipation (craving);
2) binge/intoxication (most pronounced with psychostimulants and ethanol but not present with nicotine);
3) withdrawal/negative affect (most pronounced with opioids and alcohol but common to all drugs of abuse) (see Figure 2).

Finally, drug addicts also find it difficult to reduce or terminate drug use, even when they desire to do so. Drug addicts are highly vulnerable to relapse even after long abstinence and well after symptoms of withdrawal have disappeared (Robinson and Berridge 2008). **Recurrent relapse remains as the major clinical problem in the treatment of drug addiction.**

DSM-IV Substance Abuse Criteria

Substance abuse is defined as a maladaptive pattern of substance use leading to clinically significant impairment or distress, as manifested by one (or more) of the following, occurring within a 12-month period:

1. Recurrent substance use resulting in a failure to fulfill major role obligations at work, school, or home (such as repeated absences or poor work performance related to substance use; substance-related absences, suspensions, or expulsions from school; or neglect of children or household).
2. Recurrent substance use in situations in which it is physically hazardous (such as driving an automobile or operating a machine when impaired by substance use).
3. Recurrent substance-related legal problems (such as arrests for substance related disorderly conduct).
4. Continued substance use despite having persistent or recurrent social or interpersonal problems caused or exacerbated by the effects of the substance (for example, arguments with spouse about consequences of intoxication and physical fights).

Note: The symptoms for abuse have never met the criteria for dependence for this class of substance. According to the DSM-IV, a person can be abusing a substance or dependent on a substance but not both at the same time.

DSM-IV Substance Dependence Criteria

Substance dependence is defined as a maladaptive pattern of substance use leading to clinically significant impairment or distress, as manifested by three (or more) of the following, occurring any time in the same 12-month period:

1. Tolerance, as defined by either of the following: (a) A need for markedly increased amounts of the substance to achieve intoxication or the desired effect or (b) Markedly diminished effect with continued use of the same amount of the substance.
2. Withdrawal, as manifested by either of the following: (a) The characteristic withdrawal syndrome for the substance or (b) The same (or closely related) substance is taken to relieve or avoid withdrawal symptoms.
3. The substance is often taken in larger amounts or over a longer period than intended.
4. There is a persistent desire or unsuccessful efforts to cut down or control substance use.
5. A great deal of time is spent in activities necessary to obtain the substance, use the substance, or recover from its effects.
6. Important social, occupational, or recreational activities are given up or reduced because of substance use.
7. The substance use is continued despite knowledge of having a persistent physical or psychological problem that is likely to have been caused or exacerbated by the substance (for example, current cocaine use despite recognition of cocaine-induced depression or continued drinking despite recognition that an ulcer was made worse by alcohol consumption).

American Psychiatric Association. 1994. Diagnostic and Statistical Manual of Mental Disorders: DSM-IV. Washington D.C.: American Psychiatric Association. (pp. 181-183)

Figure 1 Official Substance Abuse/Dependence Criteria according to the DSM-IV.

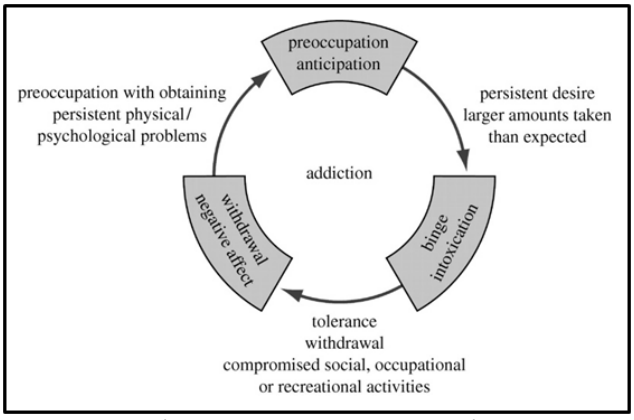

Figure 2 The addiction cycle. Preoccupation/anticipation ("craving"), binge/intoxication and withdrawal/negative affect. Reproduced from Koob and Le Moal (2008b).

2. Theories of Drug Addiction

A variety of different theoretical approaches (neurobiological, psychological, sociocultural, etc.) have been developed to understand the question of why people take drugs. Several influential hypotheses attempt to explain the progression from casual to compulsive patterns of drug use, which occurs during the development of drug addiction. This progression to drug addiction is accompanied by many drug-induced changes in the brain and associated changes in psychological functions (Robinson and Berridge 2003). What follows is a summary of some examples of the main theories of drug addiction from the field of neuroscience that incorporates the ideas previously mentioned. These include:

- The **psychomotor stimulant** view, which suggests that the reinforcing effects of drugs and their addiction liability is due to their ability to induce psychomotor activation (Wise and Bozarth 1987);
- The **hedonic** view indicating that the pleasure experienced by taking a drug and the subsequent unpleasant withdrawal symptoms are the main causes of addiction (Koob et al. 1997; Koob and Le Moal 1997; Solomon and Corbit 1974);
- The **incentive-sensitization** view, which suggests that sensitization of a neural system that attributes incentive salience causes compulsive motivation or "wanting" to take addictive drugs (Robinson and Berridge 1993; 2000);
- The view that addiction is due to **aberrant learning**, especially the development of strong stimulus-response behaviors, or habits (Berke and Hyman 2000; Everitt et al. 2001; O'Brien et al. 1992a; Tiffany 1990).

2.1 Psychomotor Stimulant Theory of Addiction

Wise and Bozarth (1987) argued that all drugs that are positive reinforcers should elicit locomotion. According to this theory, all drugs that have addiction potential (such as amphetamine, cocaine, nicotine, caffeine, opiates, barbiturates, alcohol, benzodiazepines, cannabis and phencyclidine) have psychomotor stimulant actions which are due to the activation of central dopaminergic systems. Thus, the focus of the psychomotor theory of addiction was on the mesolimbic dopamine system being critical for the psychomotor stimulation produced by all drugs of abuse and their reinforcing actions (Bozarth and Wise 1981).

2.2 The Hedonic Allostasis Theory

The explanation for drug addiction in the hedonic allostasis theory is that drugs are taken because they are pleasant; but with repeated use, neuroadaptations produced by the drug lead to tolerance (a decreasing effect with repeated dosing or when larger doses must be administered to produce the same effect) and dependence (appearance of unpleasant withdrawal symptoms upon the cessation of use). Thus, compulsive drug-taking is maintained to avoid unpleasant withdrawal symptoms (Koob et al. 1997; Koob and Le Moal 1997; 2001).

According to Solomon and Corbit (1974), hedonic, affective or emotional states, once initiated, are automatically modulated by the central nervous system (CNS) with mechanisms that reduce the intensity of hedonic feelings. The opponent process theory is defined by two opposing processes: 1)

The *a-process* consists of either positive or negative hedonic responses, which occur shortly after the presentation of a stimulus; correlates closely with the intensity, quality and duration of the reinforcer; and shows tolerance; and 2) By contrast, the *b-process* appears after the *a-process* has terminated and is slow to develop, slow to build up to an asymptote, slow to decay, and gets larger with repeated exposure. Thus, this *b-process* tends to reduce the intensity of the emotional state initially induced by the *a-process*. For example, in the context of drug dependence, Solomon argued that the first few self-administrations of an opiate drug produce a pattern of motivational changes similar to that of the *a-process* or euphoria, followed by a decline in intensity. Then, after the effects of the drug wear off, an opposing, aversive negative emotional state emerges (i. e. the *b-process*).

Based on the opponent process model of addiction, Koob and Le Moal (1997) proposed the hedonic allostasis theory, which postulates that repeated drug use promotes a transition of behavioral processes from an impulsive mode of action, which is driven by pleasure, relief, and gratification (i.e. maintained by positive reinforcement), towards a compulsive mode of action, which is driven by relief of anxiety or stress (i.e. maintained by negative reinforcement).

At the neurobiological level, Koob and colleagues (Koob et al. 1997; Koob and Le Moal 1997; 2001; 2008b) describe the hypothesized existence of a drug state (*a-state*) and a corresponding *a-process*, and a withdrawal state (*b-state*) and corresponding *b-process*. The positive *a-process* is caused by the activation of mesolimbic dopamine projections to the nucleus accumbens and amygdala that mediates the acute reinforcing effects of drugs. Therefore, repeated drug use induces tolerance or downregulation in the mesolimbic dopamine system, decreasing the drug *a-state*. Sudden cessation of drug use causes dopamine (and serotonin) neurotransimission to drop further, below normal levels, for several days at least, resulting in the dysphoric *b-state* of withdrawal. They also suggest that repeated drug use activates an additional *b-process* via the hypothalamic-pituitary-adrenal axis stress system, causing release of corticotrophin-releasing factor (CRF) in the amygdala, as well as other stress responses (Koob et al. 1997; Koob and Le Moal 1997). As a result, addicts who originally take drugs to gain a positive hedonic state are rolled into a predominantly negative hedonic state, which, according to Koob and colleagues, causes the transition to addiction (see Figure 3).

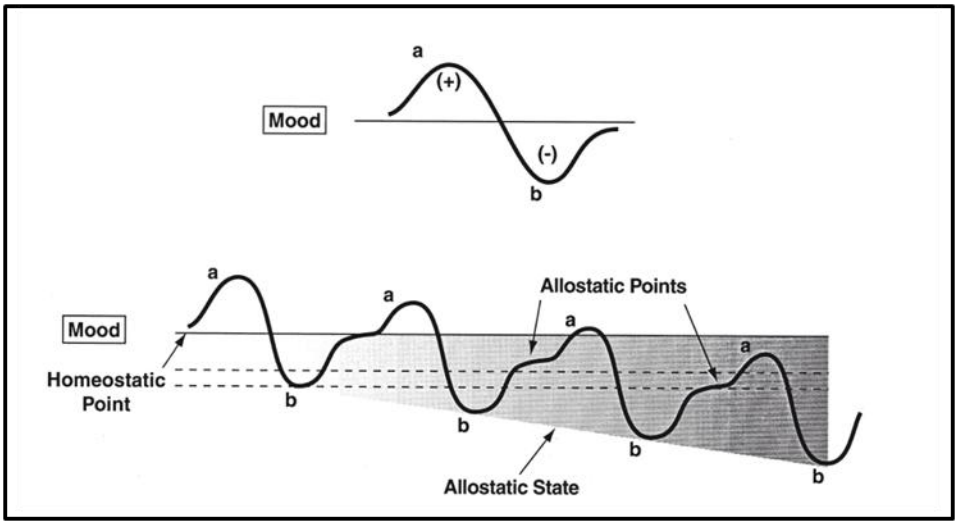

Figure 3 Hedonic allostasis theory. (Top) This diagram represents the initial experience of a drug with no prior drug history. The *a-process* represents a positive hedonic state or positive mood state and the *b-process* represents the negative hedonic state or negative mood state. (Bottom) The changes in the affective stimulus (state) in an individual with repeated frequent drug use may represent a transition to an allostatic state in the brain reward systems (a transition to addiction). Note that the apparent b-process never returns to the original homeostatic level before drug-taking begins again, thus creating a greater and greater allostatic state in the brain reward system. Reproduced from Koob and Le Moal (2001).

2.3 Incentive-Sensitization Theory of Addiction

If the compulsive pattern of drug use is not motivated by either the desire to obtain pleasure or by the desire to relieve withdrawal, then what motivates this compulsive pattern? In an attempt to answer this question, Robinson and Berridge, based on the concept of "incentive motivation"[1] originally proposed by Bindra (1978), proposed the concept of "incentive-sensitization".

The central thesis of the incentive-sensitization theory of addiction (Robinson and Berridge 1993) can be summarized in four points [see also (Berridge and Robinson 1998; Robinson and Berridge 2000; 2001; 2003; 2008)]:

1) Repeated exposure to potentially addictive drugs can, in susceptible individuals and under particular circumstances, produce long-lasting adaptations in neural systems (i.e. addictive drugs change the brain);
2) The brain systems that are changed include those that normally regulate the attribution of incentive salience [i.e. the transformation of an otherwise relatively neutral perceptual or representational event (cue) into an attractive and "wanted" incentive stimulus] to stimuli predictive of reward (mesocorticolimbic dopamine system);
3) The nature of these "neuroadaptations" is to render these brain circuits hypersensitive ("sensitized") in a way that results in pathological levels of incentive salience being attributed to drugs and drug-associated stimuli;

1Bindra's incentive motivation concept proposes that stimuli associated with rewards come to attain similar motivational properties as the rewards they predict via stimulus-stimulus associations. Following learning, these conditioned stimuli (CSs) become reward-like in that they are attractive, noticeable, and liable to be approached and even consumed like the rewards they predict. When learned CSs are later encountered, they are attributed with incentive salience, and animals are drawn to them as if they were "motivational magnets" (Bindra 1978).

4) The brain systems that are sensitized do not mediate the pleasurable or euphoric effects of drugs (drug "liking"), but instead mediate a subcomponent of reward termed incentive salience (drug "wanting"[2]).

Thus, the compulsive pattern of drug-seeking and drug-taking behavior is produced by an interaction between incentive salience mechanisms with associative learning mechanisms that direct motivation towards drug-related stimuli (see Figure 4).

Furthermore, a behavioral manifestation of sensitization to many drugs of abuse is a progressive and enduring enhancement in the motor stimulant effects elicited by repeated administration of such drugs (Stewart and Badiani 1993). Studies demonstrating sensitization involve measures of the psychomotor activating effects of drugs, such as their ability to enhance locomotor activity, rotational behavior or stereotyped motor patterns (Robinson and Becker 1986; Robinson and Berridge 1993; Segal et al. 1981; Stewart and Badiani 1993). These studies are thought to be relevant to addiction because of the assumption that the neural substrate that mediates these effects is either the same as, or at least overlaps with, the neural substrate responsible for the rewarding effects of drugs (the mesocorticolimbic dopamine system) (Wise and Bozarth 1987). Additionally, prior exposure to drugs of abuse enhances the incentive effects of drugs, measured using a variety of behavioral paradigms such as acquisition of drug self-administration (see Section 3.1.1) and conditioned place preference (see Section 3.1.3) (Lett 1989; Vezina 2004; Ward et al. 2006). Finally, further evidence for incentive-sensitization comes from studies showing that past drug treatment, which produces psychomotor sensitization, facilitates conditioned reinforcement (Di Ciano 2008; Taylor and Horger 1999).

In summary, sensitization is neither an inevitable consequence of exposure to potentially addictive drugs nor a simple pharmacological phenomenon. Both the expression and the induction of sensitization can be powerfully modulated by non-pharmacological factors, including environmental (and presumably psychological) factors associated with drug administration (Robinson and Berridge 2000; Robinson et al. 1998; Stewart and Vezina 1991).

2 A further refinement made by Robinson and Berridge is the distinction between two forms of drug wanting. One form is conscious, goal-directed, and depends upon knowledge of the relationship between specific actions and the value of their outcomes. This explicit form of wanting is manifested in conscious feeling of urge to take a drug, as well as in decision-making processes that involve recall of past outcomes of drug use. The other form of drug wanting is largely non-conscious, habitual, and does not require knowledge about the relationship between drug taking and its outcomes. This implicit form of wanting explains, for example, how relapse can occur in the absence of conscious urges to use drugs, as well as how drug seeking can escalate in the face of decreasing pleasure from drug taking because of tolerance (Robinson and Berridge 2001; Berridge and Robinson 2003).

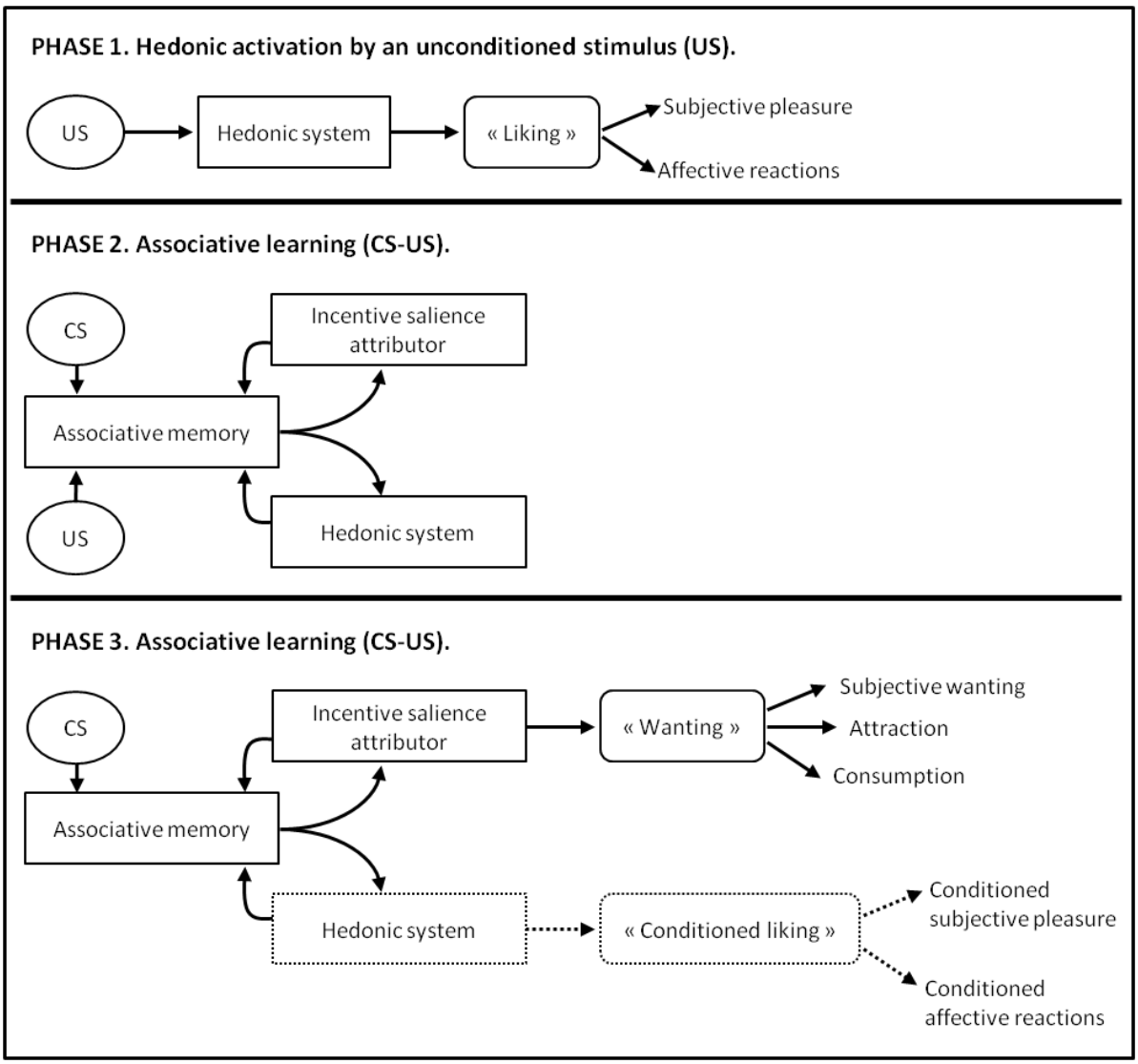

Figure 4 Incentive-sensitization model. Phase 1: The presentation of a reward or an unconditioned stimulus (US) activates the hedonic system. This activation of the hedonic system results in feeling pleasure ("liking"), which is manifested by emotional responses (like orofacial reactions in the case of a food reward) or, in humans, by testimony of such subjective pleasure. Phase 2: When a reward (US) and a conditioned stimulus (CS) are presented contiguously and contingently, an association is established between them. The CS acquires incentive value during this associative learning. Phase 3: After conditioning has been established, the presentation of the CS triggers several conditioned responses. The CS has acquired incentive value, and creates a desire ("wanting") which is manifested by the attraction to the CS. Separately, the CS can also activate the hedonic system and produce conditioned emotional responses. However, only the desire ("wanting") is capable to direct and control behavior. Modified from Berridge and Robinson (1998).

2.4 Aberrant Learning Theory of Addiction

Drug use begins by learning that drugs are rewarding due to their powerful interoceptive effects (see Section 6) that increase the desire to use the drug again. Furthermore, over time, certain cues in the environment become associated with drug use until these cues alone are sufficient to stimulate desire or craving for the drug. Thus, drugs of abuse, through their direct pharmacological actions on multiple neurotransmitter systems, alter and enhance learning systems involved in seeking rewards (Torregrossa et al. 2011).

This is the reason why it has been hypothesized that the transition to addiction results from the ability of drugs to promote aberrant learning (Berke and Hyman 2000; Di Chiara 1999; Everitt et al. 2001; Hyman and Malenka 2001; O'Brien et al. 1992b; Robbins and Everitt 1999; Tiffany 1990; Yuferov et al. 2010). The aberrant learning hypothesis of addiction suggests that drugs produce abnormally strong or aberrant associations involved in reward learning. These associations can be summarized in two types:

1) Action-Outcome (A-O): Cognitive, explicit recognition of the causal relationship between an act and its outcome.
2) Stimulus-Response (S-R): A habitual link between a specific stimulus and a specific response.

2.4.1 Action-Outcome (A-O)

When people take drugs, they learn about causal relationships between their actions and an outcome, such as a drug effect (i.e. taking the drug results in a desired effect) (Balleine and Dickinson 1998; Cardinal et al. 2002). The A-O explanation suggests that the main problem in drug addiction is that cognitively accessible memories of drug pleasure are exaggerated or altered.

2.4.2 Stimulus-Response (S-R)

The S-R hypothesis proposes that drug addiction involves a transition from behavior originally controlled by explicit and cognitively guided expectations about A–O relationships (i.e. the memory of drug pleasure) to more automatic behavior consisting primarily of S-R behaviors, or habits (Berke and Hyman 2000; Everitt et al. 2001; Robbins and Everitt 1999; Tiffany 1990).

At the neural level, Everitt and colleagues (Everitt et al. 2001) proposed that this progression from goal-directed behavior to habit is consolidated through the engagement of corticostriatal loops operating through the dorsal striatum (see Section 12) (Everitt and Wolf 2002; Robbins and Everitt 1999; White 1996).

Thus, this hypothesis explains addiction by suggesting that originally goal-directed behaviors become over-learned and habitual, to the point that they essentially become compulsive.

3. Behavioral Paradigms Assessing the Effects of Drugs in Rodents

The main behavioral paradigms currently used for the study of the positive/negative reinforcing and subjective effects of drugs can be summarized as follows (see Figure 5):

- Behavioral paradigms of the positive/negative reinforcing effects of drugs;
- Behavioral paradigm of the subjective effects of drugs.

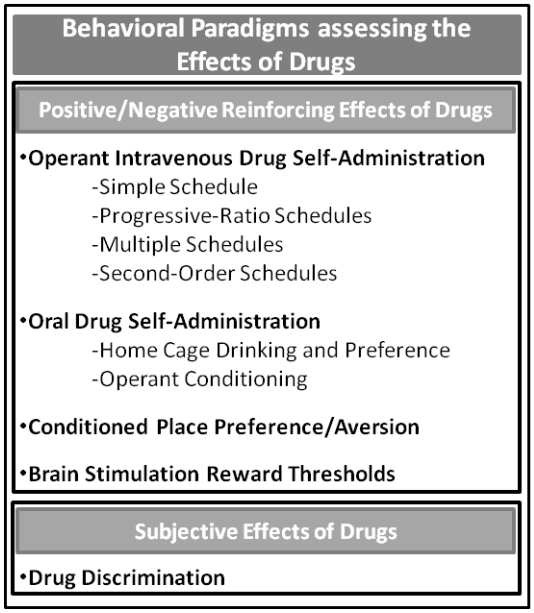

Figure 5 Table summarizing the main behavioral paradigms assessing the effects of drugs.

3.1 Behavioral Paradigms Assessing the Positive Reinforcing Effects of Drugs in Rodents

Reinforcement is said to occur when a response has an effect on the environment that makes the response more likely to be repeated in the future (Panlilio 2011). Positive reinforcement occurs when a response has the effect of producing something (i.e. positive reinforcers increase response probability because of the states they induce) (Wise and Bozarth 1987). As previously mentioned, drugs of abuse function as positive reinforcing stimuli; as a result, this function has provided the framework for currently used behavioral paradigms of addiction. The behavioral paradigms assessing the positive reinforcing effects of drugs can be divided into:

- Operant intravenous drug self-administration;
- Oral drug self-administration;
- Conditioned place preference;
- Brain stimulation reward thresholds.

3.1.1 Operant Intravenous Drug Self-Administration

The intravenous drug self-administration procedure is generally considered the most direct measure of the reinforcing properties of drugs of abuse in animals. Drugs of abuse are readily self-administered intravenously by experimental animals, and, in general, drugs that are self-administered correspond to those that have high abuse potential (Collins et al. 1984). However, not all drugs abused by humans are self-administered by experimental animals (e.g. hallucinogens). Furthermore, there are species- and strain-related differences in the degree to which a drug is self-administered (Kuzmin and Johansson 2000).

For intravenous self-administration studies, experimental animals (such as rats or mice) are prepared with indwelling intravenous catheters and trained to perform a response [typically to press a lever or to insert their nose ("nose-poke") into a specific hole] to receive response-contingent intravenous

drug infusions (see Figure 6) (Jonkman and Kenny 2012; Thomsen and Caine 2001). The number and pattern of responses required for each infusion is determined by the schedule of reinforcement imposed. Drug availability is typically signaled by an environmental stimulus. In addition to the intravenous route of administration, the intragastric or oral route can be employed.

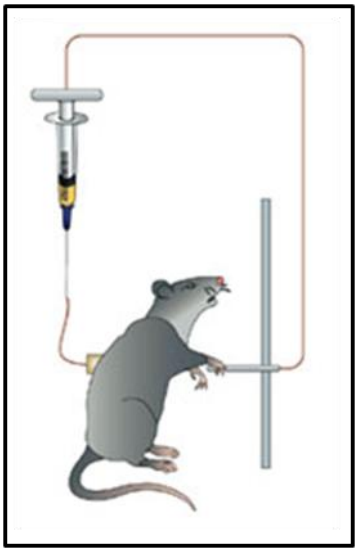

Figure 6 Intravenous self-administration of drugs. Animals can be trained to reliably lever-press to receive an infusion of drug. This model has been successfully used to measure the reinforcing properties of different drugs of abuse in rodents and primates. Reproduced from Laviolette and van der Kooy (2004).

Most studies on the reinforcing properties of drugs of abuse rely on schedules of reinforcement, in which the animal has to make a certain number of lever-presses to receive an infusion of drug. Thus, in operant conditioning, schedules of reinforcement are an important component of the learning process, since when and how often a behavior is reinforced can have a dramatic impact on the strength and rate of the response. The schedules of reinforcement can be divided into:

- Simple schedules;
- Progressive-ratio schedules;
- Multiple schedules;
- Second-order schedules.

3.1.1.1 Simple Schedules

In *fixed-ratio* (FR) schedules, the number of responses required for drug infusion is set at a fixed number. Rats on a simple schedule of reinforcement, such as FR-1, where one lever-press or one nose-poke delivers one drug infusion, will develop a highly stable pattern of drug self-administration in a limited access situation. However, as the unit dose is decreased, rats increase their self-administration rate, presumably to compensate the decrease in the unit dose. On the contrary, if the unit dose is increased, rats decrease their self-administration rate. Thus, manipulations which change the self-administration rate on this specific fixed-ratio schedule correlate with changes in the unit dose. If the dose remains consistent, changes in responding may be interpreted as changes in the reinforcing potency of the drug (Koob and Le Moal 2006).

On the other hand, in a *fixed-interval* (FI) schedule, the frequency of drug infusions is determined primarily by the interval schedule imposed and not the response rate. Rats on a fixed-interval schedule of reinforcement, where the first response is rewarded only after a specified period of time has elapsed, results in lower rates of responding that gradually increase towards the end of the interval (as the moment of delivery of the reinforcer gets closer) (Koob and Le Moal 2006). Therefore, the use of these two schedules of reinforcement can provide information regarding the motivational effect of drugs of abuse.

3.1.1.2 Progressive-Ratio Schedules

Progressive-ratio schedules are used to directly evaluate the reinforcing efficacy of a self-administered drug. In this procedure the response requirements for each successive drug reinforcement are increased, and the *breaking point* (the point at which the animal will no longer respond) is determined (Stafford et al. 1998). This schedule has been used to study the relative reinforcing efficacy of compounds both within and across drug classes (Mello et al. 1988) as well as the neural basis of drug reinforcement and drug craving (McGregor and Roberts 1995; Mello et al. 1988; Rocha et al. 1998; Stafford et al. 1998).

3.1.1.3 Multiple Schedules

Clinical definitions of drug addiction and dependence typically refer to the disruptive effects of addiction on non-drug-related activities. In a multiple schedule, self-administration is one component, and another component may involve a non-drug natural reinforcer. Behavior maintained by cocaine or food alternately in the same test session and with the same reinforcement requirements has been reported (Caine and Koob 1994). In addition to evaluating the selectivity of manipulations that apparently reduce the reinforcing efficacy of a drug, these procedures can provide information regarding those factors affecting “loss of control”, as well as behavioral and/or pharmacological therapies for the treatment of addiction.

3.1.1.4 Second-Order Schedules

In a second-order schedule, completion of an individual component (or part) of the schedule produces the terminal event (drug infusion) according to another overall schedule. Typically, completion of a unit schedule results in the presentation of a brief stimulus (e.g. visual), and completion of the overall schedule results in the delivery of the stimulus and the drug; as a result, this stimulus acquires, along the training, secondary reinforcing properties. This schedule requires extended sequences of behavior, thus, modeling the human condition in which drug taking is preceded by a series of behaviors (e.g. procurement, preparation) (Koob and Le Moal 2006).

3.1.2 Oral Drug Self-Administration

Oral self-administration has focused largely on alcohol because of the face validity of the paradigm and because intravenous self-administration of alcohol is difficult to sustain in rodents (Hyytia et al. 1996). Oral drug-self administration can be divided into:

- Home cage drinking and preference;
- Operant conditioning.

3.1.2.1 Home Cage Drinking and Preference

A simple approach to studying the motivation to consume a drug is to measure the volume consumed when a drinking bottle is available in the home cage. Usually a choice is offered between a drug solution and alternative solutions, one of which is often water, and the proportion of drug intake relative to total intake is calculated as a preference ratio (Li 2000).

3.1.2.2 Operant Conditioning

A more "motivational" approach is to have animals work to obtain drugs for oral consumption using operant procedures. The advantages of such an approach are numerous. The effort to obtain the substance can be separated from the consummatory response (e.g. drinking) and intake can easily be charted over time. In addition, different schedules of reinforcement can be used to change baseline parameters (Stewart et al. 1996; Weiss et al. 1990).

3.1.3 Conditioned Place Preference

Conditioned place preference (CPP) is a classical conditioning procedure in which administration of a drug is paired with one distinct environment and administration of placebo with another. After several environmental pairings, non-injected animals are allowed to access both environments and the time spent in each environment is assessed (see Figure 7). The animal's choice to spend more time in either environment provides a measure of the conditioned reinforcing effect of a drug. Animals exhibit a conditioned preference for an environment associated with drugs that function as positive reinforcers (i.e. spend more time in the drug-paired compared to placebo-paired environment) and avoid environments paired with aversive states (i.e. conditioned place aversion). For example, in a study where groups of rats were given pairings of one chamber with cocaine and the other with saline, followed by a period of several drug-free days during which the animals were allowed to explore both chambers freely, it was shown that a priming injection of cocaine given before a test for preference reinstates CPP, suggesting that drugs may induce relapse by renewing the incentive value of drug-associated cues (Mueller and Stewart 2000). On the other hand, in a study using morphine-dependent rats, it was shown that naloxone was able to induce conditioned place aversion in these rats but not in placebo rats, suggesting that the desire to avoid the naloxone-precipitated withdrawal state contributes to the compulsive nature of opiate abuse (Frenois et al. 2002). Therefore, this procedure permits assessment of the conditioning of drug reinforcement and can provide information regarding the positive and negative reinforcing effects of drugs.

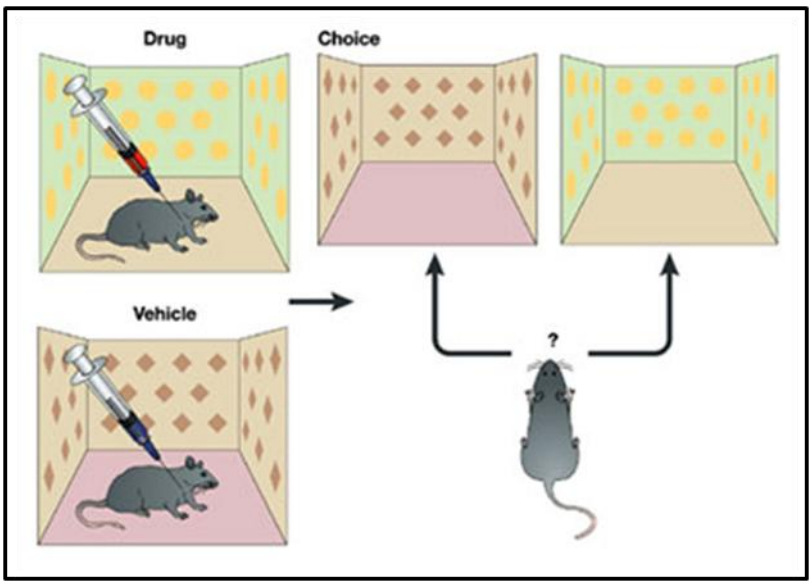

Figure 7 Conditioned place preference (CPP). In this procedure, animals receive a drug and are placed in an environment that has a specific odor, color and/or texture. On the following day, the animal receives the vehicle instead of the drug and is placed in another conditioning environment. After several pairings, animals are given the opportunity to spend time in both environments. Reproduced from Laviolette and van der Kooy (2004).

3.1.4 Brain Stimulation Reward Thresholds

Electrical self-stimulation of certain brain areas is rewarding for animals and humans as demonstrated by the fact that subjects will readily self-administer the stimulation (Olds and Milner 1954). The high reward value of intracranial self-stimulation (ICSS) has led to the hypothesis that ICSS directly activates neuronal circuits that are usually activated by conventional reinforcers (e.g. food, water and sex). In the ICSS procedure, the animal is working to directly stimulate presumed reward circuits in the brain, which provides a mean of quantifying the rewarding effects of drugs of abuse or the degree of dysphoria produced by drug withdrawal (see Figure 8). In a study where rats were given differential access to intravenous cocaine self-administration, it was shown that repeated prolonged access to cocaine (six hours or more) produces an escalation in drug intake which is not observed with limited access (one hour) to cocaine. After each self-administration session, ICSS thresholds were measured, and it was shown that the thresholds remained unchanged and stable in drug-naive rats and in short access rats, whereas they were progressively elevated in long access rats, suggesting a process of escalation by which decreased reward function contributes to increased cocaine intake, which in turn decreases brain reward function (Ahmed et al. 2002). Therefore, ICSS provides a unique tool to investigate the influence of various substances on reward and reinforcement processes.

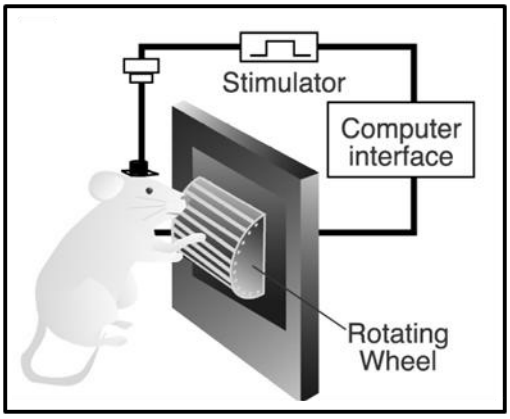

Figure 8 Intracranial self-stimulation paradigm (ICSS). In the ICSS paradigm, animals are trained to spin a wheel to receive a current through electrodes implanted in the brain. ICSS can be utilized in rodents (rats, mice) to understand how pharmacological or molecular manipulations affect the function of brain reward systems. Reproduced from Roberts and Koob (1997).

3.2 Behavioral Paradigms Assessing the Negative Reinforcing Effects of Drugs in Rodents

In contrast to positive reinforcement, negative reinforcement occurs when the response becomes more likely because it eliminates something aversive (i.e. negative reinforcers increase response probability by terminating a condition or central state that we then infer to be aversive) (Wise and Bozarth 1987). Some drugs can produce negative reinforcement by providing relief from pain, anxiety or stress. Since withdrawal from chronically administered drugs of abuse is usually unpleasant, avoiding or escaping from this state can be negatively reinforcing (Panlilio 2011). For example, morphine-dependent monkeys will press a lever that prevents or terminates injections of opioid antagonists that precipitate withdrawal symptoms (Goldberg et al. 1971). Thus, animal paradigms assessing the negative reinforcing effects of drugs of abuse include the same paradigms used to assess the positive reinforcing effects of drugs (described above).

3.3 Behavioral Paradigm Assessing the Subjective Effects of Drugs in Rodents: Drug Discrimination

The drug discrimination (DD) paradigm measures the discriminative stimulus properties of a drug, which are thought to model subjective effects reported by humans (Preston and Bigelow 1991). Specifically, the drug discrimination paradigm tests and classifies drugs according to their similarity or dissimilarity to previously experienced training drugs (Stolerman and Shine 1985).

The use of the DD paradigm in studies of drug addiction is based on two hypotheses:

1) The same components of a drug's actions subserve discriminative stimulus effects in animals and subjective effects in humans (Colpaert 1999; Glennon et al. 1991; Platt et al. 2001; Preston and Bigelow 1991).
2) The discriminative stimulus effects of drugs may contribute to drug taking in intermittent users and to relapse of addiction in former drug addicts. Furthermore, discriminative stimuli can signal the availability of a reinforcer and therefore set the occasion to engage

in those behaviors that enable consumption of the reinforcing drug (Stolerman 1992; Stolerman et al. 2002).

In a typical experiment, using a fixed schedule of reinforcement, animals are trained to perform a particular response (e.g. pressing one lever designated the drug-associated lever) following administration of a fixed drug dose and to press another lever following administration of saline. Most commonly, an appetitively motivated operant procedure is used in which animals are food or water deprived. Responding on the training-condition appropriate lever results in the delivery of food or water. Training is continued until the animal reliably selects the appropriate lever after drug or saline administration. Once trained, tests of stimulus generalization or antagonism are implemented to determine whether other doses of the training drug or another drug treatment produces stimulus effects qualitatively similar to or different from that of the training drug (Colpaert 1999; Porter and Prus 2009; Solinas et al. 2006) (see Figure 9).

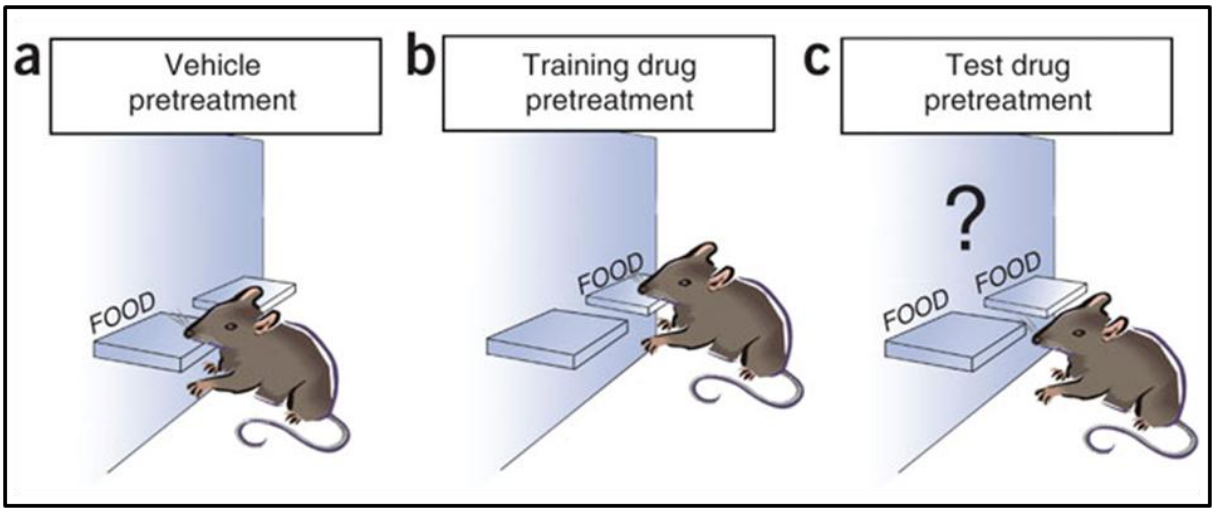

Figure 9 Drug discrimination procedure. (a,b) During training, one lever results in food delivery in sessions following a vehicle injection, and the other lever results in food delivery in sessions following an injection of the training drug. (c) During test sessions, the rat receives a test drug alone (generalization test) or in combination with the training drug (blockade test) to determine which lever it will press. To prevent bias, responding on either lever results in food delivery during test sessions. Alternatively, this could be achieved by not reinforcing either lever. However, this is generally avoided in order to prevent extinction of the operant behavior and disturbance of discrimination in subsequent sessions. Reproduced from Solinas et al. (2006).

3.4 Reliability and Predictability of the Behavioral Paradigms Assessing the Effects of Drugs in Rodents

A major advantage of the behavioral paradigms is the translation of the human condition to the animal model (face validity) and the translation of the neurobiological measures back to the human condition in order to predict vulnerability (predictive validity) (Koob and Le Moal 2006).

In the case of drug self-administration, the dependent variable provides a reliable measure of the motivation to obtain drugs (e.g. the amount of work an animal will perform to obtain the drug) or, in an alternative framework, in demonstrating that drugs function as powerful reinforcers. Responding maintained by drugs as reinforcers is stable across sessions and can be altered predictably by

neurotransmitter antagonists. Drugs with high reinforcement potential in experimental animals have reinforcing effects in humans as measured by both operant and subjective reports (Lamb et al. 1991).

In the case of conditioned place preference, drugs that produce conditioned preferences for the drug-associated environment are those that function as positive reinforcers in other paradigms. Conditioned aversions also are observed in response to drugs that are negative reinforcers or produce aversive or dysphoric states in human subjects (Hand et al. 1988; Mucha and Herz 1985).

Finally, in the case of drug discrimination, the dependent variable is very reliable as a measure of the interoceptive effects of drugs. Stimulus generalization gradients are stable once drug discrimination is acquired and neurotransmitter antagonists alter the stimulus effects of various drugs predictably. Drugs that produce discriminative stimulus effects that generalize to known drugs of abuse have been shown to have abuse liability (Glennon et al. 1991).

4. Animal Models of Drug Addiction

While no animal model in the field of drug addiction fully emulates the human condition, animal models do permit investigation of specific elements of the process of drug addiction and are considered to be among the best available models of neuropsychiatric diseases (Kalivas et al. 2006). Thus, animal models have been established and validated, and provide a valuable means to investigate models of different stages of the addiction cycle, models of psychological constructs such as positive and negative reinforcement[3], and models of actual symptoms of addiction (Koob and Le Moal 1997; Koob et al. 1998). What follows is a condensed description of some animal models that have been shown to be reliable for various stages of the addictive process. However, since ***relapse*** is the subject of my PhD, and is the most difficult aspect to treat in drug addiction, it will be explained in further detail in the section below.

4.1 Acquisition of Drug Self-Administration

Acquisition of drug self-administration is defined as the initial use of a drug, a transition from early occasional taking to an increase over a period of hours, days or weeks to a constant rate of intake over time (Carroll and Meisch 2011). Drugs that are self-administered by animals correspond well with those that have high abuse potential in humans. Furthermore, individual differences in the response to pyschostimulants and other drugs of abuse in general have been demonstrated in humans (de Wit et al. 1986) and laboratory animals (Piazza et al. 1989; Piazza and Le Moal 1996). The importance of individual differences in humans is accepted in clinical practice and is demonstrated in animal studies, where "vulnerable" rats have a higher chance of developing addiction than "resistant" rats, independently of the quantity of drug available (as appears to occur in the real world) (Piazza et al. 2000).

[3] A reinforcer is defined operationally as "any event that increases the probability of a response" and often is used interchangeably with "reward". In general, drugs function as positive reinforcers by virtue of their rewarding effects, and reward often connotes additional attributes of a drug (e.g. pleasure) that cannot be easily defined operationally.

4.2 Escalation in Drug Self-Administration

Drug intake escalation can be defined as a progressive increase in individual drug consumption over time that becomes excessive, overwhelming and difficult to control (Ahmed 2011), which is one of the major behavioral phenomena characterizing the development of addiction. Furthermore, this transition from drug use to drug addiction can be found in animal models of prolonged access to intravenous cocaine self-administration, where with 1 hour of cocaine access, rats' drug intake remained low and stable, whereas with 6 hours of cocaine access, rats' drug intake gradually escalated over days (Ahmed and Koob 1998). These findings provided an animal model for studying the development of excessive drug intake.

4.3 Environmental Modulation of Drug Taking

Drugs and environment play a central role in human addiction (Badiani et al. 2011). Interestingly, it has been shown that the context can modulate the reinforcing effects of drugs. Specifically, resident animals (animals housed in the self-administration chambers) took more heroin than non resident animals (animals transferred to the self-administration chambers before the test sessions), whereas the contrary effect was observed for cocaine (Caprioli et al. 2007). Furthermore, these findings correlate with the human situation where heroin abusers prefer to take heroin at home, whereas cocaine abusers prefere to take cocaine outside home. This animal model represents a starting point to begin exploring more specific therapies for different types of addiction.

4.4 Compulsive Quality of Drug-Seeking

In an attempt to model drug addiction in animals, Deroche-Gamonet and colleagues (2004) measured three addiction-like behavioral criteria in rats: 1) increased motivation to take the drug, 2) inability to refrain from drug-seeking and 3) maintained drug use despite aversive consequences. These authors found that, after 40 days of cocaine self-administration, 17% of animals developed these three addiction-like criteria, which were reflected in: 1) increased break points under a progressive ratio of cocaine reinforcement, 2) persistent responding during signaled periods of drug unavailability and 3) persistence of instrumental nose-poke response for cocaine even when it was punished by mild footshocks. These findings provided an animal model to the addiction vulnerable subgroup of human subjects (less than 20% of the population) (Anthony et al. 1994).

5. Reinstatement Model of Relapse

In the context of drug addiction, relapse refers to the reinitiation of drug-seeking and drug-taking after abstinence (Stewart 2008). Relapse can be studied using an animal model: the reinstatement model of relapse.

The reinstatement model of relapse was developed about thirty years ago (de Wit and Stewart 1981; 1983; Shaham et al. 2003) and is arguably the most valid animal model of relapse. This model provides a well characterized preclinical model with relevance toward understanding drug relapse in humans, since conditions that are reported to provoke relapse in humans will also reinstate extinguished drug taking behavior in laboratory animals (Shaham et al. 2003).

Studies carried out in humans and animals have demonstrated that a return to drug use can be precipitated by three distinct types of stimuli (de Wit 1996; Duncan et al. 2007; See 2002; Shaham et al. 2000; Shalev et al. 2002; Spealman et al. 2004; Stewart 2004; Weiss 2005):

1) exposure to an environmental stimulus (i.e. a discrete or contextual cue) that is strongly associated with the drug experience (McFarland and Ettenberg 1997; Meil and See 1997),
2) exposure to stressors (Ahmed and Koob 1997; Shaham et al. 1997b);
3) exposure to a pharmacological stimulus (i.e. the drug itself or a pharmacologically related agent) that induces some component of the drug experience (De Vries et al. 1998; de Wit and Stewart 1981; 1983; Shaham et al. 1994; Stewart et al. 1984).

In the reinstatement model of relapse (de Wit and Stewart 1981; 1983; Epstein et al. 2006; Spealman et al. 2004; Stewart 2004), animals are first trained to self-administer an addictive drug by pressing one of two levers. The animals are then exposed to a period of drug abstinence (the drug is no longer available). During this abstinence period, animals may simply be left in their home cages (Fuchs et al. 2006; Grimm et al. 2001), or be re-exposed to the context where the drug was previously delivered (the operant chambers), with the only difference being that lever pressing no longer results in drug delivery (period of extinction training); learning under this period of extinction training is reflected by decreased rates of lever pressing. When animals reduce responding to very low levels during this period, tests for reinstatement can begin. During these tests for reinitiation of drug seeking, lever pressing can be reinstated using three types of stimuli: presentation of an environmental stimulus previously associated with drug administration, exposure to a stressor, or direct administration of the drug itself (de Wit and Stewart 1983; Schmidt et al. 2005; See 2002; Shaham et al. 2003; Zhou et al. 2005). During this period of reinstatement, animals are given access to the levers, but the drug remains unavailable (see Figure 10).

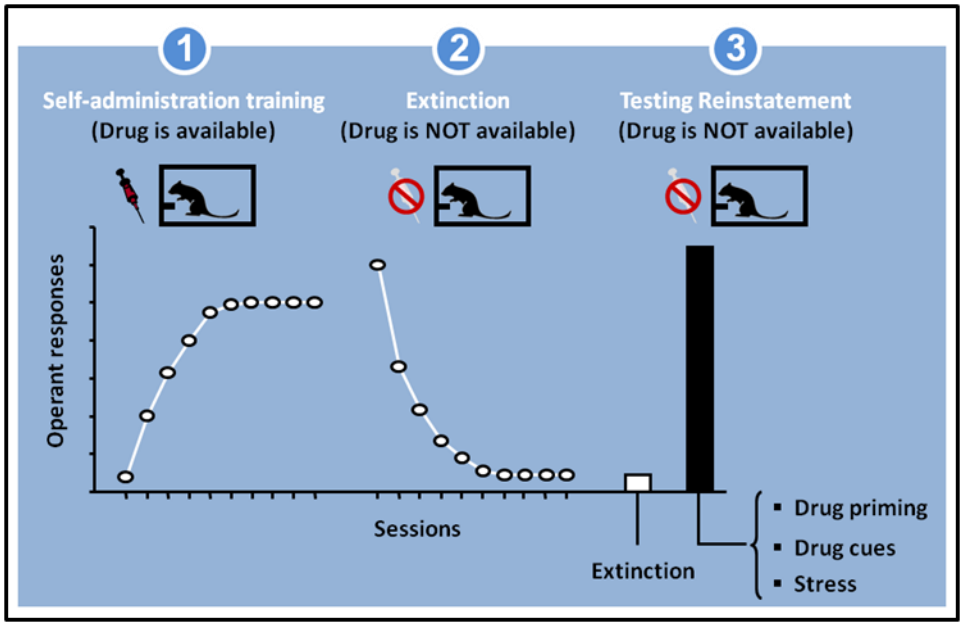

Figure 10 The reinstatement model of relapse. 1) In the first phase, animals are trained to self-administer a drug by means of lever pressing (drug delivery is accompanied by a cue –typically a light and/or a tone-). 2) In the second phase, animals go to extinction training during which no drug is administered in response to lever pressing. 3) Finally, in the reinstatement phase, animals are presented with the cue that had accompanied each drug infusion during self-administration, a mild stressor (typically foot shock), or the drug itself. Modified from Keiflin (2008).

Reinstatement of drug-seeking has been studied using several variants of this model. The difference between these variants is the temporal organization of the three phases (training, extinction and reinstatement) (Shalev et al. 2002). In the between-session variant, the three phases are spread over several days. In the within-session variant, the three phases take place on the same day, during the same session. Finally, in the between-within variant, the training phase lasts several days, but the phases of extinction and reinstatement are held on the same day, during the same session (see Figure 11).

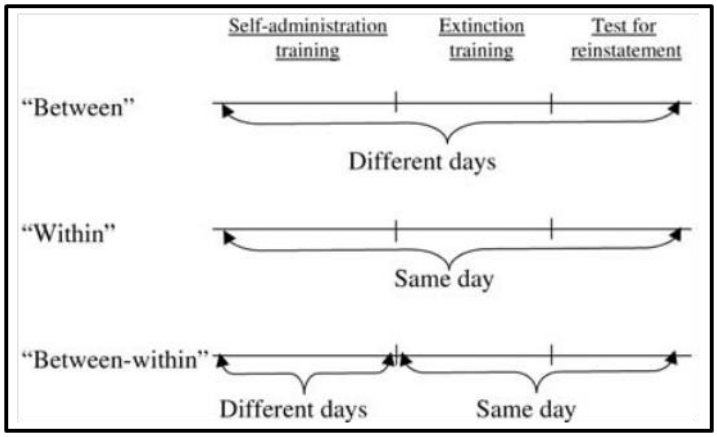

Figure 11 Timeline of the between, within and between-within session reinstatement procedures. Reproduced from Shalev et al. (2002).

5.1.1 Cue and Context-Induced Reinstatement

Contexts or environments where drugs are used can serve as conditioned stimuli (cues), eliciting expectations, thoughts, neural and neurochemical responses, emotional and motivational responses, and behavioral responses such as approach (Stewart 2008). Discrete stimuli such as odours, visual stimuli, people, music, etc. can have similar effects. Although these stimuli are paired with the effects of drugs whenever they are self-administered, their effectiveness can best be studied using classical conditioning procedures where environments or discrete stimuli are explicitly paired with injections of a drug. Discrete stimuli paired either with passive infusions (classical conditioning) or with response-contingent presentation of a drug (instrumental learning) can come to serve as conditioned reinforcers, maintaining behavioral responses such as lever pressing in the absence of drugs. Finally, cues that predict the availability/non-availability of drugs (discriminative stimuli) can differentially control the occurrence of drug-seeking/-taking behaviors (Carter and Tiffany 1999; Childress et al. 1993; Crombag et al. 2008; Di Ciano and Everitt 2003; Shaham et al. 2003; Stewart 2008).

Evidence for the ability of drug-associated stimuli to function as conditioned reinforcing stimuli can be observed in a study in rats by Weiss (2000) where drug-associated stimuli that signaled response-contingent availability of intravenous cocaine (white noise) versus saline (illumination of the house light), elicited drug-seeking behavior specifically during periods of white noise. Furthermore, after extinction of cocaine-taking behavior, re-exposure to the cocaine-associated stimulus, but not to a non-reward stimulus, produced strong recovery of responding on the previously active lever in the absence of drug availability. Similarly, cues associated with the availability of oral alcohol self-administration can also reinstate responding (Ciccocioppo et al. 2001). Therefore, animal models for

cue reinstatement show that discriminative stimuli predictive of drug availability elicit strong recovery of extinguished drug-seeking behavior in the absence of drug availability.

5.1.2 Stress-Induced Reinstatement

Human studies show that relapse to drug-taking is more likely to happen under situations of stress (Brown et al. 1995; Kosten et al. 1986; Mattoo et al. 2009). Animal models for stress reinstatement show that several stressors, such as a footshocks, food deprivation (Shalev et al. 2000) and administration of corticotrophin-releasing factor (CRF) (Shaham et al. 1997b) reinstate drug-seeking behavior in rats in the absence of drug availability (Shaham et al. 2000; Shaham et al. 2003).

In cocaine-trained rats (Ahmed and Koob 1997; Erb et al. 1996) and in experiments where rats were trained to self-administer alcohol (in a two-bottle choice) (Le et al. 2000), heroin (Shaham and Stewart 1995b) or nicotine (Buczek et al. 1999) intravenously, footshock stress reinstated drug-seeking behavior. Furthermore, footshock-induced reinstatement appears to be selective for drug, in the sense that footshock is ineffective in reinstating food-seeking behavior in animals trained to self-administer food (Ahmed and Koob 1997). These findings show that exposure to stress effectively reinstates drug seeking in animals experienced in the self administration of drugs of abuse from different pharmacological classes.

5.1.3 Drug-Induced Reinstatement

The reinstatement of drug craving or seeking by priming injections of the abused or training drug, drugs of a similar class, or drugs that activate pathways in common with the training drug is a robust phenomenon in humans and laboratory animals (Jaffe et al. 1989; Meyer 1988; Robinson and Berridge 1993; Stewart 2008; Stewart et al. 1984).

Animal models for drug-primed reinstatement show that exposure to psychostimulants (de Wit and Stewart 1981), opiates (de Wit and Stewart 1983), nicotine (Shaham et al. 1997a) and alcohol (Le et al. 1998) can elicit strong recovery of extinguished drug-seeking behavior in the absence of the availability of the training drug. The priming effect occurs across several routes of administration, including intravenous, oral, subcutaneous, intraperitoneal and intramuscular. It is not necessary that the priming dose is given by the same route as the previously self-administered drug (Carroll and Comer 1996). Furthermore, systemic injections of compounds of drug classes other than the training drug are generally less effective in reinstating drug self-administration behavior (Gerber and Stretch 1975), suggesting that the effectiveness of compounds in reinstating drug self-administration decreases as their discriminative stimulus similarity to the training drug decreases. After priming with the drug, the latency to reinitiate responding, or the amount of responding on the previously active lever, are used to reflect the motivation for drug-seeking behavior, and are usually studied after a few hours or a few daily sessions in the absence of the drug (Stewart et al. 1984); however, a number of studies in rats have shown that drug-seeking behavior can also be elicited after (up to six weeks) drug-free period (Chiamulera et al. 1996; Erb et al. 1996; Shaham et al. 1994). These findings indicate the validity of drug-induced reinstatement, since as in the human situation, drug addicts might go without drug taking for weeks or even months before relapsing.

Priming phenomena have also been investigated in humans (de Wit 1996). Studies with priming effects in humans have been conducted using ethanol (Bigelow et al. 1977; Hodgson et al. 1979;

Ludwig et al. 1974), heroin (Meyer et al. 1978), cocaine (Jentsch and Taylor 1999) and nicotine (Chornock et al. 1992), showing that in regular drug users, preloads of drugs increase both self-reported desire for the drug and actual consumption of the drug.

A number of mechanisms of action have been proposed to explain reinstatement induced by the drug itself (de Wit 1996; Katz and Higgins 2003). One hypothesis is based on the ***incentive properties*** of drugs of abuse (especially psychostimulants). It has been proposed that re-exposure to the drug increases and reactivates the incentive value of stimuli previously associated with the drug (e.g. the lever), which incites the animal to approach and interact with these stimuli, and consequently leads to compulsive drug use and relapse upon exposure to these stimuli (Goddard and Leri 2006; Mueller and Stewart 2000; Robinson and Berridge 1993; Stewart 2000; Stewart et al. 1984). Another hypothesis proposes that the ***discriminative stimulus effects*** of drugs play a prominent role in reinstatement induced by re-exposure to the drug (Banks et al. 2007; Bickel and Kelly 1988; de Wit 1996; Katz and Higgins 2003). In fact, it has been demonstrated that drugs can be recognizable stimuli and as result, identified by the individual (which is illustrated in the drug discrimination procedure). In the case of drug self-administration studies (where the drugs are the reinforcers), the long-lasting effects induced by the drugs could also constitute an internal contextual stimuli, which is present during drug self-administration sessions but absent during extinction sessions. Thus, the interoceptive effects induced by the drug may act as a stimulus, signaling to the rat that the drug is now available or that pressing the active lever will lead to drug infusions (Preston and Bigelow 1991). As a consequence, re-exposure to the self-administered drug or to another drug producing similar discriminative effects signals the end of the extinction period and places the animal “back” in drug self-administration sessions.

5.1.3.1 Cross-Reinstatement

There is evidence from animal studies that drugs other than the previously self-administered drug may also reinstate drug-seeking behavior (De Vries et al. 1998). According to the incentive-sensitization view of addiction (see Section 2.3) (Robinson and Berridge 1993), drugs can prime responding for each other because they share the property to activate the mesolimbic dopaminergic system which becomes sensitized upon repeated drug use in a long-lasting manner. In other words, it has been proposed that the ability of opiates and psychostimulants to reinstate responding is related to their ability to cause behavioral and neurochemical sensitization (Stewart and Vezina 1988).

However, the ability of a drug to reinstate responding may be also due to the fact that it shares discriminative stimulus properties with the previously self-administered drug (Stolerman 1992). In studies with rats trained to self-administer cocaine or heroin (de Wit and Stewart 1981; 1983), experimenter-delivered injections of cocaine reinstated responding in rats previously trained to self-administer cocaine, and injections of heroin reinstated responding in animals previously trained to self-administer heroin; however, injections of cocaine did not reinstate responding in rats trained to self-administer heroin, and heroin did not reinstate responding in rats trained to self-administer cocaine, suggesting that only drugs that have interoceptive effects similar to those of the self-administered drug restore responding. Thus, the discriminative stimulus effects of drugs are often pharmacologically selective, such that only drugs sharing pharmacological mechanisms of action with the training drug elicit training drug-appropriate responding (Holtzman 1985).

6. Interoceptive Discriminative Stimulus Properties of Drugs of Abuse

Interoception comprises sensing the physiological condition of the body, the conscious representation of the internal state, and the initiation of motivated action to homeostatically regulate the internal state (Paulus et al. 2009). Interoception includes a range of sensations such as temperature, pain, itch, tickle, muscle tension, hunger, thirst, air hunger, and intestinal tension, which together provide an integrated sense of the body's physiological condition (Craig 2002; Paulus et al. 2009). These bodily states have hedonic value because they are all associated with different subjective qualities that are either pleasant or unpleasant and are distinguishable from somatic sensations (e.g. joint-position) which do not have inherent hedonic value (Naqvi and Bechara 2008). This aspect of "sensing the physiological condition of the body" is important for drug addiction, since, in addition to serving as reinforcers, drugs of abuse can also function as stimuli which set the occasion for behavior (Bickel and Kelly 1988; Cameron 2001). For example, a drug state (such as the one induced by the pharmacological effects of nicotine) can serve as an interoceptive cue for the presence of a response-outcome relation (Bevins and Palmatier 2004; Wilkinson et al. 2006).

Apart from their direct CNS effects, nearly all drugs of abuse exert powerful effects on the periphery: visceral effects on the cardiovascular system, the airway, and the gut; taste sensations and pain sensations; and distortions in sensory processing (Everitt and Robbins 2005; Naqvi and Bechara 2010). It is widely known that cocaine, amphetamine, marijuana, and nicotine are all potent stimulators of the autonomic nervous system, eliciting increases in heart rate and blood pressure, along with peripheral vasoconstriction (Naqvi and Bechara 2010). Furthermore, aversive effects of opiates, such as nausea and constipation, are mediated by peripheral receptors (Bechara and van der Kooy 1985). Snorting cocaine elicits a bitter taste, a harsh sensation in the nose and throat, and increases in heart rate and blood pressure (Naqvi and Bechara 2008); these sensations might explain why cocaine users report cocaine-like subjective effects within seconds, even before the drug has time to reach the brain, cross the blood-brain barrier and interact with its central pharmacological targets (Wise and Kiyatkin 2011). Cigarette smoking has a strong taste and highly salient sensory effects within the airway, which may be more rewarding to smokers than the direct CNS effects of nicotine (Naqvi and Bechara 2010; Westman et al. 1996). Additionally, the peripheral effects of smoked nicotine may, like those of cocaine, be quickly relayed to the brain, since nicotinic receptors are highly concentrated in the lungs and, therefore, nicotine accumulates in the brain over minutes rather than seconds (Rose et al. 2010). Use of heroin, which involves violating the body envelope with a needle (Naqvi and Bechara 2008), also has well-known peripheral actions that could signal receipt of the drug before it arrives in the brain and could contribute a conditioned component to the rewarding effects of the drug in experienced users (McDonald and Siegel 2004). Summarizing, bodily effects play an important role in the subjective or "discriminative" effect ("feeling") of taking a drug (Everitt and Robbins 2005) (see Figure 12). As pointed out by Cameron (2001): "Interoception is a psychosomatic phenomenon par excellence, connecting, as it does, body to brain to behavior and thought". Thus, the conditioned interoceptive cues of addictive drugs may play a more important part in addiction than has been widely considered.

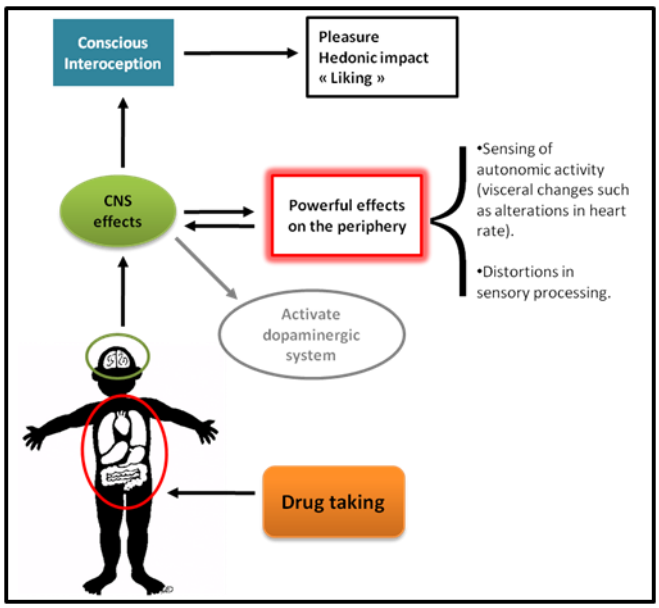

Figure 12 A model for the role of the interoceptive discriminative stimulus properties of drugs of abuse. Interoceptive effects of drug taking are signaled in the brain. The representation of these interoceptive effects in the brain gives rise to conscious interoception, which allows, for example, the ability to perform sensory discrimination. This representation is then fed to brain regions that translate the interoceptive effects of drug use into conscious pleasure. Dopamine (DA) release from VTA neurons, facilitated by the direct CNS effects of drug taking, modulates this conscious pleasure. Dopamine may also be important for learning processes that cause the interoceptive effects to become pleasurable in the first place.

7. Learning and Conditioning

Rewards are often predicted and wanted, as well as liked, especially when preceded by learned cues (Smith et al. 2011). Reward controls goal-directed behavior and involves learning, since it acts as a positive reinforcer; obtaining it, in turn, is associated with pleasant feelings which give incentive value to the goal-object (Martin-Soelch et al. 2001). For example, attraction to food in the fridge when hungry involves learned predictions of tasty food, motivation to eat, and experiencing pleasure while eating (Smith et al. 2011). Therefore, the expectation of a reward can have powerful effects on learning and performance (Savage and Ramos 2009).

The associative learning theory characterizes learning as the formation of an association between mental representations of events in the world. Animals learn to predict variations in external state by storing information about stimuli, responses and outcomes (Rescorla 1991). The phenomena of learning can be dissociated in two basic paradigms of conditioning:

1) Pavlovian (classical)
2) Instrumental (operant)

Pavlovian and instrumental learning usually occur in parallel during the learning and performance of a task. Both forms of conditioning concern the ways that animals learn to predict and respond to important events in their environments, like the delivery of appetitive (food or water) and aversive (electric shocks) stimuli (Dayan and Balleine 2002).

7.1 Pavlovian Conditioning

Pavlovian conditioning mechanisms are responsible for the formation of associations between conditioned and unconditioned stimuli, usually as a result of simple temporal coincidence. Thus, learning to anticipate a reward can occur by associating a discrete stimulus with an outcome. The outcomes are provided whatever the animals do, so any changes in behavior presumably reflect innately specified reactions to predictions of the outcomes (Dayan and Balleine 2002). A predictive cue such as a conditioned stimulus or discriminative stimulus can enter into associations with either the general motivational properties of the reward or the sensory specific properties of reward (Delamater 2007; Holland 2004). Therefore, reward expectancies produced by Pavlovian procedures can have affective, motivational and sensory discriminative properties that mediate instrumental behavior (Urcuioli 2005).

7.2 Instrumental Conditioning

Whereas Pavlovian conditioning enables an animal to anticipate motivationally significant events, it is instrumental conditioning that allows control over these events in the service of its needs and desires (Balleine and Dickinson 1998). Instrumental learning mechanisms play a role in learning when specific instrumental responses made by the animal impact the probability of a reward (response-contingent reinforcement) (Berridge and Robinson 2003).

Studies of instrumental conditioning have established that, in rats, actions such as lever pressing are controlled by two dissociable associative processes (Dickinson 1985; Dickinson and Balleine 1994; Dickinson et al. 1995):

1) A goal-directed process;
2) A stimulus-response habit mechanism.

7.2.1 Goal-directed

By definition, goal-directed behavior is performed to obtain a desired goal. Goals refer to desired (or anticipated) outcomes (or end states). These goals can be the consequence of physiological needs, such as thirst and hunger, as well as various other “needs” or motives, such as restoring personal hygiene, making friends, etc (Aarts and Dijksterhuis 2000).

Although all instrumental behaviors are essential to achieving their corresponding goals, they are not necessarily purposely goal-directed. Much of our behavior starts out as intentional, goal-directed actions, but with repetitive practice these actions are transformed into stimulus-response habits (Balleine and Dickinson 1998). Indeed, this transition can be demonstrated operationally in the laboratory. Instrumental conditioning in rodents provides a very accurate model of goal-directed action in humans (Balleine 2005).

Dickinson and Balleine (1994) proposed that a behavior is goal-directed if:

- it is sensitive to the contingency between action and outcome;
- it is directly related to the current motivational value of the outcome.

It turns out that under certain training conditions, instrumental lever pressing in rodents meets both of these conditions. Not only are rodent actions sensitive to changes in the value of the goal or outcome with which they are associated but they are also highly sensitive to changes in their causal consequences. Simply stated, if the anticipated outcome is desirable, a goal-directed action should be more probable, but if the anticipated outcome is aversive, performance should be less probable (Ostlund and Balleine 2008b). Furthermore, rats will stop responding if performance no longer delivers the instrumental outcome (i.e. extinction conditions) (Balleine 2005).

There are several factors that determine whether instrumental responding will be under goal-directed or habitual control. Most notably, the appearance of habitual control is facilitated by the amount of instrumental training (Adams 1982; Dickinson 1985; Dickinson et al. 1995; Holland 2004; Killcross and Coutureau 2003; Nordquist et al. 2007). Studies of instrumental conditioning in rats have shown that during early learning, behavior is goal-directed, the initiation of the response is under the direct control of the current value of the reinforcer or outcome and performance is influenced by changes in outcome value and by the instrumental contingency between response and outcome. As training proceeds, however, instrumental performance shifts to a stimulus-response process. Actions become habitual, and responding is characterized by direct initiation of responding by a stimulus or context presentation so that performance is insensitive to changes in instrumental contingency and independent of the current value of the reinforcer[4] (Adams 1982; Balleine and Dickinson 1998; Dickinson 1985; Dickinson et al. 1995) (see Figure 13). Thus, goal-directed and habit learning processes are simultaneously engaged, but the fact that one or the other predominates depends on the circumstances during training (Balleine 2005). As a consequence, assessment of changes in operant responding after outcome devaluation is usually used to differentiate goal-directed from habit-driven behavior (see Section 7.3) (Adams 1982; Dickinson 1985; Dickinson and Balleine 1994).

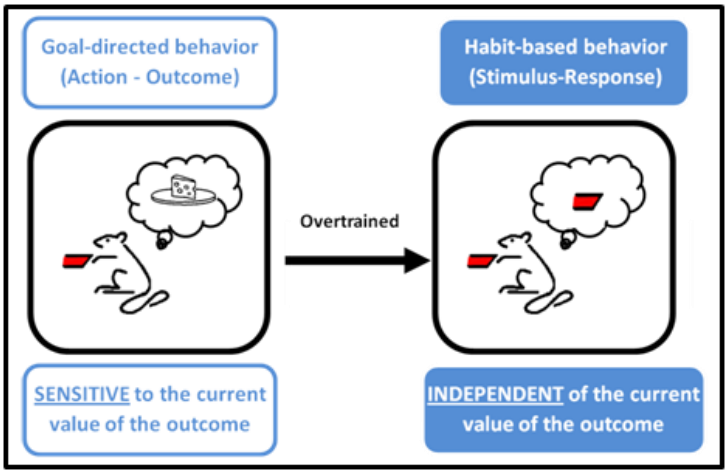

Figure 13 Instrumental behavior. An instrumental behavior, which starts out as an action controlled by knowledge about the outcome, with repeated practice becomes a response independent of the current value of the outcome and simply triggered by the stimuli in whose presence, has been repeatedly performed.

[4] It has long been recognized that instrumental procedures determine not only response-outcome contingencies but also Pavlovian relationships between the contextual stimuli and the reinforcer. Consequently, in determining the status of instrumental performance, it is important to control for any Pavlovian influence (Jonkman et al. 2010).

Thus, the control of simple instrumental action, such as lever pressing for food by a hungry rat, is complex and mediated by at least three different learning processes (Balleine and Dickinson 1998):

1) *A contingency learning process*. This process enables the animal to encode the relation between an action and a reward.
2) *An incentive learning process*. This process allows the animals to assign an appropriate value to a reward and learn how this value is modulated by its motivational states.
3) *A stimulus-response mechanism*. Instrumental responding can be controlled by this process which appears to predominate after extended training.

7.2.2 Habits

Habit, to most of us, has multiple connotations. A habit is a behavior that we do often, almost without thinking. Habits can be evaluated as relatively neutral, or as "good" (desirable) or as "bad" (undesirable). Yet during much of our waking lives, we act according to our habits, from the time we wake up to the time we fall asleep. Whether good, bad, or neutral, habits can have great power over our behavior (Graybiel 2008). Habits allow us to perform actions in a mindless, automatic fashion which allows economy of cognitive resources.

When a behavior has been performed many times in the past, future behavior becomes increasingly under control of an automatized process, whereas a behavior executed less frequently is (still) guided by evaluative interpretations and considerations. Habitual behavior is automatic, and defined as a function of relative frequency of past performance. It is determined by past behavior and not mediated by attitudes, intentions, or other concepts referring to more deliberate or conscious processes. This is characteristic of most of the daily activities of humans, the notion being that with sufficient practice, performance on any task, from tooth brushing to piano playing, can be automatic. So, it is actually people's unthinking routines - or habits - that form the bedrock of everyday life. Without habits, people would be doomed to plan, consciously guide, and monitor every action (Graybiel 2008; Neal et al. 2006).

7.2.2.1 What is Habit Learning?

In the field of learning theory, the term habit refers to stimulus–response learning (S-R) (Dickinson 1985; Ostlund and Balleine 2008b). For example, let's consider a hungry rat pressing a lever for a food pellet. Stimulus-response learning assumes that the delivery of the food pellets strengthens or reinforces an association between environmental stimuli (context, sight of the lever) and the lever-press response. As a consequence, when returned to the training situation, the rat should automatically perform the lever-press response without the need of any planning since the associations have already been established. Importantly, these associations are no encoded as a consequence (or goal) of the action (Adams and Dickinson 1981).

That is, a habitual response should be elicited and performed by its associated stimuli even if, in the meantime, the consequences of that response have become noxious or aversive (Everitt and Robbins 2005; Ostlund and Balleine 2008b).

7.2.2.2 Characteristics of Automatic Processes

Several properties have been proposed as key features of automatic processing. However, the following list (Tiffany 1990) focuses on five characteristics of automaticity, with the greatest potential relevance for addictive behavior:

1) *Speed*: With practice, task performance speeds up, with a corresponding decline in performance variability, representing a transition from non-automatic to automatic processes in the control of task performance.
2) *Autonomy*: An automatized act can be carried out without initiation through intention. Under proper stimulus conditions, an automatized action may be initiated involuntarily. The presentation of a stimulus may be sufficient to cause the enactment of an automatized action sequence.
3) *Lack of control*: With appropriate eliciting stimuli, it may be difficult to inhibit the elicitation of an automatic process, and once initiated, the process may be difficult to impede or diminish. Automatic processes are easily triggered by external stimulus conditions and somewhat less penetrable by executive cognitive processes. Once initiated, an automatized process may have a ballistic quality, in that it is difficult to impede and tends to run on to completion.
4) *Effortlessness*: The fast, coherent, and consistent performance of well-practiced individuals on certain tasks is generally described as being easy and non-demanding.
5) *Conscious awareness*: Awareness, or its absence, can only be inferred by the verbal report of the individual. These processes can be enacted without concurrent activation of cognitive structures that permit an accurate or veridical description of the mechanics of the automatized functioning.

Thus, in addition to habits being learned, repetitive, sequential, context-triggered behaviors, habits can be defined experimentally as being performed not in an action-outcome way (Adams and Dickinson 1981; Balleine and Dickinson 1998).

7.3 Outcome Devaluation Paradigm

Both goal-directed and habit-based behaviors appropriately explain why hungry rats will press a lever for a food reward: either 1) because the rat wants food and has learned that it can be obtained by pressing a lever (A-O association) or, 2) because this response has been elicited by some environmental stimulus (S-R association).

The traditional assay for distinguishing goal-directed from habit-based behavior is the reinforcer or outcome devaluation paradigm (Adams 1982; Adams and Dickinson 1981). Briefly, following instrumental training the value or significance of the outcome is modified (devalued[5]) without changing the general motivational state of the animal. After changing the value of the outcome, the capacity of the animal to integrate the knowledge acquired during the instrumental training with that gained from the changed value of the outcome (outcome devaluation procedure), is assessed in non-reinforced extinction conditions (in the absence of rewards) to test for the effects of the revaluation

[5] It is easy to devalue ingestive reinforcers (food), but it is much more difficult to devalue intravenously self-administered drugs such as cocaine (Everitt and Robbins 2005).

on the previously learned association, and avoid new learning (Adams 1982). Thus, if behavior tested in extinction is sensitive to devaluation, responding must be mediated by a representation of the outcome and therefore be goal-directed. By contrast, if responding is insensitive to devaluation and therefore independent of the current value of the outcome, the behavior is habitual.

Three methods are commonly used for outcome devaluation (see Figure 14):

1) Specific satiety procedure;
2) Conditioned taste aversion procedure;
3) Motivational shifts.

7.3.1 Specific Satiety Procedure

In a specific satiety procedure, rats are pre-fed on a particular food, such that they develop a temporary, specific satiation for this particular food. Consumption tests show that such a procedure selectively devalues the pre-fed food in comparison to other, non-pre-fed foods (Balleine and Dickinson 2000; Corbit et al. 2001; Coutureau and Killcross 2003; Killcross and Coutureau 2003; Yin et al. 2005a; Yin et al. 2005b). Interestingly, very similar effects have been found in human subjects (Valentin et al. 2007).

7.3.2 Conditioned Taste Aversion Procedure

In a conditioned taste aversion procedure, gastric illness is induced when a gustatory stimulus is paired with agents which produce nausea and gastric upset (i.e. with an injection of lithium chloride). As a result, this gustatory stimulus acquires secondary properties which can be described as “conditioned nausea” (Garcia and Koelling 1966).

Adams and Dickinson (1981) demonstrated that after training rats to press a lever for sucrose, devaluing the sucrose by pairing its consumption with gastric illness (induced by an injection of lithium chloride) caused a reduction in lever pressing in an extinction test (i.e. in the absence of the now devalued outcome), suggesting that rats were able to integrate learning during training with the experienced change in the value of the sucrose. Similar devaluation effects have been demonstrated when inducing conditioned taste aversion with lithium chloride (Adams 1982; Adams and Dickinson 1981; Balleine and Dickinson 1992; Holland 2004; Yin et al. 2004).

7.3.3 Motivational Shifts

Motivational shifts refer to shifts between different motivational states. Motivational shifts can either devalue or enhance the value of outcomes. For example, hungry rats are trained to press a lever for food, and then their motivational state is shifted to satiety by allowing them ad libitum consumption of standard food chow in the home-cages. This manipulation renders the once valuable food reward less valuable. Opposite shifts (from training when sated to testing when hungry) enhance the value of the instrumental outcome, and shifts between different motivational states (between hunger and thirst) can be used to change the value of one outcome (food pellets) while maintaining the value of another (sucrose solution) (Balleine 1992; Balleine and Dickinson 2000).

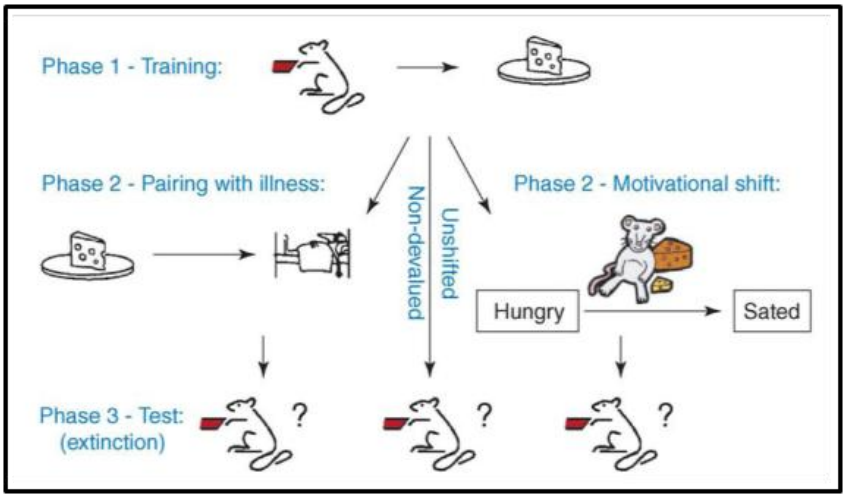

Figure 14 Outcome devaluation procedure. Phase 1: Rats are first trained to press a lever for food. Phase 2: The value of the outcome is changed, either by pairing its consumption with illness (left) or inducing a motivational shift, such as from hunger to satiety (right). Phase 3: The behavior of the rats is tested under extinction conditions (no outcomes available), and is compared with that of rats who have not undergone Phase 2. Reproduced from Niv et al. (2006).

8. Habit Theory of Drug Addiction

Investigations of normal habit learning have immediate relevance for the field of addiction research. Habits induced by exposure to drugs of abuse can dominate behavior and produce states of craving and drug-seeking that persist for years (Graybiel 2008).

The importance of habits in drug addiction was based on the observation by Tiffany that "drug-use behaviors tend to be relatively fast and efficient, readily enabled by particular stimulus configurations (i.e. stimulus bound), initiated and completed without intention, difficult to impede in the presence of triggering stimuli, effortless, and enacted in the absence of awareness" (Tiffany 1990) (see Section 7.2.2.2). Thus, habits do not depend on awareness but are directly triggered by drug-associated stimuli such as paraphernalia, locations, internal states, friends or the drug itself. So, according to this perspective, habits might play an important role in drug addiction. As a result, Everitt and colleagues (see below) have conducted research and developed a habit theory of drug addiction.

This theory postulates that drugs of abuse subvert natural learning processes (such as pavlovian and instrumental learning mechanisms), thereby facilitating the development of habitual control over drug-seeking and drug-taking (Everitt and Robbins 2005; Robbins and Everitt 1999; Vanderschuren and Everitt 2004).

Everitt and Robbins (2005) have suggested that exposure to drugs of abuse accelerates the process of behavioral automatisms and excessively strengthens the stimulus-response associations (Robbins and Everitt 1999; Vanderschuren and Everitt 2004). So, while occasional consumption of drugs of abuse is driven by its appetitive effects, compulsive drug-taking would reflect the pathological activation of behavioral automatisms in response to certain discriminative stimuli, regardless of the consequences. Thus, compulsive drug-taking behavior is automatic (not driven by the desire to consume drugs), which would explain drug use despite knowledge of adverse consequences.

In accordance with this habit theory of drug addiction, it has been shown that exposure to cocaine or amphetamine (psychostimulants) accelerates the formation of behavioral automatisms (Nelson and Killcross 2006; Nordquist et al. 2007; Schoenbaum and Setlow 2005). Another study in favor of the involvement of behavioral automatisms in drug addiction has been provided by Vanderschuren and Everitt (Vanderschuren and Everitt 2004). These authors reported that after prolonged self-administration of cocaine, rats continue to seek drugs despite the presentation of an aversive stimulus contingent with the response. They concluded that the persistency of drug-seeking behavior despite adverse consequences is the result of the activation of behavioral automatisms. These experiments strongly suggest that exposure to drugs of abuse (psychostimulants) triggers neural mechanisms that facilitate the initiation of inflexible habitual instrumental behavior.

9. Neurobiology of Drug Addiction

9.1 Neurobiology of Drugs of Abuse

The key questions in addiction are why some susceptible individuals undergo a transition from casual drug use to compulsive patterns of drug use, and why addicts find it so difficult to stop using drugs. To answer these questions it is necessary to know how drugs affect the brain. Thus, much research on the transition to addiction has aimed to identify and characterize brain systems that mediate the rewarding effects of potentially addictive drugs and how these brain systems are changed by drug use. It is well accepted that addictive drugs usurp neural circuitry normally involved in pleasure, incentive motivation, and learning (Berridge and Robinson 1998; Di Chiara 1999; Kelley and Berridge 2002).

Drugs of abuse are a chemically heterogeneous group with very distinct molecular targets. Each drug binds to its distinct initial protein target in the brain and periphery and elicits a distinct combination of behavioral and physiological effects upon acute administration. However, evidence from animal and human models has shown that, despite having very different primary molecular targets in the brain, all drugs of abuse converge on a common circuitry in the brain's limbic system: the mesolimbic dopamine pathway (Koob and Le Moal 2001; Nestler 2001; Volkow et al. 2004; Wise 2004), which includes dopaminergic neurons in the ventral tegmental area (VTA) of the midbrain and their targets in the limbic forebrain, especially the nucleus accumbens (NAc). Furthermore, all drugs of abuse share certain common effects after both acute and chronic exposure (Nestler 2005): they share the common action of increasing the concentration of dopamine (DA) released from mesocorticolimbic projections (Bassareo and Di Chiara 1997; Di Chiara and Imperato 1988; Luscher and Ungless 2006; Pettit and Justice 1989; Weiss et al. 1992). Therefore, the mesolimbic DA system has a general role in the reinforcing effects of drugs (Everitt et al. 2001) (see Figure 15).

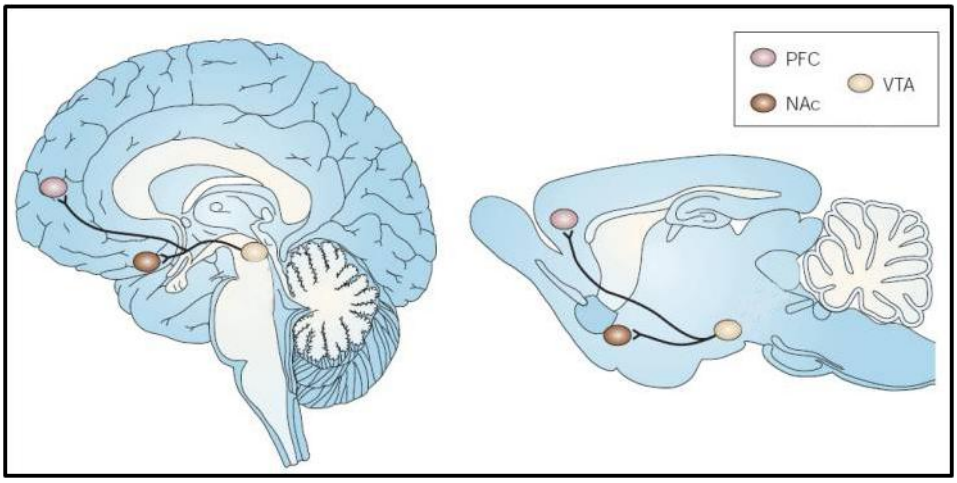

Figure 15 The ventral tegmental area (VTA) and its efferent and afferent systems. Human (left) and rat (right) brains, showing the mesolimbic and mesocortical dopamine pathways, which originate in the VTA and send ascending projections to the nucleus accumbens (NAc) and prefrontal cortex (PFC), respectively. These pathways are strongly activated by drugs of abuse and are implicated in their rewarding and aversive psychological properties. Modified from Laviolette and van der Kooy (2004).

An individual drug may have more than one molecular target; however, the following classification focuses only on those mechanisms that are directly responsible for the increase in dopamine concentration. Luscher and Ungless (2006) define three classes of addictive drugs:

1) Class I: Drugs that activate $G_{i/o}$-coupled receptors [morphine and other opioids, delta-9-tetrahydrocannabinol (THC), and ϒ-hydroxybutyrate (GHB)];
2) Class II: Drugs that mediate their effects via ionotropic receptors (nicotine, benzodiazepines, and ethanol);
3) Class III: Drugs that bind to transporters of biogenic amines [cocaine, amphetamine, methamphetamine (and their many derivates), and methylenedioxymetamphetamine (MDMA, ecstasy)] (see Figure 16).

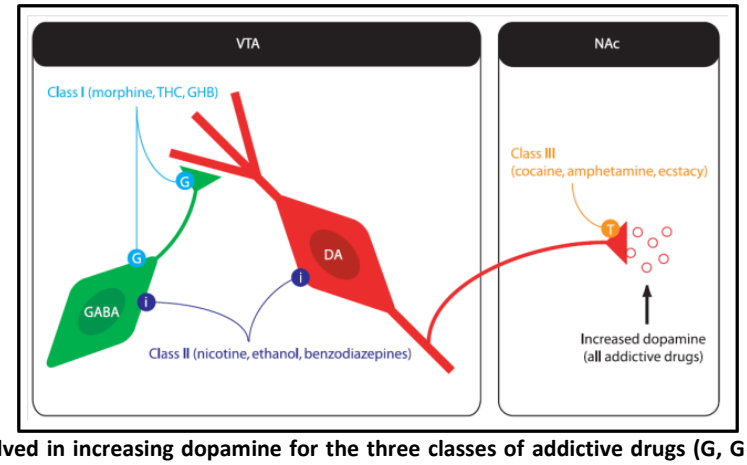

Figure 16 Targets involved in increasing dopamine for the three classes of addictive drugs (G, $G_{i/o}$-coupled receptors; I, ionotropic receptors/ion channels; T, monoamine transporters). Class I: In the VTA, the dominant mechanism of action of these drugs is inhibition of GABA neurons leading to a net disinhibition of dopamine neurons and increased dopamine release. Class II: Addictive drugs that bind to ionotropic receptors and ion channels can have combined effects on dopamine neurons and GABA neurons, eventually leading to enhanced release of dopamine. Class III: Addictive drugs interfering with monoamine transporters block the reuptake of dopamine, or stimulate non-vesicular release of dopamine, causing an accumulation of extracellular dopamine in target structures. Reproduced from Luscher and Ungless (2006).

9.1.1 Opiates

Opiates, including heroin[6], exert their effects by binding to three opioid receptor types (mu, delta and kappa) (De Vries and Shippenberg 2002) and mimicking the actions of endogenous opioid peptides, the endorphins, endomorphins, enkephalins, and dynorphins. Stimulation of the μ-opioid receptor (MOR) subtype is largely responsible for the reinforcing effects of opiates, although some data suggest that delta receptors also play a role in opiate reinforcement (De Vries and Shippenberg 2002; Mello and Negus 1996). MOR are ubiquitous in the CNS, with extensive expression most notably in the ventral tegmental area (VTA), striatum, bed nucleus of the stria terminalis (BNST), and amygdala (Svingos et al. 1996). MOR activation modulates neuronal activity in three primary ways: postsynaptic inhibition, axoaxonic inhibition, and via presynaptic autoreceptors (Yaksh 1997). Activation of MOR by heroin and other opiates disinhibits GABA neurons in the VTA, consequently increasing DA release in the nucleus accumbens and prefrontal cortex (PFC) (Kelley et al. 1980; Pickel et al. 2002).

Furthermore, the discriminative stimulus effects of opioids are centrally mediated (Locke and Holtzman 1985; Valentino et al. 1981), with a crucial role of MOR in this behavior, but opioid agonists such as heroin and morphine have many actions in the CNS, which could potentially serve as the basis for the discrimination of its presence (Shaham and Stewart 1995a; Solecki et al. 2005).

Finally, repeated use of opiates alters the activity of NAc medium spiny neurons that are primary targets of dopamine neurons (De Vries and Shippenberg 2002). Specifically, chronic exposure to opiates causes long-lasting decreases in dendritic arborization and in the density of dendritic spines on cells in brain regions associated with incentive motivation, reward and learning (i.e. nucleus accumbens and prefrontal cortex) (Robinson and Kolb 2004). Repeated use of opiates also induces neuroadaptations in other brain regions (e.g. basolateral amygdala, locus coeruleus, BNST) and neurotransmitter systems (e.g. glutamate, noradrenaline, CRF) (Kreek and Koob 1998). Each of these adaptations may be involved in the expression of the characteristic motivational/somatic withdrawal symptoms that occur upon cessation of the drug. In rodents, this opiate withdrawal syndrome consists of a constellation of measurable behaviors, such as diarrhea, “wet dog” shakes, lacrimation and body weight loss (Schulteis et al. 1994). However, in the absence of any observable signs of withdrawal, rats will avoid cues and contexts previously paired with opiate withdrawal (Frenois et al. 2005). In addition, opiate withdrawal is also characterized by an aversive motivational state reflected by negative affective symptoms, such as anhedonia, dysphoria, irritability and anxiety, which appear before the full expression of somatic withdrawal [nausea, muscle cramps, diarrhea, sweating, fever (“cold turkey”)] (Robbins and Everitt 1999).

6 Heroin, or diacetylmorphine, is a semisynthetic drug produced by chemical modification of the natural alkaloid morphine. The addition of the acetyl group allows heroin to penetrate the blood-brain barrier more quickly than morphine but once in the brain, heroin is rapidly de-acetylated into the active metabolite 6-monoacetylmorphine (6 MAM) which is then de-acetylated again to form morphine. Like morphine, therefore, heroin has a greater affinity for the μ-opioid receptor, whose activation is associated with the induction of analgesia, respiration depression, miosis, reduced gastrointestinal motility and euphoria (Bertalmio et al. 1992; Kamendulis et al. 1996; Leri et al. 2003).

9.1.2 Nicotine

Nicotine sustains tobacco addiction by acting on nicotinic acetylcholine receptors (nAChRs[7]) in the brain. When nicotine binds nAChRs they become cation-permeable and depolarise the cell. Nicotine increases dopamine through a complex interplay of actions at these ionotropic receptors on GABA and dopamine neurons, and glutamatergic inputs to dopamine neurons (Benowitz 2010; Fagen et al. 2003; Mansvelder et al. 2002; Mansvelder and McGehee 2000; Rice and Cragg 2004; Zhang and Sulzer 2004). Nicotine also increases activity in the prefrontal cortex, thalamus and visual system, reflecting activation of corticobasal ganglia–thalamic brain circuits (part of the reward network), and releases dopamine in the striatum (Brody 2006). Insular hypocretin transmission also plays a role in the expression of the motivational properties of nicotine (Hollander et al. 2008).

Nicotine is a reinforcing substance in both humans and animals (Picciotto 1998). In humans, acute nicotine administration produces positive effects like mild euphoria and lightly enhanced cognition (Markou 2008). Smokers use it to modulate levels of arousal and to control mood (e.g. to reduce stress and anxiety). Smoking cigarettes improves concentration, reaction time and performance of certain tasks (Hughes and Hatsukami 1986; Knott et al. 2009). These subjective positive effects support intravenous nicotine self-administration in several species, including rats, mice, non-human primates and humans (Markou and Paterson 2001; Picciotto and Corrigall 2002). Additionally, nicotine (similar to other psychomotor stimulants) enhances the reward value of other stimuli (Harrison et al. 2002).

On the other hand, with repeated nicotine exposure, the GABA system becomes desensitized, leading to a net shift in the action of nicotine to the DA neurons (this is partly mediated by increased glutamatergic input to the DA system). The shift in the functional balance between GABA and DA neuronal populations in the VTA might lead to a dysregulated DA signal in the VTA, which in turn leads to the aversive psychological effects of nicotine craving and withdrawal, and/or to the potentiation of the incentive salience of nicotine and its compulsive use (Laviolette and van der Kooy 2004). Hence, within hours upon cessation of nicotine exposure, a nicotine withdrawal syndrome emerges, which is characterized by depressed mood, dysphoria, insomnia, anxiety, restlessness, irritability, cognitive deficits, decreased heart rate and weight gain (Robbins and Everitt 1999; Shiffman and Waters 2004). In rats, nicotine withdrawal is characterized by increases in somatic signs of withdrawal and affective changes (i.e. increased anxiety-like behavior and reward deficits) analogous to the effects observed in humans (Markou 2008). Furthermore, chronic exposure to nicotine (and cocaine) causes long-lasting increases in dendritic arborization and in the density of dendritic spines on cells in brain regions associated with incentive motivation, reward and learning (i.e. nucleus accumbens and prefrontal cortex) which could contribute to the sensitization state observed after stimulant exposure (Robinson and Kolb 2004). Changes in nAChR receptor number and functions (with different receptors showing differential changes) (Benowitz 2010) and adaptations in glutamate transmission also occur.

7 Each nAChR is composed of five subunits of varying types combined in a variety of stoichiometries; differences in subunit composition yield a number of different nAChR subtypes (Gotti et al. 2007). Current evidence suggests that the reinforcing and reinforcement-enhancing effects of nicotine are mediated by several nAChR subtypes, all of which contain the critical β2 subunit (Besson et al. 2006; Dani et al. 2001; Paterson 2009).

9.1.3 Cocaine

Cocaine is a powerful stimulant drug obtained from the plant *Erythroxylum* coca that has multiple pharmacological actions in both the central and peripheral nervous systems. The hydrochloride (HCl) salt of cocaine dissociates in solution at physiological pH, and free cocaine readily crosses the blood brain barrier. Intravenous (IV) cocaine HCl has almost immediate behavioral and cardiovascular effects, which have been assumed to result from its rapid entry into the brain (Knuepfer and Branch 1992; Tella et al. 1990). However, the central pharmacological effects of cocaine HCl are not particularly fast (Stuber et al. 2005).

In the central nervous system (CNS), cocaine blocks dopamine (DA), noradrenaline (NA), and serotonin (5-HT) uptake through inhibition of their respective transporters. Blocking of the dopamine transporter (DAT) leads to an increase of dopamine concentrations in the nucleus accumbens (Ritz et al. 1990). Therefore, its addiction liability derives primarily from its ability to increase dopamine concentration (Wise et al. 2008).

Cocaine produces euphoria, increases activity, facilitates performance (particularly in situations of fatigue) and decreases appetite (Fischman and Schuster 1982). Acute administration of cocaine has profound metabolic effects on the ventral striatum (Lyons et al. 1996; Porrino 1993) in rodents (it increases cerebral metabolism) (London et al. 1986) and primates (glucose utilization is decreased in the limbic system) (Lyons et al. 1996).

On the contrary, the repeated use of cocaine produces significant neuroadaptations in both structure and function throughout the brain (Porrino et al. 2007). For example, repeated exposure to cocaine causes decreases in the concentration of dopamine D2 receptors (Nader et al. 2002), a long-lasting enhancement in its ability to elevate extracellular glutamate in both the NAc and the VTA (Kalivas and Duffy 1998; Pierce et al. 1996; Reid and Berger 1996; Schmidt and Pierce 2010), and a long-lasting reduction in its capacity to elevate extracellular DA in the PFC (Sorg et al. 1997). Furthermore, chronic abusers of cocaine show reduced rates of frontal metabolism (Volkow et al. 1992), exhibit lack of judgment, disinhibition, difficulty making decisions, unreliability, euphoria, apathy, irritability, and also report depression (Bolla et al. 1998; Cadet and Bolla 1996; Rogers and Robbins 2001).

Summarizing, when drugs of abuse are administered repeatedly, many of their effects begin to change (Badiani and Robinson 2004). However, these neuroadaptations appear to be complicated since different effects (in dopamine function) have been reported for the same drug (because of the difference in drug doses and routes of administration) (Nestler 2005). Nonetheless, according to Koob and Le Moal, Di Chiara and colleagues, and Wise (Di Chiara et al. 2004; Koob and Le Moal 2001; Wise 2004), chronic exposure to any drug of abuse cause an impaired dopamine system as a homeostatic response to repeated drug activation of the dopamine system (tolerance), a reduction in the baseline levels of dopamine function, and sensitization in the dopamine system. Chronic drug exposure is also associated with changes in central corticotrophin-releasing factor (CRF) systems, which mediates the negative emotional symptoms along with many of the somatic symptoms that occur on drug withdrawal (Nestler 2005). In addition, chronic drug exposure causes reduced baseline activity of various regions of frontal cortex (cortical hypofrontality), which are involved in impulsivity and compulsivity (Volkow et al. 2004). Thus, neuroadaptation refers largely to the processes by

which initial drug effects are either enhanced (i.e. sensitization) or attenuated (i.e. tolerance) by repeated drug exposure. In addition, some neuroadaptive changes may be permanent (and dependent on the amount of prior drug exposure), causing long-lasting memories related to drug experience, such that even after prolonged periods of drug withdrawal (months or years), stressful events or exposure to drug-associated cues can trigger intense craving, and in many cases reinstatement of drug use (i.e. relapse) (Pierce and Kalivas 1997; Roberts and Koob 1997).

9.2 Neural Substrates of Reinstatement

The three different stimuli (drug cues, stressors and the drug itself) which have been shown to induce relapse, engage distinct neural circuits, particularly in terms of the circuitry involved in sensory perception. Hence, an addict can easily distinguish between the environmental cues, such as a crack pipe, the effects of taking cocaine, or the stressful life experience, which all precipitate craving and may cause relapse. Furthermore, a component of the different perceptions is the subjective valence that the drug addict attributes to the experience. In this manner, while the drug itself delivers a positive reward, the stressor is interpreted as a negative experience by the drug addict. Although the three types of stimuli are experienced by the drug addict as perfectly different from each other, they all produce a related interoceptive and emotional state (see Section 6) that increases the possibility of performing the same drug-seeking behavior. Given the common behavioral response of drug addicts to all three stimuli, it can be hypothesized that while the neural circuits mediating the perception and conscious interpretation of the stimuli are different, the circuitry mediating the emotional impulse and the initiation of drug-seeking behavior is largely coincident (Kalivas and McFarland 2003).

Thus, according to Kalivas and McFarland (2003) reinstatement of drug-seeking behavior by drug cues, stressors, or the drug itself (drug priming) converge on the medial prefrontal cortex (anterior cingulate cortex) and output through the core of the nucleus accumbens. Cue-induced reinstatement appears to be dependent on activation of the basolateral amygdala and medial prefrontal cortex via a glutamatergic innervation of the nucleus accumbens core. Dopamine innervations of the basolateral amygdala and prefrontal cortex also may have a role (Kalivas and McFarland 2003; Shaham et al. 2003). The extended amygdala (central nucleus of the amygdala and lateral BNST) appears to play an important role in stress-induced reinstatement. Finally, drug-induced reinstatement (from studies with cocaine-trained rats) involves a frontal cortex (cingulate cortex) glutamatergic projection to the nucleus accumbens core, and dopamine projections both to the prefrontal cortex and nucleus accumbens shell (Kalivas and McFarland 2003; Shaham et al. 2003) (see Figure 17).

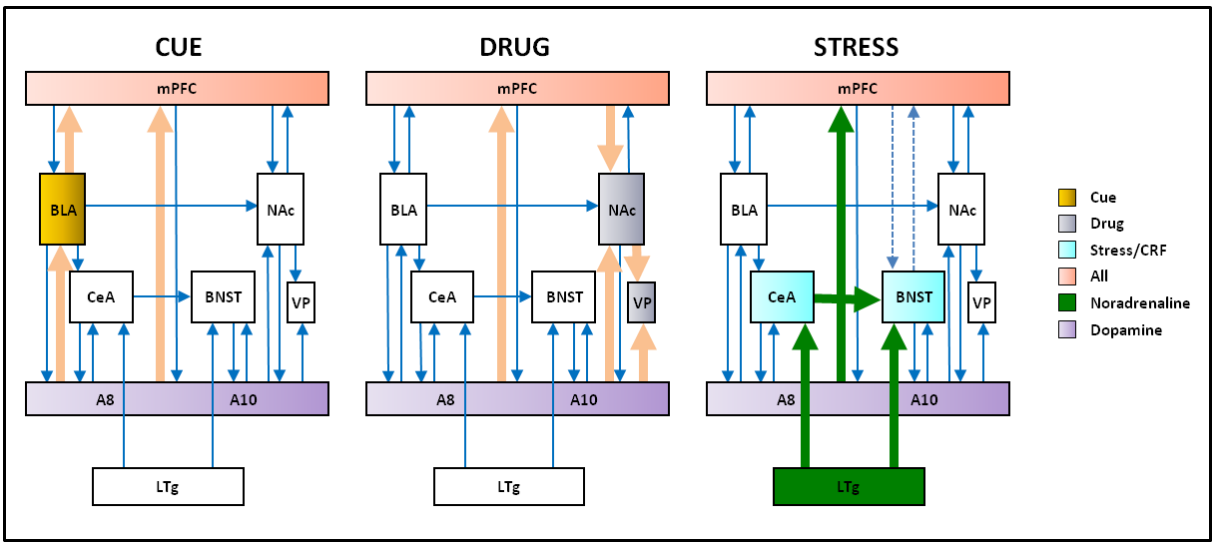

Figure 17 Hypothetical brain circuits critical for the induction of reinstatement of drug-seeking behavior by cues, drugs, and footshock stressor. Most of the data described in this figure is from studies with cocaine-trained rats. In each case, thick arrows indicate pathways that may be involved in reinstatement; thin arrows indicate some of the existing direct anatomical connections and dashed arrows indicate indirect connections. A8 and A10 dopamine (DA) cell groups, BLA basolateral amygdala, BNST bed nucleus of the stria terminalis, CeA central amygdala, CRF corticotropin-releasing factor, LTg noradrenaline cell groups of the lateral tegmental nuclei, mPFC medial prefrontal cortex, NAc nucleus accumbens, VP ventral pallidum. Modified from Shaham et al. (2003).

9.3 Neural Substrates of Habit-based Behaviors in Drug Addiction

9.3.1 Goal-directed vs Habit-based Behaviors

The distinction between two types of behavior (goal-directed vs habit-based) is also paralleled by a distinction between two different neural pathways to action selection. It is known that different cortico-basal ganglia circuits support learning and performance of goal-directed and habit-based behaviors (Balleine and Dickinson 1998; Corbit and Balleine 2003; Killcross and Coutureau 2003; Yin and Knowlton 2004; Yin et al. 2004; 2005a; 2006; Yin et al. 2005b).

Several studies suggest that goal-directed and habit-based behaviors are coordinated on the medial prefrontal cortex. Rats with lesions to the prelimbic medial prefrontal cortex display habit-based instrumental performance that is insensitive to outcome devaluation even with only limited amounts of training (Balleine and Dickinson 1998; Killcross and Coutureau 2003); conversely, animals with lesions to the more ventral infralimbic cortex fail to develop habitual responding despite extended training (Coutureau and Killcross 2003; Killcross and Coutureau 2003). Furthermore, after extended training that produces habitual behavior in rats, goal-oriented behavior can be reinstated if the infralimbic prefrontal cortex is inactivated (Coutureau and Killcross 2003). This finding suggests that the circuits controlling goal-directed behavior may be actively suppressed when behavior becomes habitual (Coutureau & Killcross 2003). Similarly, lesions or inactivation of the dorsolateral but not dorsomedial striatum preserve otherwise habitual actions in a goal-directed mode (in overtrained rats), whereas lesions or pharmacological manipulations of the dorsomedial striatum disrupt the formation of A-O associations (Balleine 2005; Yin et al. 2004; 2006; Yin et al. 2005b). These findings suggest a neural distinction between systems that control goal-directed actions (controlled by the dorsomedial striatum) and S-R habits (controlled by the dorsolateral striatum) (see Figure 18). Furthermore, in a recent study by Lingawi and Balleine (Lingawi and Balleine 2012), and based on

studies where it was shown that the amygdala (specifically the basolateral nucleus) plays a critical role in the acquisition of goal-directed behaviors (Balleine et al. 2003; Ostlund and Balleine 2008a); these authors identified another nucleus of the amygdala (specifically the central nucleus) involved in the acquisition of habits.

Corroborating this dissociation, neuropsychological and neuroimaging studies in humans indicated that goal-directed learning is mediated by the prefrontal cortex, whereas habit learning relies on an intact striatum (Knowlton et al. 1996; Valentin et al. 2007). Thus, behavior develops from being a declarative process (involving prefrontal cognitive processes) into a habitual behavior (utilizing working memory circuitry), which can be physiologically adaptive since well-learned behaviors proceed without conscious involvement, and if the stimulus or context changes, cognitive processes interrupt the habit by developing a new appropriate adaptive behavior (Barnes et al. 2005). However, in the case of drug-seeking, the capacity of cognitive processes to interrupt the drug-seeking habit is impaired (Everitt and Robbins 2005; Kalivas and Volkow 2005).

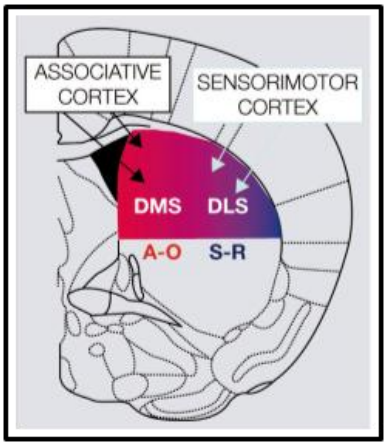

Figure 18 Scheme showing the striatal regions involved in goal-directed behavior (A-O) and habit-based behavior (S-R). Reproduced from Hilario and Costa (2008).

9.3.2 Neurobiological Substrates of Habit-based Behaviors in the Context of Drug Addiction

At the neurobiological level, the habit theory of drug addiction is based on the identification by Haber and colleagues (in primates) (Haber et al. 2000) of a continuum between the ventral and the dorsal region of the striatum (from the nucleus accumbens to the dorsolateral striatum), through spiral connections with the mesencephalon (see Figure 19). The ventral part of the striatum, connected to a set of limbic structures, is involved in the motivational component of behavior (Balleine 2005; Yin et al. 2005b). In contrast, the dorsal part of the striatum (connected to the motor cortex) and its dopaminergic innervations, are involved in behavioral automatisms (Faure et al. 2005; Yin et al. 2004; 2005a). Thus, these spiraling loops permit the progressive automaticity of behavior during extended training (Everitt and Robbins 2005).

Furthermore, there is data supporting this notion. It has been shown that exposure to psychostimulants induces sensitization of dopaminergic transmission in the ventral and dorsal part of the striatum (Paulson and Robinson 1995; Vanderschuren and Kalivas 2000). This potentiation of dopaminergic transmission in the dorsal striatum explains the acceleration of behavioral

automatisms in animals pre-exposed to drugs of abuse (Nelson and Killcross 2006; Nordquist et al. 2007). Interestingly, interactions between ventral and dorsal domains of the striatum (in particular, projections from the nucleus accumbens core to the dorsolateral striatum), mediated by dopaminergic transmission, have been shown to be critical in habitual cocaine-seeking (Belin and Everitt 2008). Moreover, in agreement with this neurobiological framework, it has been shown that self-administration of cocaine in monkeys induces neuroadaptations in the ventral striatum that, with prolonged self-administration, extend gradually to the dorsal striatum (indicating automaticity of self-administration behavior) (Porrino et al. 2004). Taken together, the above results provide an anatomical basis for the behavioral shift (from voluntary drug use to more habitual and compulsive use) that may occur in human addicts.

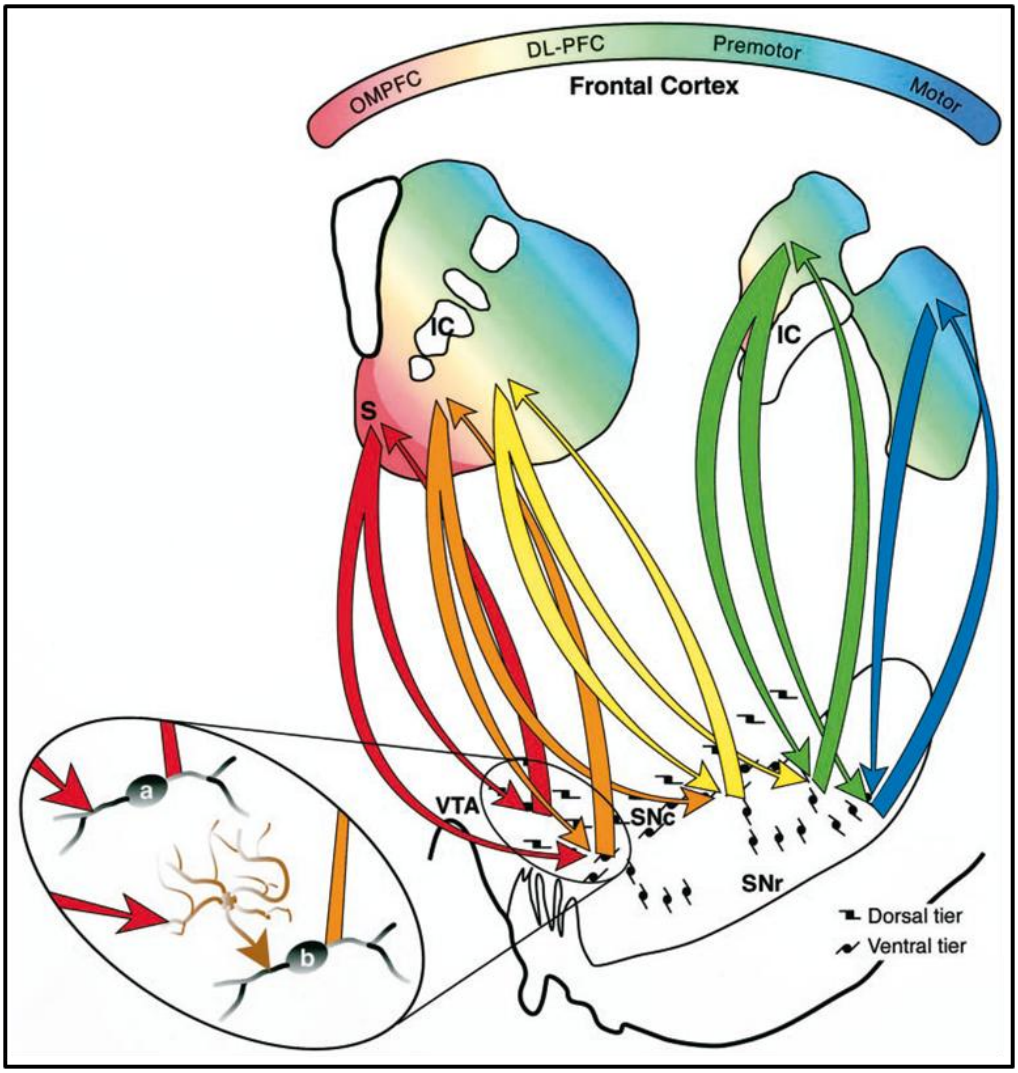

Figure 19 Illustration of spiraling connections between the striatum and the mesencephalon. GABAergic projections from the striatum exert an inhibitory feedback on dopaminergic neurons (neuron "a" on the illustration). This inhibitory feedback is "closed" because it only involves two regions mutually connected. However, the striatal neurons also establish non-reciprocal connections with certain neurons from the mesencephalon. Thus, the striatal neurons inhibit certain local interneurons (GABAergics) from the mesencephalon (neuron "b" on the illustration). This results in a disinhibition of certain dopaminergic neurons, which contribute to propagate the message to different regions of the striatum. These non-reciprocal connections between the striatum and the mesencephalon (spiral connections) permit a continuum between the ventral and dorsal part of the striatum, regions implicated in motivated and automatic behavior, respectively. Reproduced from Haber et al. (2000).

Problem Statement

Significant progress has been made towards elucidation of the different types of stimuli that can precipitate relapse in drug-taking behavior. However, until we understand the factors that determine drug-seeking and drug-taking behavior, it is unlikely that we will be able to provide effective treatments.

Although the factors responsible for the resumption of drug-taking in human addicts are not completely understood, acute re-exposure to the drug has been identified as a major determinant of relapse. Furthermore, in an animal model of relapse, it has been shown that one of the most powerful events for reinstating drug-seeking or drug-taking behavior is a priming injection of the drug itself (Stewart 2000). Nevertheless, the reasons why this situation makes the drug addict very vulnerable are still under debate and several theories are opposed.

Relapse happens in different ways. One theory of relapse suggests that relapse happens through incentive motivation, and another suggests that relapse happens through discriminative signals. Drugs of abuse have motivational effects on individuals, increasing the incentive value of environmental stimuli. When the drug addict is sensitized to these stimuli, a pathological motivation for the drug is generated: an intense desire called craving. Addicts are motivated to take drugs (i.e. they crave drugs) and have a strong desire to re-experience the positive hedonic effects of drugs (i.e. to achieve remembered pleasure) (Robinson and Berridge 2000). Furthermore, drugs of abuse, once consumed, besides their incentive motivational effects, can also function as discriminative stimuli, which reset behavioral automatisms leading the individual to resume drug consumption (de Wit 1996). Thus, according to the incentive motivation view, relapse is a motivated and voluntary process that corresponds to a desire to consume drugs and to the organization of behavior to reach the goal (the drug). However, according to the discriminative signals view, relapse is *not* a voluntary process but an automatic response to certain stimuli previously associated with drug consumption, which leads to stimulus-response association (independent of craving).

Recently, hypotheses concerning the development of drug addiction have centered on the progressive adaptations in the brain with the concept that control over drug use evolves from voluntary drug use (action-outcome) to more habitual (stimulus-response) and compulsive use (Belin et al. 2009; Everitt and Robbins 2005; Vanderschuren and Everitt 2005). In support of this habit theory of drug addiction, previous studies in rats have shown that sensitization to cocaine or amphetamine accelerates the formation of habits during behavioral training for a food reward (Nelson and Killcross 2006; Nordquist et al. 2007; Schoenbaum and Setlow 2005), suggesting that the neuroadaptative changes that these drugs induce are involved in habit formation.

However, supporting evidence for the habit theory of drug addiction has been collected solely with cocaine and amphetamine (psychostimulant drugs). Since relapse is a common feature of all drugs of abuse, it is important to know whether this control over drug use (from voluntary to more habitual use) applies to all drugs of abuse. Despite their different actions in the nervous system, cocaine, heroin and nicotine are drugs of abuse that to some points have common mechanisms, such as activating the dopaminergic system and increasing locomotion. Therefore, these three drugs may use common mechanisms to produce behavioral automatisms.

In our research group, it has been shown that the discriminative stimulus properties of cocaine play a crucial role in relapse. In a study that assessed the relative contribution of the incentive, discriminative and reinforcing properties of cocaine in food-seeking reinstatement, it was demonstrated that when present during operant conditioning for food presentation, the stimulus *cocaine* acquires conditioned properties that can then promote reinstatement of the extinguished operant behavior, which was previously oriented towards food (and not towards drugs) (Keiflin et al. 2008a). These data clearly demonstrate that in this particular setting, which enables the dissociation of the incentive and discriminative properties of cocaine, the discriminative properties (and not the incentive properties) of cocaine alone are able to reinstate an instrumental response.

Furthermore, by using a devaluation procedure, preliminary data from our laboratory has shown that re-exposure to the cocaine stimulus reinstates an extinguished food-seeking behavior previously trained under the influence of cocaine, even when the food outcome has been made aversive. This property seems to be unique to cocaine since the reinstatement of food-seeking induced by the food itself or by an environmental discriminative stimulus remains sensitive to the value of the outcome. The expression of such drug-related habits, independent of volition, could explain the rapid loss of control of former addicts when they re-experience the drug. However, is this true for other drugs of abuse, such as heroin and nicotine?

Aim

The experiments presented in this thesis were conducted with the aim of elucidating whether different drugs of abuse (cocaine, heroin and nicotine), with different interoceptive properties, can trigger habit-based behavior by way of their discriminative stimulus properties.

To this end, the following questions will be approached:

- **Question 1**
  Are the discriminative stimulus properties of heroin and nicotine different from those of cocaine?
  In order to identify the behavioral nature of drug-induced reinstatement, we will assess the relative contribution of the different properties of cocaine, heroin and nicotine (incentive, discriminative and reinforcing) in sucrose-seeking reinstatement.

- **Question 2**
  A further step is to understand the nature of the reinstatement: goal-directed or habit-based behavior. Thus, if we found out that the discriminative stimulus properties of cocaine, heroin and nicotine play a role in reinstatement, we can ask: do the discriminative stimulus properties and/or the neuroadaptations produced by cocaine, heroin and nicotine lead to habit-based behavior?
  In order to identify if reinstatement is a goal-directed or a habit-based behavior, we will assess whether re-exposure to cocaine, heroin and nicotine can reinstate extinguished food-seeking behavior even when the food outcome has been made aversive by a devaluation procedure. The devaluation procedure allows to change the appetitive value of the food to an aversive one. Therefore, if reinstatement of food-seeking is a goal-directed behavior, the new representation of the food should prevent reinstatement, whereas if it is a habit-based behavior, reinstatement should happen independently of the new food value.

- **Question 3**

  Does pretreatment with different drugs of abuse lead to an acceleration of habit-based behavior formation?

  In order to identify the way that drugs of abuse lead to the formation of habits, we will examine the effects of cocaine, heroin and nicotine sensitization on the temporal transition from action-outcome processes to stimulus-response habits for a food reward after outcome devaluation.

These studies are a continuation of a previous thesis in the laboratory and some of the work presented here was done with the assistance of a technician and two Masters students.

Materials and Methods

Each experiment reported in this thesis addresses a specific issue and therefore requires an experimental protocol of its own. The experimental protocols used are each described in the relevant chapter. Only the animal housing and experimental devices common to all experiments are described here.

1. Subjects

Male Wistar rats (HAN) were used as experimental subjects (Charles River Laboratories, France). Upon arrival at the laboratory, the age of these animals was from 5 to 6 weeks old and their weight was between 175 and 225 g. Rats were housed in collective cages in groups of 4. Animals were allowed at least one week familiarization with the vivarium before training began. The conditions of food access varied and will be specified in each chapter. Water was always available *ad libitum*. The rats were weighed three times per week to ensure that their body weight was not reduced below 90%. The home cages were located in a temperature (22 °C) and humidity (60%) controlled animal facility and subjected to a 12 h reverse light/dark cycle (lights on from 20:00 to 08:00 h). All experiments were conducted during the dark phase of the light/dark cycle (activity phase in rats) and were performed in accordance with the European directive (86/609/EEC) and with approval of the Centre National de la Recherche Scientifique (CNRS), concerning the use of laboratory animals. All efforts were made to minimize the number of animals used and their suffering.

2. Apparatus

2.1 Operant Conditioning Chambers

Operant conditioning for liquid sucrose and/or food pellet reinforcement was conducted in twelve standard operant conditioning chambers (30 cm height × 40 cm length × 36 cm depth, Imetronic, Pessac, France) located in an experimental room equipped with white noise generators. Each experimental chamber was individually housed in wood attenuation boxes fitted with ventilation fans and had two clear Plexiglass walls on the front and back sides and two opaque panels on the left and right sides. The floor consisted of 6 mm diameter steel bars spaced 15 mm apart, center-to-center. Two retractable levers (0.03 N force required for activation) were located on either side of the left panel (7 cm above the floor) counterbalanced as active or inactive across left and right. Illumination of a soft house-light bulb (2W) signaled the start of the session.

2.1.1 Operant Conditioning for Sucrose Solution

Each response on the active lever started an external pump fitted with syringes connected with Silastic tubing to a dipper located midway between the two levers in which a 0.2 ml volume of a 5% sucrose solution was delivered in 0.8 s. Responses on the inactive lever were recorded but were without consequence. In order to count each passage of an animal from one side of the cage to the other (crossovers), each experimental chamber was also equipped with two pairs of infrared beams.

Number of lever presses, sucrose solution deliveries, and crossovers were automatically recorded (Imetronic software, Pessac, France) (see Figure 20).

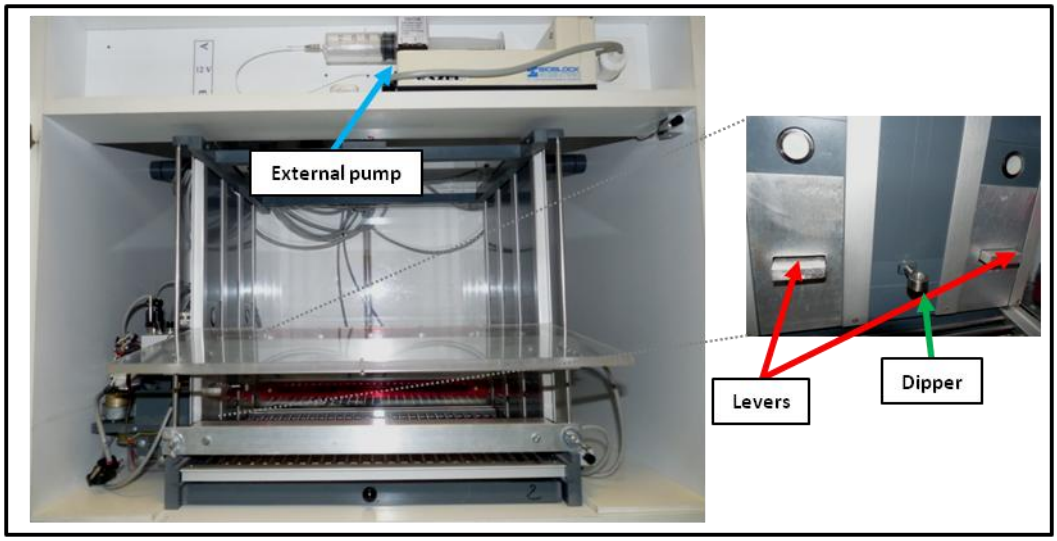

Figure 20 Operant conditioning cages for sucrose solution reinforcement.

2.1.2 Operant Conditioning for Food Pellets

The cages described for sucrose solution reinforcement were the same cages used for food pellet reinforcement. However, in this case, each response on the active lever activated a pellet dispenser located outside the cage allowing the immediate delivery of the food pellets into the food magazine located midway between the two levers. Responses on the inactive lever were recorded but were without consequence. Number of lever presses, food pellets distributed, and crossovers were automatically recorded (Imetronic software, Pessac, France) (see Figure 21).

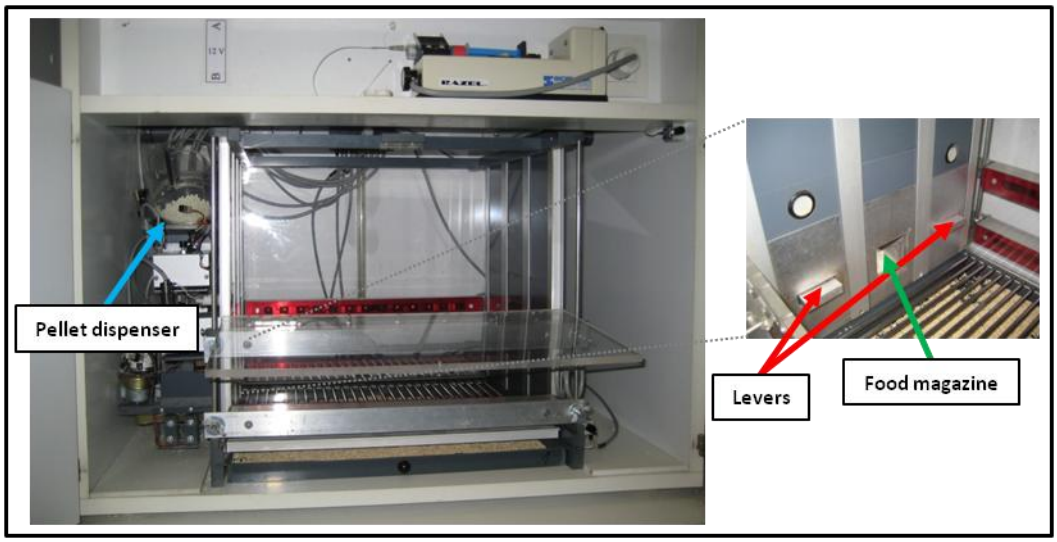

Figure 21 Operant conditioning cages for food pellet reinforcement.

2.2 Locomotor Activity Cages

In addition to the operant conditioning chambers, rats were also exposed to locomotor activity cages. Locomotor activity was conducted in twenty-four locomotor activity cages (35 cm height × 25 cm length × 25 cm depth, Imetronic, Pessac, France) located in a dark experimental room equipped with white noise generators, where neither sucrose solution, food pellets nor levers were present. The door, floor, and ceiling of each experimental cage consisted of wire mesh and the side walls were made of 10 mm thick clear Plexiglass (see Figure 22).

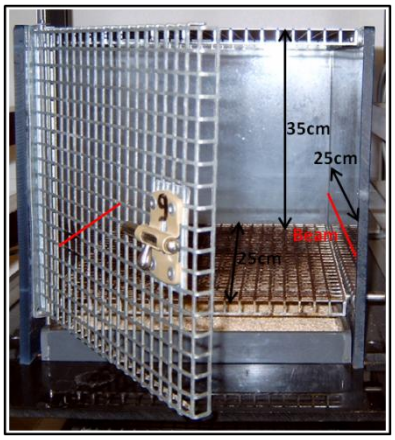

Figure 22 Locomotor activity cages.

3. Material

3.1 Drugs

- Cocaine hydrochloride (Cooperative Pharmaceutique Française, France) at doses of 7.5 mg/kg, 10 mg/kg, and 30 mg/kg.
- Heroin (3,6-diacetylmorphine HCl) (Francopia Sanofi, France) at a dose of 0.25 mg/kg.
- (-) Nicotine hydrogen tartrate (Sigma, St. Louis, MO, USA) at a dose of 0.4 mg/kg.
- *d*-Amphetamine sulphate at a dose of 2 mg/kg.

All drugs were dissolved in sterile physiological saline (0.9% NaCl). Cocaine, heroin and amphetamine doses are expressed as the weight of the salt, whereas the nicotine dose is expressed as the base. The pH of the nicotine solution was adjusted to approximately 7.0 with NaOH before use. Cocaine and amphetamine were administered intraperitoneally and heroin and nicotine were administered subcutaneously. Injection volumes were 1 ml/kg body weight in all experiments. Control treatments consisted of an equivalent volume of saline. Injection routes and doses were selected from previous reports in the literature (Keiflin et al. 2008b; Nelson and Killcross 2006; Rogers et al. 2008; Schoenbaum and Setlow 2005; Shoaib 2006).

3.2 Food Outcomes

- For Experiments 1, 2 and 3, rats were given sucrose solution [D(+)-Sucrose GPR RECTAPUR] as food outcome.

- For Experiments 4, 5, 6 and 7, rats were given food pellets [Pellets formula “P”: Test Diet 1811155 (5TUL) AIN-76A, rodent tablet 45 mg] as food outcome.
- For Experiment 8, rats were given two types of food pellets [Pellets formula “F”: Test Diet 1811251 (5TUT), sucrose tablet 45 mg and Pellets formula “GB”: BioServ F0165 (Dustless Precision Pellets), rodent grain-based diet 45 mg] as food outcome.

4. Statistical Analysis

Data are presented as mean ± standard error of the mean (SEM). Statistics were carried out using STATISTICA version 7.1 for Windows (StatSoft France, 2005). $p<0.05$ was considered statistically significant.

Question 1:
Are the discriminative stimulus properties of heroin and nicotine different from those of cocaine?

Previous work in our team has shown that the discriminative stimulus properties of cocaine play a crucial role in the reinstatement of extinguished responding for a non-drug reinforcer (food pellets) (Keiflin et al. 2008a). In order to evaluate whether this property is a common feature of all drugs of abuse, we compared cocaine to other drugs of abuse (heroin and nicotine) and expanded this study to another type of reinforcer (5% sucrose solution).

We aimed to answer this question in two parts. In the first part of this chapter, analysis was carried out on data from an experiment conducted previously in our laboratory, which examined the involvement of the discriminative stimulus properties of heroin and cocaine in the reinstatement of an extinguished lever pressing response for sucrose. The second part of this chapter examines the involvement of the discriminative stimulus properties of nicotine and cocaine in the reinstatement of an extinguished lever pressing response for sucrose.

The two experiments presented in this chapter resulted in the preparation of a manuscript which is submitted to the journal *Psychopharmacology* for publication. A summary of the experiments is given below, followed by the prepared manuscript in full.

1. Experiment 1:

Study of the discriminative stimulus properties of cocaine and heroin in reinstatement.

Methods summary

Rats were equally exposed to two different environments (operant cages and individual cages) for two daily 30 min sessions across the whole experiment. During the acquisition phase, the rats were trained to press one of two levers (the active lever) for sucrose solution (5%), under a FR-5 schedule of reinforcement, until they reached a level above 100 lever presses per session.

Subsequently, during the conditioning phase, rats were divided into different groups, “inside” and “outside”. The “inside” groups received a non-contingent peripheral injection of either cocaine (10 mg/kg; i.p.), heroin (0.25 mg/kg; s.c.) or NaCl (1 ml/kg; i.p.) before being placed in the operant cages (i.e. named “inside” because the animals would be under the effect of the drug *inside* the operant cage only), whereas the “outside” groups received either cocaine (10 mg/kg; i.p.), heroin (0.25 mg/kg; s.c.) or NaCl (1 ml/kg; i.p.) before being placed in the individual cages, where neither sucrose solution nor levers were present (i.e. named “outside” because they would be under the effect of the drug *outside* the operant cage only). In this way, the discriminative stimulus properties of cocaine and heroin would be associated with operant responses for the “inside” groups, but not for the

"outside" groups. To equate the exposure to injections, the "inside" groups also received NaCl injections in the individual cages, and the "outside" groups also received NaCl injections in the operant cages.

After 10 days of drug treatment, rats were subjected to a period of extinction (14 days), during which lever presses no longer resulted in sucrose solution delivery, and drug injections were replaced by NaCl injections. During this period, rats were still exposed to both environments. Extinction sessions continued until lever presses reached a level below 10 per session, at which point the sucrose-seeking behavior of the rats was considered to be extinguished. Subsequently, reinstatement of this behavior was tested (still under extinction conditions) by non-contingent injections of cocaine (10 mg/kg; i.p.) and heroin (0.25 mg/kg; s.c.) for all groups (cocaine, heroin and NaCl) in both environments ("inside" and "outside"). Each drug-induced reinstatement test was conducted over two days: a saline day and a drug day. Finally, all groups (cocaine, heroin and NaCl) in both environments ("inside" and "outside") were tested with no sucrose solution delivery and with non-contingent sucrose solution delivery over two consecutive days (see Figure 23).

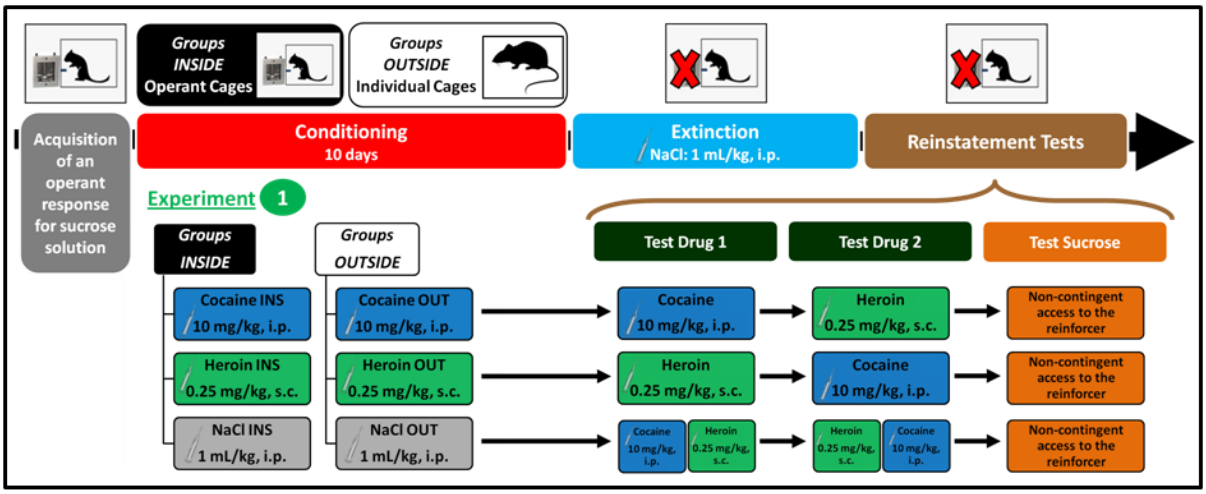

Figure 23 Experimental protocol for Experiment 1. Study of the discriminative stimulus properties of cocaine and heroin in reinstatement.

Results summary

This experiment demonstrated that:

1. Non-contingent injections of cocaine reinstate lever pressing for sucrose in rats that previously performed the operant responses under the effects of cocaine, but not under the effects of heroin.

2. Non-contingent injections of heroin do not reinstate lever pressing for sucrose, neither in cocaine nor in heroin pretreated rats.

3. Non-contingent sucrose solution delivery reinstates lever pressing for sucrose in all groups, independently of drug treatment (cocaine, heroin or NaCl) and of the environment where the drug was experienced ("inside" or "outside" the operant cage), indicating that reinstatement can be effectively induced in all groups.

The present results replicate those from the work of Keiflin (Keiflin et al. 2008a) –regarding cocaine- and extend them to another natural reinforcer (5% sucrose solution). These results also indicate that, when comparing different drugs of abuse, the role that discriminative stimulus properties of drugs play in reinstatement can be critical for certain drugs of abuse, such as cocaine, but not for others, such as heroin.

2. Experiment 2:

Study of the discriminative stimulus properties of cocaine and nicotine in reinstatement.

We have shown that the discriminative stimulus properties of cocaine play a crucial role in reinstatement. However, this does not extend to all drugs of abuse, since our results showed that heroin does not play such a role. This raised the question of whether cocaine is unique in comparison to other drugs of abuse such as heroin or nicotine regarding the involvement of its discriminative stimulus properties in reinstatement. For this reason, the second experiment of my PhD examined the role of the discriminative stimulus properties of nicotine in reinstatement, compared with cocaine.

Methods summary

The experimental procedure was exactly as described for Experiment 1, except nicotine (0.4 mg/kg; s.c.) was tested instead of heroin (see Figure 24).

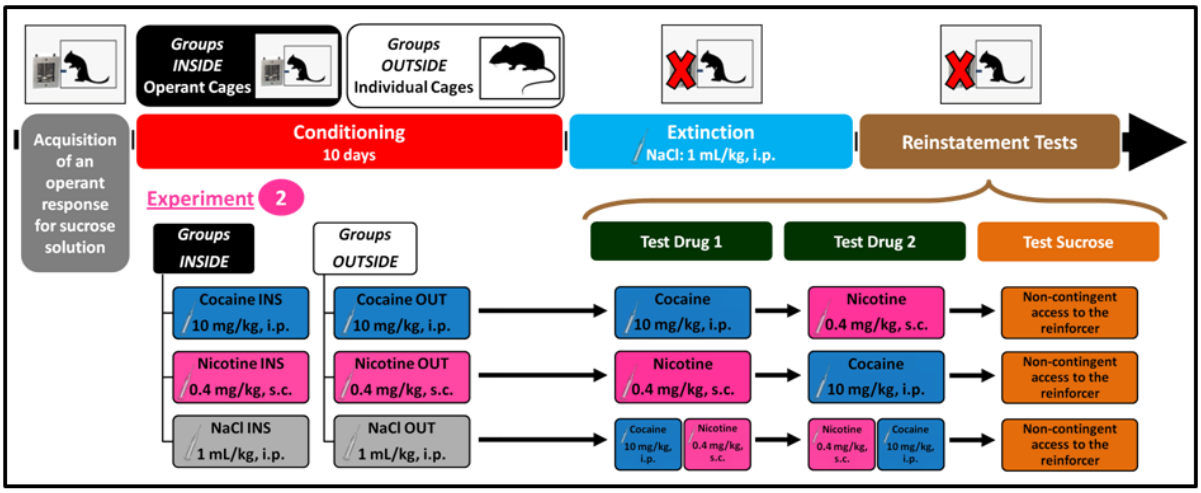

Figure 24 Experimental protocol for Experiment 2. Study of the discriminative stimulus properties of cocaine and nicotine in reinstatement.

Results summary

This experiment demonstrated that:

1. Non-contingent injections of cocaine reinstate lever pressing for sucrose in rats that previously performed the operant responses under the effects of cocaine, but not under the effects of nicotine.

2. Non-contingent injections of nicotine reinstate lever pressing for sucrose in rats that previously performed the operant responses under the effects of nicotine, but not under the effects of cocaine.

3. Non-contingent sucrose solution delivery reinstates lever pressing for sucrose in all groups, independently of drug treatment (cocaine, nicotine or NaCl) and of the environment where the drug was experienced ("inside" or "outside" the operant cage).

The present results indicate that, when comparing different drugs of abuse, the discriminative stimulus properties of cocaine and nicotine play a significant role in driving reinstatement of an extinguished operant behavior not directed towards the drug itself but towards a natural reinforcer (5% sucrose solution), indicating that the animals are using the discriminative stimulus properties of the drugs to guide their behavior.

In conclusion, the results from Experiment 1 and Experiment 2 indicate that the role of discriminative stimulus properties of drugs in relapse can be critical for certain drugs of abuse, such as cocaine and nicotine, but not for others such as heroin. This dissociation suggests that different neuropsychological processes might underlie the relapse process. A better understanding of how the discriminative stimulus properties of drugs of abuse lead to reinstatement will help to understand the relapse process and could provide new directions for relapse prevention therapies.

The work from Experiment 1 and Experiment 2 resulted in the writing of a manuscript which is submitted to *Psychopharmacology* for publication.

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The discriminative stimulus properties of cocaine and nicotine, but not heroin, play a critical role in reinstatement.

María del Carmen González Marín, Caroline Vouillac, Ronald Keiflin and Martine Cador

RATIONALE: Relapse remains the major problem for the treatment of addiction. Using an animal model of drug relapse, it has been shown that a rodent can reinstate drug-seeking behavior when re-exposed to the drug itself. Drugs, in addition to possessing incentive motivational effects, can function as discriminative stimuli and guide behavior. **OBJECTIVE**: We compared, in two separate experiments, the contribution of the discriminative stimulus properties of heroin, nicotine and cocaine to reinstatement of sucrose-seeking behavior. **METHODS:** In Experiment 1, rats were trained to lever press for a sucrose solution while they were under the effects of a non-contingent peripheral injection of cocaine (10 mg/kg; i.p.) or heroin (0.25 mg/kg; s.c.). Additional control groups were trained to lever press for sucrose solution while under saline and received peripheral injections of cocaine and heroin 5 hours later in individual cages, where neither sucrose nor levers were present. Then, rats were subjected to a period of extinction (sucrose-delivery and drug injections ceased). Reinstatement of instrumental sucrose-seeking behaviors under extinction conditions was tested by non-contingent injections of cocaine (10 mg/kg; i.p.) and heroin (0.25 mg/kg; s.c.) and sucrose solution delivery. In experiment 2, the same protocol was followed, except nicotine (0.4 mg/kg; s.c.) was tested instead of heroin. **RESULTS:** Non contingent injections of cocaine and nicotine reinstated lever pressing only in rats that previously performed the operant responses under the effects of cocaine and nicotine respectively, while heroin does not reinstate lever pressing in any group. When the reinforcer itself (sucrose) was non-contingently presented, lever pressing was reinstated in all groups independently of the drug treatments and the environment where the drug state was experienced. **CONCLUSIONS:** These data indicate that the discriminative stimulus properties of cocaine and nicotine, but not heroin, can play a critical role in the reinstatement of an instrumental response.

Introduction

The development of drug addiction involves a transition from casual (recreational) drug taking towards loss of control over this behavior and, ultimately, compulsive drug taking, characterized by a high rate of relapse (Le Moal and Koob 2007), despite the knowledge of adverse consequences (Everitt and Robbins 2005; Koob 2009; Robinson and Berridge 2008).

Relapse to drug taking remains the major problem for the treatment of drug addiction (Stewart 2008). Studies using an animal model of drug relapse (the reinstatement model), have demonstrated that the resumption of drug seeking can be induced by re-exposure to cues previously associated with drug exposure, by acute exposure to stressors, and by re-exposure to the drug itself (Shaham et al. 2003).

Nevertheless, the reasons for such vulnerability are still debated across several hypotheses. According to the incentive sensitization theory (Robinson and Berridge 1993), addiction is the result of sensitized neural systems, primarily the mesolimbic dopamine system. This sensitized state potentiates the capacity of acute drug exposure to intensify the incentive motivational effects of drugs and drug-associated stimuli.

Drugs of abuse, in addition to serving as reinforcers, can also function as discriminative stimuli which "set the occasion" for behavior. Methodologically, the drug sets the occasion on which a response (e.g. lever press) will be reinforced. In this context, the pharmacological (interoceptive) effects, or discriminative stimulus properties of the drug, can function as a conditioned stimulus (CS) and enter into association with other appetitive stimuli (e.g. sucrose) that will activate brain networks for conscious feelings, and for the motivation that guides subject's behavior (Besheer et al. 2004). Therefore, exposure to a drug itself (a drug prime) may be effective in producing reinstatement because the interoceptive stimulus properties produced by the drug set the occasion for further drug seeking behavior (Flagel et al. 2009).

In our research group, we previously assessed the relative contribution of the different properties of cocaine (incentive, discriminative and reinforcing) in cocaine-induced reinstatement. In order to dissociate the discriminative properties from the reinforcing properties of cocaine, which occur when cocaine is self-administered, rats were trained to press a lever for a non-drug reward (food pellets) and experienced cocaine either during the operant conditioning sessions or in another environment without food access. The ability of non-contingent cocaine to reinstate lever pressing associated with food delivery was then assessed after a period of extinction. It was shown that the discriminative stimulus

properties of cocaine play a crucial role in reinstatement since, when present as a stimulus during operant conditioning, cocaine acquires conditioned properties which can then promote reinstatement of an extinguished operant behavior previously oriented towards food pellets (Keiflin et al. 2008a). This study demonstrated that the cocaine-generated interoceptive stimuli can acquire conditioned properties that guide animal's behavior. Since relapse is a common feature of all drugs of abuse, it is important to know whether this applies to all drugs of abuse.

Many drugs of abuse, including cocaine, heroin and nicotine, while having very different primary molecular targets in the brain, have the mutual effect of increasing the concentration of dopamine (DA) released from the mesocorticolimbic DA system (Kalivas et al. 2009; Koob 2009; Luscher and Ungless 2006; Wise 2009; Wolf et al. 2004), which mediates the reinforcing and incentive effects of these drugs (Berridge 2007; Koob and Volkow 2010; Nestler 2005; Pierce and Kumaresan 2006; Robinson and Berridge 1993; Veeneman et al. 2011; Wise 2004).

In order to evaluate whether cocaine's conditioned properties generated by the interoceptive stimuli that guide an animal's behavior are a common feature of all drugs of abuse, we compared cocaine with heroin and nicotine, and extended the study to another type of reinforcer (5% liquid sucrose). Therefore, in the present study, we assessed the relative contribution of the different properties of cocaine, heroin and nicotine (incentive, discriminative and reinforcing) in the drug-induced reinstatement paradigm. In order to clearly dissociate the discriminative stimulus properties from the reinforcing properties of the drug, rats were trained to self administer a non-drug reward (5% liquid sucrose). In Experiment 1, rats were trained to lever press for a sucrose solution while they were under the effects of cocaine or heroin. Additional control groups were trained to lever press for sucrose solution while under saline and experienced 5 hours later cocaine or heroin in individual cages, where neither sucrose nor levers were present. Reinstatement of instrumental sucrose-seeking behaviors under extinction conditions was tested by non-contingent injections of cocaine and heroin and sucrose solution delivery. In experiment 2, the same protocol was followed, except nicotine was tested instead of heroin. If the discriminative stimulus properties of the drugs are important for a relapse process, the groups which have received the drug during the learning of the instrumental responding will reinstate the behavior (though the behavior is not directed towards the drug but towards the sucrose solution) in comparison to the group which have received the same amount of drug but outside the operant responding sessions. In addition, we expect that both groups will reinstate when sucrose solution will be delivered.

Materials and Methods

Subjects

105 male Wistar rats (175-225 g; Charles River Laboratories, France) were housed in groups of four per cage on a 12 h reverse light/dark cycle (lights on from 20:00 h to 08:00 h) in a temperature (22°C) and humidity (60%) controlled room. Rats were acclimated to handling and allowed to adapt to the environment for a minimum of four days prior to the start of the experiment. Experiments were conducted during the dark phase of the light/dark cycle. Water was available ad libitum and food was restricted to 18 g chow per day to maintain rats at 90% of their free-feeding weight. Food was distributed in the home cages everyday at 19:00 h. All experimental procedures were performed in accordance with the European directive (86/609/EEC) and with the approval of the Centre National de la Recherche Scientifique.

Drugs

Cocaine hydrochloride (Cooperative Pharmaceutique Française, France), heroin (3,6-diacetylmorphine HCl) (Francopia Sanofi, France) and (-) nicotine hydrogen tartrate (Sigma, St. Louis, MO, USA) were dissolved in sterile physiological saline (0.9%). The cocaine and heroin doses are expressed as the weight of the salt. The nicotine dose is expressed as the base, and pH was adjusted to approximately 7.0 before use. Cocaine (10 mg/kg) was administered intraperitoneally and heroin (0.25 mg/kg) and nicotine (0.4 mg/kg) were administered subcutaneously. Injection volumes were 1 ml/kg. Injection routes and doses were selected from previous reports in the literature (Keiflin et al. 2008b; Rogers et al. 2008; Shoaib 2006).

Apparatus

Operant conditioning chambers. Operant conditioning for sucrose reinforcement was conducted in twelve standard operant conditioning chambers (30 cm height × 40 cm length × 36 cm depth, Imetronic, Pessac, France) located in an experimental room equipped with white noise generators. Each experimental chamber was individually housed in wood noise attenuation boxes fitted with ventilation fans and had two clear Plexiglass walls on the front and back sides, and two opaque panels on the left and right sides. The floor consisted of 6 mm diameter steel bars spaced 15 mm apart, center-to-center. Two retractable levers (0.03 N force required for activation) were located on either side of the left panel (7 cm above the floor) counterbalanced as active or inactive across front and back. Illumination of a soft house light bulb (2W) signaled the start of the session. Each response on the active lever started an external pump fitted with syringes connected with Silastic tubing to a dipper, located midway between the two levers, in which a 0.2 ml volume of a 5% sucrose solution was delivered in 0.8 s. Responses on the inactive lever were recorded but were without consequence. In order to count each passage of an animal from one side of the cage to the other (crossovers), each experimental chamber was also equipped with two pairs of infrared beams. Number of lever presses, sucrose solution deliveries, and crossovers were automatically recorded (software Imetronic, Pessac, France).

Standard individual chambers. In addition to the operant conditioning chambers, rats were also exposed to standard individual chambers which were very different from the operant chambers (35 cm height × 25 cm length × 25 cm depth, Imetronic, Pessac, France) located in a dark experimental room equipped with white noise generators, where neither sucrose solution nor levers were present. The door, floor, and ceiling of each experimental cage consisted of wire mesh and the side walls were made of 10 mm thick clear Plexiglass.

Experimental Procedure

Experiment 1: The contribution of the discriminative stimulus properties of cocaine and heroin in sucrose-seeking reinstatement.

Operant conditioning and extinction. Rats were equally exposed to two different environments in two daily 30 min sessions. The first environment was the operant conditioning chamber and the second environment was the standard individual chamber as described above. Sessions were run at the same time of the day, 5-6 days per week.

Acquisition

Rats were randomly assigned to one operant conditioning chamber and were trained to press one of the two levers (the active lever) for sucrose solution (5%) delivery. Rats were initially trained under a fixed ratio 1 (FR-1) schedule of reinforcement until they reached a level above 100 lever presses per session; afterwards, the response requirement was raised to FR-5 for two days before the start of the conditioning sessions.

Conditioning

After the second FR-5 session, rats were divided into two groups, “inside” and “outside”, which were each further divided into three goups (cocaine, heroin, saline). The “inside” groups received a non-contingent peripheral injection of cocaine (10 mg/kg; i.p.) (n=11), heroin (0.25 mg/kg; s.c.) (n=7) or saline (n=5) immediately prior to the conditioning sessions in the operant chambers, i.e. the aimals were under the drug state *inside* the operant chambers. In contrast, the “outside” groups received a peripheral injection of cocaine (10 mg/kg; i.p.) (n=12), heroin (0.25 mg/kg; s.c.) (n=10) or saline (n=5) immediately prior to the conditioning sessions in the standard individual chambers, i.e. they were under the drug state *outside* the operant chambers. To equate the exposures to injections, the “inside” groups received saline injections in the standard individual cages, and the “outside” groups received saline injections in the operant chambers.

Extinction

After 10 days of drug treatment, rats were subjected to a period of extinction during which lever presses no longer resulted in sucrose delivery. During this period, rats continued to be exposed to both environments but drug injections were replaced by saline injections. Extinction sessions continued for a minimum of 10 days and until the responding rate fell below 10 per session for 3 consecutive days on the previously active lever.

Reinstatement tests

Following extinction, rats underwent three reinstatement tests, with a minimum of 3 days of extinction between each test. Test sessions were conducted every third day (except when this fell on the first session of the week), providing that subjects maintained the criterion of below 10 lever presses during the previous extinction sessions.

Cocaine-induced reinstatement

Reinstatement of instrumental sucrose-seeking behavior under extinction conditions was tested by a single, non-contingent injection of cocaine for all rats. The cocaine-induced reinstatement test was conducted over two sessions: a saline session and a drug session, separated by 24 h. The first session included a single saline injection administered immediately prior to placing the rat in the operant chamber for a 1 h test session, whereas the second session included a single cocaine injection (10 mg/kg; i.p.) immediately prior to the test session. During each 1 h test session, the house light was illuminated and lever presses had no programmed consequences. Operant responses and locomotor activity (crossovers) were recorded during both reinstatement sessions.

Heroin-induced reinstatement

The reinstatement procedure was exactly the same as for cocaine-induced reinstatement, except heroin (0.25 mg/kg; s.c.) was injected non-contingently.

Note that during the first drug-induced reinstatement test, the drug and dose administered was the same as that administered during the conditioning sessions. Cocaine was administered to the cocaine groups (“inside” and “outside”) and heroin was administered to the heroin groups (“inside” and “outside”). On the contrary, in the second drug-induced reinstatement test, cocaine was administered to the heroin groups (“inside” and “outside”) and heroin was administered to the cocaine groups (“inside” and “outside”). The saline groups received both drugs in a counterbalanced way.

Sucrose-induced reinstatement

The effect of non-contingent sucrose solution delivery was assessed in all rats. The sucrose-induced reinstatement test was conducted over two sessions, as in the drug-induced reinstatement tests. The first session was a control extinction session during which sucrose solution was not delivered. During the second session, 1.2 ml of a sucrose solution (5%) was delivered non-contingently over the first 60 s.

Experiment 2: The contribution of the discriminative stimulus properties of cocaine and nicotine in sucrose-seeking reinstatement.

The experimental procedure was exactly as described for experiment 1, except nicotine (0.4 mg/kg; s.c.) was tested instead of heroin.

Conditioning

After the second FR-5 session, rats were divided into two groups, “inside” and “outside”, which were each further divided into three goups (cocaine, nicotine, saline). The “inside” groups received a non-contingent peripheral injection of cocaine (10 mg/kg; i.p.) (n=12), nicotine (0.4 mg/kg; s.c.) (n=12) or saline (n=7) immediately prior to the conditioning sessions in the operant chambers, i.e. the aimals were under the drug state *inside* the operant chambers. In contrast, the “outside” groups received a peripheral injection of cocaine (10 mg/kg; i.p.) (n=6), nicotine (0.4 mg/kg; s.c.) (n=11) or

saline (n=7) immediately prior to the conditioning sessions in the standard individual chambers, i.e. they were under the drug state *outside* the operant chambers. To equate the exposures to injections, the "inside" groups received saline injections in the standard individual cages, and the "outside" groups received saline injections in the operant chambers.

The experiment then continued to progress exactly as outlined in Experiment 1.

Cocaine-induced reinstatement and sucrose-induced reinstatement

The procedures used for cocaine and sucrose reinstatement tests were identical to those described in Experiment 1.

Nicotine-induced reinstatement

The reinstatement procedure was exactly the same as for cocaine-induced reinstatement except nicotine (0.4 mg/kg; s.c.) replaced cocaine.

Note that during the first drug-induced reinstatement test, the drug and dose administered was the same as that administered during the conditioning sessions. Cocaine was administered to the cocaine groups ("inside" and "outside") and nicotine was administered to the nicotine groups ("inside" and "outside"). On the contrary, in the second drug-induced reinstatement test, cocaine was administered to the nicotine groups ("inside" and "outside") and nicotine was administered to the cocaine groups ("inside" and "outside"). The saline groups received both drugs in a counterbalanced way.

Statistical Analyses

Throughout the experiments a total of 15 rats were excluded due to a failure to meet the acquisition criteria (100 lever presses per session). Operant responses were analyzed with repeated measures analysis of variance (ANOVA). Data from both experiments were analyzed using separate ANOVAs. Between-subjects factors were treatment (three levels: cocaine, heroin, saline; or cocaine, nicotine, saline) and conditioning environment (two levels: inside, outside). Within-subjecs factors were lever (two levels: active, inactive), challenge (three levels: cocaine, heroin, sucrose; cocaine, nicotine, sucrose) and session. Locomotor activity data were analyzed in the same way, with between-subjects factors of treatment (cocaine, heroin, saline; cocaine, nicotine, saline) and conditioning environment (inside, outside), and within-subjects factors of challenge (cocaine, heroin, sucrose; cocaine, nicotine, sucrose) and session. Post hoc analyses were carried out using the Newman-Keuls (NK) test. The level of $p<0.05$ was the criterion for statistical significance. Group data are presented as the mean ± SEM in the text and figures.

Results

Experiment 1: Contribution of the discriminative stimulus properties of cocaine and heroin in sucrose-seeking reinstatement.

Conditioning and extinction of the operant behavior

Figure 1a and b depict the level of operant responses across the conditioning sessions. During the conditioning phase, the global ANOVA revealed no significant interaction between treatment x conditioning environment x levers x sessions ($F(18, 396)=1.27$, n.s.) indicating that operant responses did not differ significantly between groups. However, rats injected with cocaine, heroin and NaCl differed in their operant responses on the active lever during the conditioning phase; rats treated with heroin and NaCl showed higher levels of responding across sessions than rats treated with cocaine, as indicated by a significant treatment x levers x sessions interaction ($F(18, 396)=2.24$, $p<0.002$; Fig 1a,b). Furthermore, cocaine, heroin and NaCl had differential effects on operant responses on the active lever depending on whether the drug state was experienced inside or outside the operant cages; rats experiencing cocaine, heroin or NaCl *outside* the operant cages showed higher levels of responding across sessions than rats experiencing the drug state *inside* the operant cages, as indicated

by a significant conditioning environment x levers x sessions interaction ($F(9,396)=3.27$, $p<0.000$; Fig 1a,b). During extinction sessions, the global ANOVA revealed no significant interaction between treatment x conditioning environment x levers x sessions ($F(26,572)=0.83$, n.s.). In comparison to inactive lever responding, operant responses on the active lever progressively decreased until reaching the extinction criteria after 14 sessions, as indicated by a significant levers x sessions interaction ($F(13,572)=76.45$, $p<0.000$; Fig 1c,d).

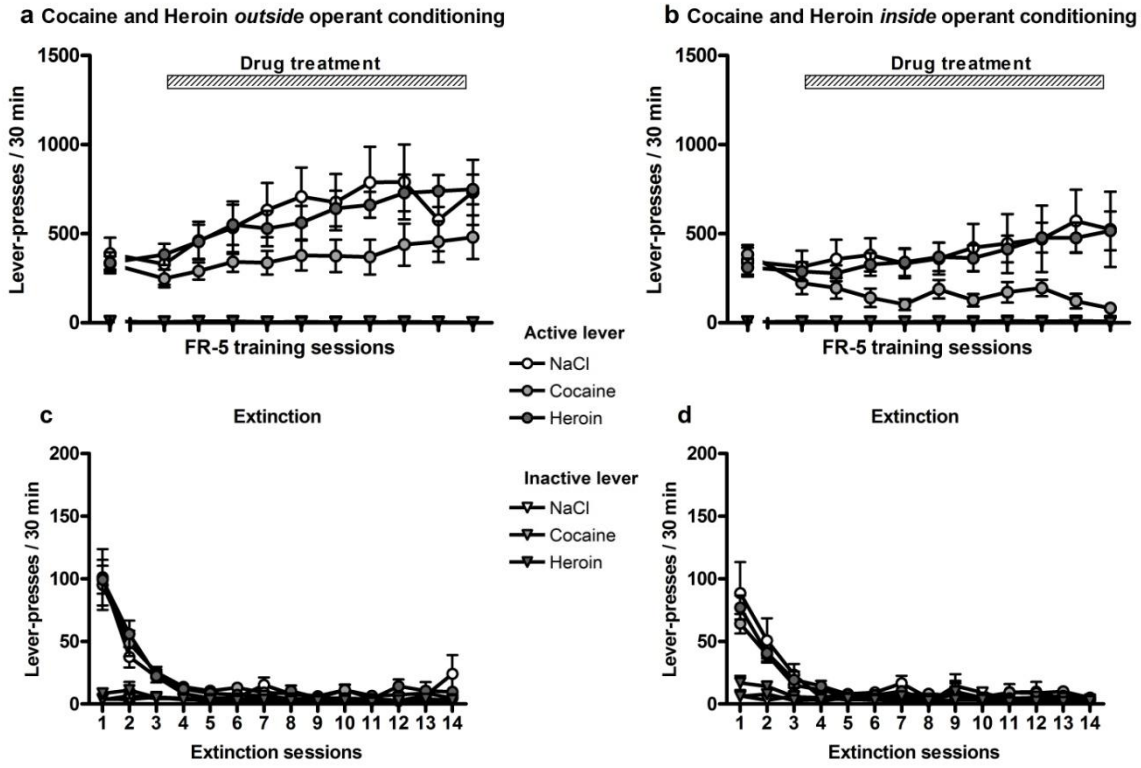

Fig. 1 Conditioning and extinction of an operant sucrose-reinforced behavior; each point represents mean (± SEM) responses per session on the active or inactive lever. **a** and **b** show the acquisition of the operant behavior for rats exposed to different drug treatments (NaCl 1ml/kg, cocaine 10 mg/kg or heroin 0.25 mg/kg) either *outside* (**a**) or *inside* (**b**) the operant cages. **c** and **d** show the extinction of the operant behavior for these same animals previously exposed to NaCl, cocaine or heroin either *outside* (**c**) or *inside* (**d**) the operant cages.

Cocaine reinstates lever pressing in rats that previously performed the operant responses under the effect of cocaine, but not under the effect of heroin

Figure 2a shows the total number of lever presses on the active and inactive levers during the cocaine-induced reinstatement test. The global ANOVA indicated significant main effects of treatment ($F(2,44)=4.55$, $p<0.015$), levers ($F(1,44)=12.31$, $p<0.001$), challenge sessions ($F(1,44)=8.54$, $p<0.005$) and a significant interaction between treatment x conditioning environment x levers x challenge sessions ($F(2,44)=7.42$, $p<0.001$). When compared to control saline injection, the effect of a priming injection of cocaine on operant responding depended on the previous drug history of the rats. The priming injection of cocaine induced a strong reinstatement of lever pressing on the previously sucrose-reinforced lever only in rats that previously performed the operant responses under the effects of cocaine (NK test, $p<0.000$ when compared to their respective control saline injection). No reinstating effect of cocaine was observed in drug naive rats (group NaCl) as well as in rats that had previously experienced cocaine *outside* the operant cages. In heroin pretreated rats, a priming injection of cocaine did not reinstate lever pressing, neither in rats that previously performed the operant responses under the effects of heroin nor in rats that had previously experienced heroin *outside* the operant cages (NK test, n.s.). Figure 2b shows the locomotor activity recorded during the saline and cocaine challenges of the reinstatement phase. The global ANOVA did not indicate a significant

treatment x conditioning environment x challenge sessions interaction (F(2, 44)=0.06, n.s.). However, a priming injection of cocaine induced a motor hyperactivity in all groups of rats in comparison to their respective control saline injection, as indicated by a significant main challenge test effect (F(1,44)=91.40, p<0.000).

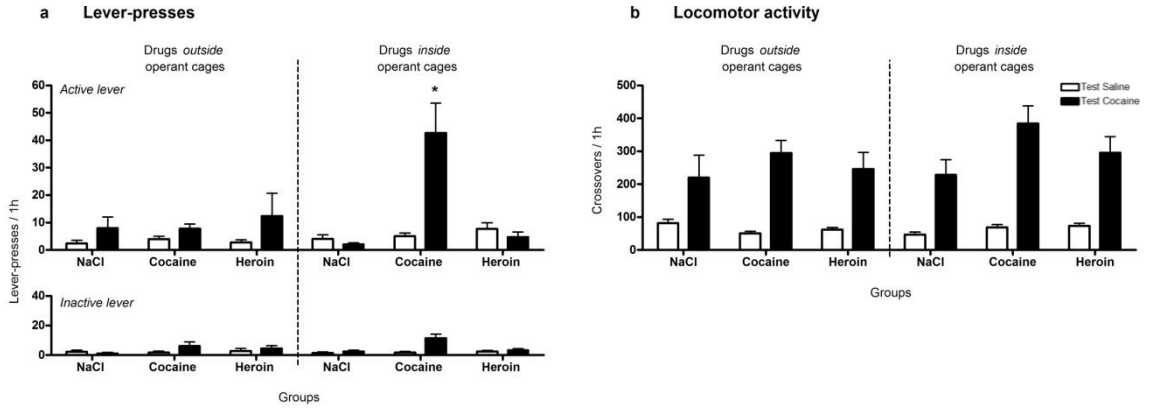

Fig. 2 Cocaine-induced reinstatement test following extinction of an operant sucrose-reinforced behavior. The effects of non-contingent cocaine 10 mg/kg were assessed for rats that had previously experienced NaCl, cocaine or heroin (1 ml/kg, 10 mg/kg and 0.25 mg/kg, respectively) either *outside* or *inside* the operant cages. **a** Histograms represent mean (± SEM) responses on the active or inactive lever. **b** Histograms represent mean (± SEM) crossovers recorded in the operant conditioning chambers during the test. * significantly different from control saline injection (p<0.05).

Heroin does not reinstate lever pressing in cocaine or heroin pretreated rats

Figure 3a shows the total number of lever presses on the active and inactive levers during the heroin-induced reinstatement test. The global ANOVA indicated no significant interaction between treatment x conditioning environment x levers x challenge sessions (F(2,44)=0.00, n.s.). When compared to control saline injection, a priming injection of heroin did not reinstate lever pressing in any group. The ANOVA indicated only a significant levers effect (F(1,44)=7.84, p<0.007). Figure 3b shows the locomotor activity recorded during the saline and heroin challenges of the reinstatement phase. The global ANOVA did not indicate a significant treatment x conditioning environment x challenge sessions interaction (F(2, 44)=1.67, n.s.). However, a priming injection of heroin induced a motor hyperactivity in all groups of rats in comparison to their respective control saline injection, as indicated by a significant challenge test effect (F(1,44)=53.67, p<0.000).

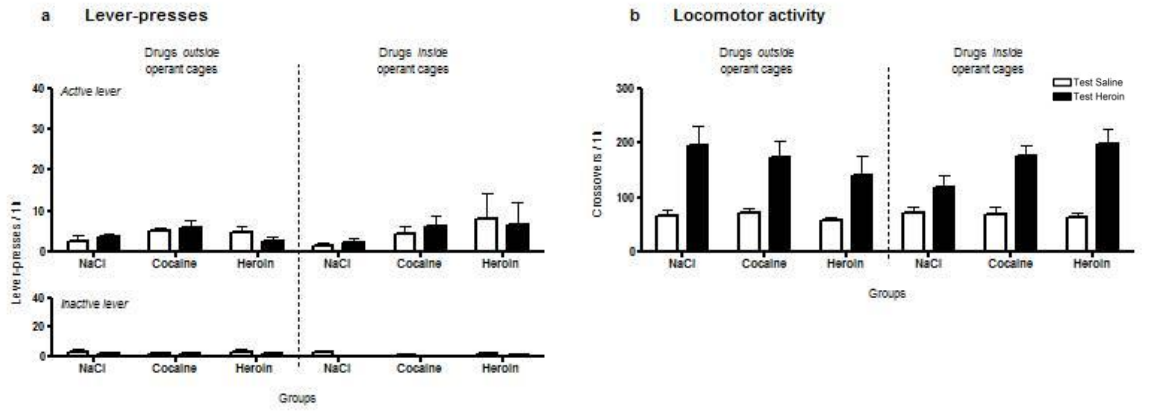

Fig. 3 Heroin-induced reinstatement test following extinction of an operant sucrose-reinforced behavior. The effects of non-contingent heroin 0.25 mg/kg were assessed for rats that had previously experienced NaCl, cocaine or heroin (1 ml/kg, 10 mg/kg and 0.25 mg/kg, respectively) either *outside* or *inside* the operant cages. **a** Histograms represent mean (± SEM) responses on the active or inactive lever. **b** Histograms represent mean (± SEM) crossovers recorded in the operant conditioning chambers during the test.

Non contingent sucrose delivery reinstates lever pressing in all groups, independently of drug treatment (cocaine or heroin)

Figure 4a shows the total number of lever presses on the active and inactive levers during the sucrose-induced reinstatement test. When compared to control session, the non-contingent delivery of sucrose solution prompted reinstatement of lever pressing on the previously sucrose-reinforced lever in all groups of rats, as indicated by a significant levers x challenge sessions interaction ($F(1,44)=44.03$, $p<0.000$). However, this reinstatement did not differ significantly between the "inside" and the "outside" rats as indicated by the non significant treatment x conditioning environment x levers x challenge sessions interaction ($F(2,44)=0.53$, n.s.). Figure 4b shows the locomotor activity recorded during the control and sucrose challenges of the reinstatement phase. The global ANOVA did not indicate a significant main effect of treatment ($F(2,44)=0.85$, n.s.), conditioning environment ($F(1,44)=0.00$, n.s.) and challenge sessions ($F(1,44)=2.84$, n.s.), but a significant interaction between all of these factors ($F(2,44)=5.04$, $p<0.010$). Post hoc NK comparisons indicated that only rats pretreated with NaCl "outside" the operant cages and rats pretreated with heroin "inside" the operant cages showed a motor hyperactivity when compared to the rest of the groups; nevertheless, this motor hyperactivity was more intense in the control session than in the sucrose challenge session.

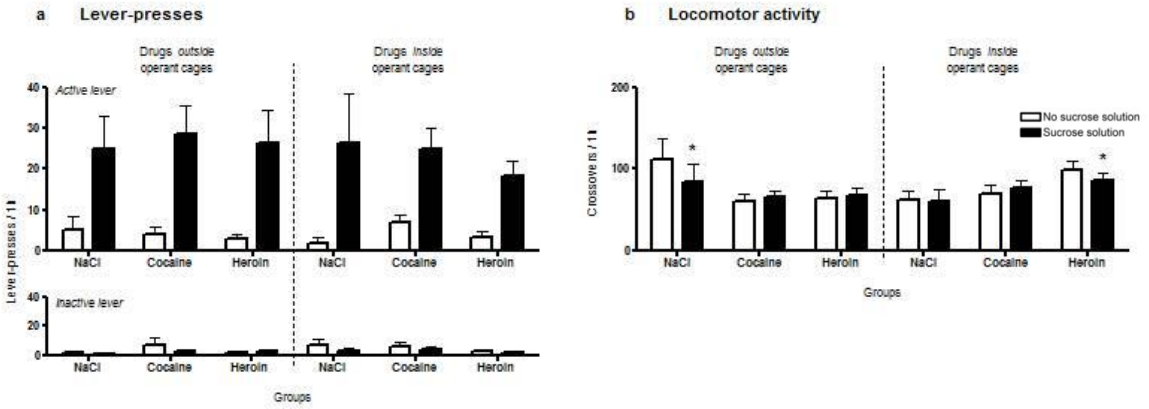

Fig. 4 Sucrose-induced reinstatement test following extinction of an operant sucrose-reinforced behavior. The effects of non-contingent sucrose-delivery were assessed for rats that had previously experienced NaCl, cocaine or heroin (1 ml/kg, 10 mg/kg and 0.25 mg/kg, respectively) either *outside* or *inside* the operant cages. **a** Histograms represent mean (± SEM) responses on the active or inactive lever. **b** Histograms represent mean (± SEM) crossovers recorded in the operant conditioning chambers during the test. * significantly different from control saline injection ($p<0.05$).

Experiment 2: Contribution of the discriminative stimulus properties of cocaine and nicotine in sucrose-seeking reinstatement.

Conditioning and extinction of the operant behavior

Figure 5a and b depict the level of operant responses across the conditioning sessions. During the conditioning phase, the global ANOVA revealed significant effects of treatment ($F(2,49)=6.62$, $p<0.002$), levers ($F(1,49)=283.68$, $p<0.000$), sessions ($F(9,441)=24.27$, $p<0.000$) and a significant interaction between treatment x conditioning environment x levers x sessions ($F(18,441)=2.53$, $p<0.000$). Rats injected with cocaine, nicotine and NaCl differed in their operant responses on the active lever during the conditioning phase; rats treated with NaCl showed higher levels of responding across sessions than those treated with cocaine and nicotine, as indicated by a significant treatment x levers x sessions interaction ($F(18,441)=3.60$, $p<0.000$; Fig 5a,b). Post hoc NK comparisons indicated that drug treatment had a significant effect on responding in rats performing the operant behavior while under the effects of the drugs. In comparison to rats treated with NaCl, cocaine treatment decreased operant responding on the active lever across sessions (starting from session 4) (Fig 5b; NK test, $p<0.000$), whereas nicotine treatment showed this decreased only in the last two sessions of the conditioning when compared to rats treated with NaCl (Fig 5b; NK test, $p<0.011$). During extinction sessions, the global ANOVA revealed no significant treatment x conditioning environment x levers x

sessions interaction (F(26,637)=0.92, n.s.). In comparison to inactive lever responding, operant responses on the active lever progressively decreased until reaching the extinction criteria after 14 sessions, as indicated by a significant levers x sessions interaction (F(13,637)=146.33, p<0.000; Fig 5c,d). These observations reproduce the data obtained in "Experiment 1".

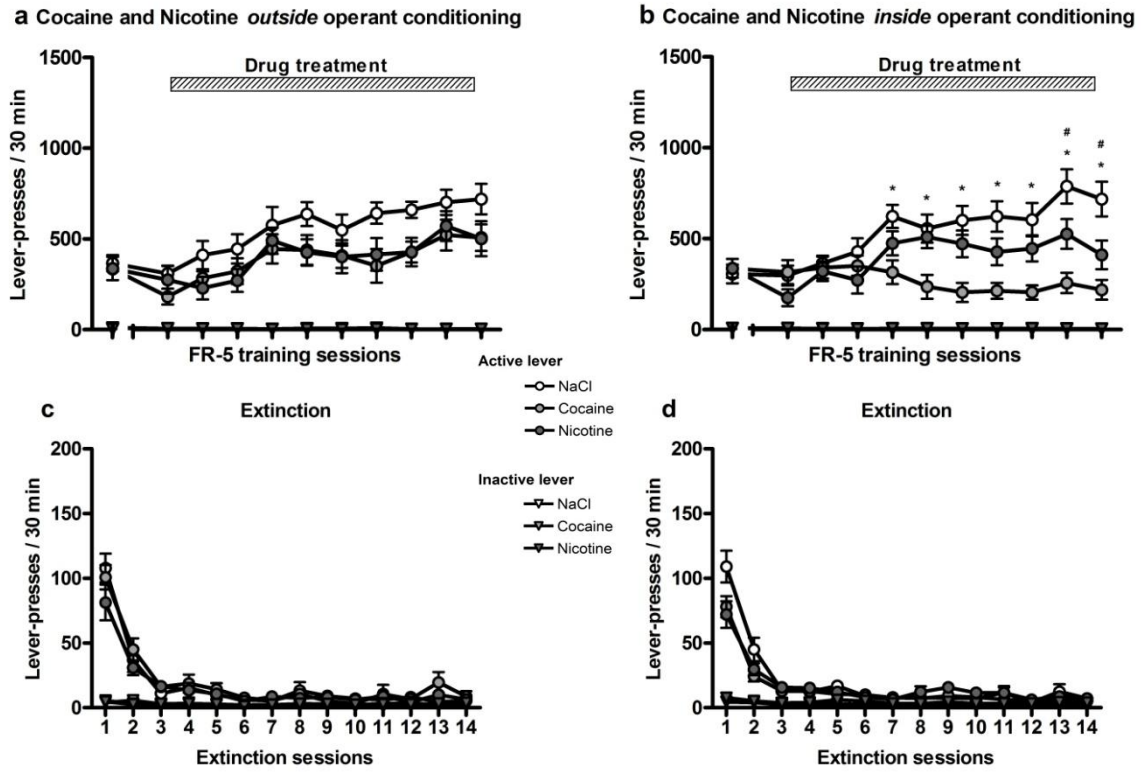

Fig. 5 Conditioning and extinction of an operant sucrose-reinforced behavior; each point represents mean (± SEM) responses per session on the active or inactive lever. **a** and **b** show the acquisition of the operant behavior for rats exposed to different drug treatments (NaCl 1ml/kg, cocaine 10 mg/kg or nicotine 0.4 mg/kg) either *outside* (**a**) or *inside* (**b**) the operant cages. **c** and **d** show the extinction of the operant behavior for these same animals previously exposed to NaCl, cocaine or nicotine either *outside* (**c**) or *inside* (**d**) the operant cages. * Cocaine group is significantly different from NaCl group (p<0.05). # Nicotine group is significantly different from NaCl group (p<0.05).

Cocaine reinstates lever pressing in rats that previously performed the operant responses under the effect of cocaine, but not under the effect of nicotine

Figure 6a shows the total number of lever presses on the active and inactive levers during the cocaine-induced reinstatement test. The global ANOVA indicated significant main effects of levers (F(1,49)=24.19, p<0.000), challenge sessions (F(1,49)=6.88, p<0.011) and a significant interaction between treatment x conditioning environment x levers x challenge sessions (F(2,49)=3.19, p<0.049). When compared to control saline injection, the effect of a priming injection of cocaine on operant responding depended on the previous drug history of the rats. The priming injection of cocaine induced a strong reinstatement of lever pressing on the previously sucrose-reinforced lever only in rats that previously performed the operant responses under the effect of cocaine (NK test, p<0.000 when compared to their respective control saline injection). No reinstating effect of cocaine was observed in drug naive rats (NaCl group) as well as in rats that had previously experienced cocaine *outside* the operant cages. In nicotine pretreated rats, a priming injection of cocaine did not reinstate lever pressing in rats that previously performed the operant responses under the effects of nicotine or in rats that had previously experienced nicotine *outside* the operant cages (NK test, n.s.). Figure 6b shows the locomotor activity recorded during the saline and cocaine challenges of the reinstatement phase. A priming injection of cocaine induced a motor hyperactivity in all groups of rats in comparison to their respective control saline injection, as indicated by a significant main challenge test effect

(F(1,49)=54.66, p<0.000). However, this motor hyperactivity did not differ between "outside" and "inside" groups as indicated by a non significant treatment x conditioning environment x challenge sessions interaction (F(2, 49)=0.99, n.s.). These observations reproduce the data obtained in Experiment 1.

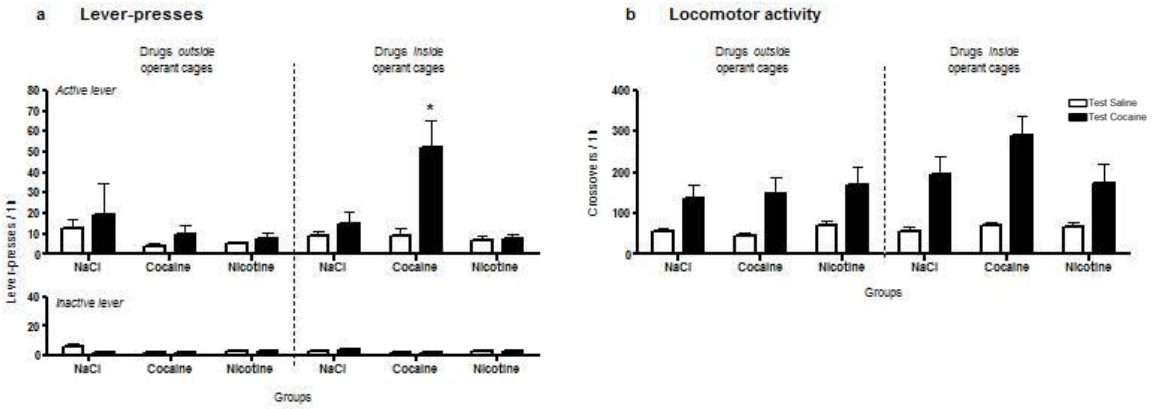

Fig. 6 Cocaine-induced reinstatement test following extinction of an operant sucrose-reinforced behavior. The effects of non-contingent Cocaine 10 mg/kg were assessed for rats that had previously experienced NaCl, cocaine or nicotine (1 ml/kg, 10 mg/kg and 0.4 mg/kg, respectively) either *outside* or *inside* the operant cages. **a** Histograms represent mean (± SEM) responses on the active or inactive lever. **b** Histograms represent mean (± SEM) crossovers recorded in the operant conditioning chambers during the test. * significantly different from control saline injection (p<0.05).

Nicotine reinstates lever pressing in rats that previously performed the operant responses under the effect of nicotine, but not under the effect of cocaine

Figure 7a shows the total number of lever presses on the active and inactive levers during the nicotine-induced reinstatement test. The global ANOVA indicated significant main effects of treatment (F(2,49)=17.98, p<0.000), conditioning environment (F(1,49)=9.27, p<0.003), levers (F(1,49)=48.91, p<0.000), challenge sessions (F(1,49)=4.39, p<0.041) and a significant interaction between all of these factors (F(2,49)=10.34, p<0.000). When compared to control saline injection, the effect of a priming injection of nicotine on operant responding depended on the previous drug history of the rats. The priming injection of nicotine induced a strong reinstatement of lever pressing on the previously sucrose-reinforced lever only in rats that previously performed the operant responses under the effect of nicotine (NK test, p<0.000 when compared to their respective control saline injection). No reinstating effect of nicotine was observed in drug naïve rats (NaCl group) or in rats that had previously experienced nicotine *outside* the operant cages. In cocaine pretreated rats, a priming injection of nicotine did not reinstate lever pressing in rats that previously performed the operant responses under the effects of cocaine nor in rats that had previously experienced cocaine *outside* the operant cages (NK test, n.s.). Figure 7b shows the locomotor activity recorded during the saline and nicotine challenges of the reinstatement phase. A priming injection of nicotine induced a motor hyperactivity in all groups of rats in comparison to their respective control saline injection, as indicated by a significant main challenge test effect (F(1,49)=22.12, p<0.000). However, this motor hyperactivity did not differ between "outside" and "inside" groups as indicated by a non significant treatment x conditioning environment x challenge sessions interaction (F(2,49)=0.40, n.s.). The global ANOVA also indicated a significant treatment x challenge sessions interaction (F(2,49)=8.58, p<0.000). Post hoc NK comparisons on this interaction revealed that this motor hyperactivity was more intense in rats previously treated with nicotine than in rats previously treated with NaCl and cocaine, when compared to the control saline session.

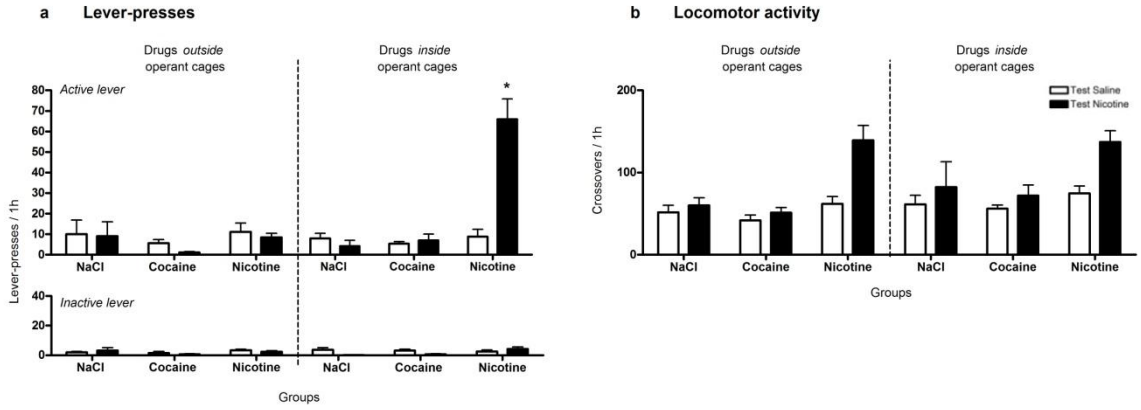

Fig. 7 Nicotine-induced reinstatement test following extinction of an operant sucrose-reinforced behavior. The effects of non-contingent nicotine 0.4 mg/kg were assessed for rats that had previously experienced NaCl, cocaine or nicotine (1 ml/kg, 10 mg/kg and 0.4 mg/kg, respectively) either *outside* or *inside* the operant cages. **a** Histograms represent mean (± SEM) responses on the active or inactive lever. **b** Histograms represent mean (± SEM) crossovers recorded in the operant conditioning chambers during the test. * significantly different from control saline injection ($p<0.05$).

Non contingent sucrose delivery reinstates lever pressing in all groups, independently of drug treatment (cocaine or nicotine)

Figure 8a shows the total number of lever presses on the active and inactive levers during the sucrose-induced reinstatement test. When compared to the control session, the non-contingent delivery of sucrose solution prompted reinstatement of lever pressing on the previously sucrose-reinforced lever in all groups of rats, as indicated by a significant levers x challenge sessions interaction ($F(1,49)=88.65$, $p<0.000$). However, this reinstatement did not differ significantly between the "inside" and the "outside" groups as indicated by the non significant treatment x conditioning environment x levers x challenge sessions interaction ($F(2,49)=1.81$ n.s). Figure 8b shows the locomotor activity recorded during the control and sucrose challenges of the reinstatement phase. The global ANOVA indicated no significant interaction between treatment x conditioning environment x challenge sessions ($F(2,49)=1.09$, n.s.). The non-contingent delivery of sucrose solution had no differential effects on locomotor activity, as indicated by a non significant challenge sessions effect ($F(1,49)=0.52$, n.s.). The global ANOVA also indicated a significant treatment x challenge sessions interaction ($F(2,49)=7.40$, $p<0.001$). Post hoc NK comparisons on this interaction indicated that rats pretreated with nicotine showed a different level of activity than rats pretreated with NaCl and cocaine, when compared to the control session.

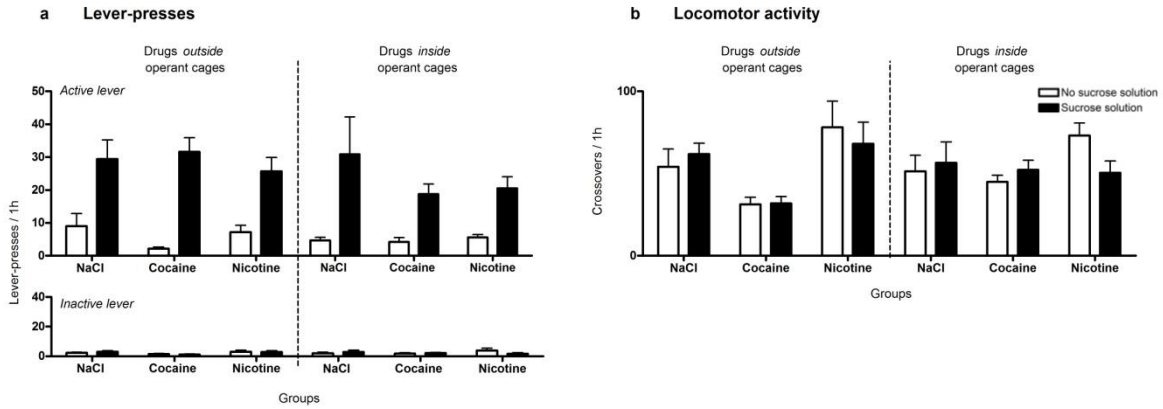

Fig. 8 Sucrose-induced reinstatement test following extinction of an operant sucrose-reinforced behavior. The effects of non-contingent sucrose-delivery were assessed for rats that had previously experienced NaCl, cocaine or nicotine (1 ml/kg, 10 mg/kg and 0.4 mg/kg, respectively) either *outside* or *inside* the operant cages. **a** Histograms represent mean (± SEM) responses on the active or inactive lever. **b** Histograms represent mean (± SEM) crossovers recorded in the operant conditioning chambers during the test.

Discussion

This study demonstrates that cocaine and nicotine, but not heroin, when present as a stimulus during operant conditioning for a non-drug reward, acquire discriminative properties which can then promote reinstatement of operant responding for the same non-drug reward after a period of extinction. The present results are in agreement with those previously obtained with cocaine and food pellets (Keiflin et al. 2008a), and demonstrate that the effect extends to another natural reinforcer (5% sucrose solution). Importantly, our findings demonstrate that not all drugs of abuse can serve as discriminative stimuli to promote reinstatement of an operant response.

Rats that had previously experienced cocaine, heroin or nicotine either *inside* or *outside* the operant cages expressed increased locomotor activity during the reinstatement session when challenged with any of these drugs prior to the session. This indicates that the specificity of the reinstatement observed in rats that had previously performed the operant responding under the effects of cocaine and nicotine, and the lack of reinstatement in the heroin rats, is not due to a decreased sensitivity to the psychomotor effects of the drugs, or to a different degree of locomotor sensitization. Furthermore, when the reinforcer itself (5% sucrose solution) is non-contingently presented, all groups reinstated lever pressing, independently of their drug history, indicating that reinstatement can be efficiently induced in all groups with an appropriate challenge. This finding shows that: 1) the absence of cocaine and nicotine-induced reinstatement seen in rats that had experienced the effect of these drugs *outside* the operant cages, and 2) the absence of heroin-induced reinstatement in rats that had experienced heroin either *inside* or *outside* the operant cages, is not a consequence of forgetting the original operant task, since these same rats reinstated the extinguished operant behavior when challenged with the original reinforcer (5% sucrose solution).

Decreasing effects of cocaine and nicotine, but not heroin, in operant responding during conditioning

The highly palatable sucrose solution used as a reinforcer in this experiment led to very high levels of responding. However, the groups that experienced cocaine and nicotine during operant conditioning showed much lower levels of instrumental responses when compared to the NaCl group; whereas the group that experienced heroin during operant conditioning did not show such a decrease. This decrease in operant responding can be explained by the classical disruptive effects of psychostimulants on a high rate of operant responding (McMillan and Katz 2002). In accordance, the rats that experienced the effects of the drug *outside* the instrumental sessions did not show this decrease in their level of responding.

Altogether, it seems that rats trained under the effects of cocaine showed the lowest levels of instrumental responding during the conditioning phase. Nevertheless, this decrease in responding was not reflected in reinstatement, since these rats, along with the rats trained under nicotine, showed cocaine and nicotine-induced reinstatement, respectively. This is in accordance with the study by Keiflin et al. (2008a), where it was shown that cocaine induced reinstatement of responding to food pellets in all rats, despite the fact that cocaine treatment dose-dependently (5, 10 or 15 mg/kg) decreased instrumental responding during the conditioning phase. Interestingly, in the present study, rats trained under heroin did not show a decrease in responding during the conditioning phase, perhaps because exposure to opiates is associated with an increase in consumption of highly palatable food and an increase in the willingness to work for it (Kelley and Berridge 2002). Surprisingly, these rats were the only ones that did not show drug-induced reinstatement.

The high level of response we see during the first couple of extinction sessions is a classical response during extinction, a response to the sucrose-predictive cues - the levers and sucrose dipper - that were present during the conditioning phase and continued to be present during the extinction sessions (Wise and Kiyatkin 2011).

This high level of responding will continue until the rats make a new association between the interoceptive stimulus poperties of saline and the operant response, as suggested by Wise and Kiyatkin (2011). In our experiment, this association was made after approximately 4 sessions for all the groups, independently of drug treatment, although in general, the *inside* groups showed lower levels of responding at the first extinction session than the *outside* groups. One possible explanation could be

that, since the *inside* groups were those which experienced the effects of the drugs in the operant cage during the conditioning (where they lever press less than the *outside* groups), they decrease operant responding during extinction as well; perhaps as a reflection of the association between drug effects, operant cage and lever presses experienced during the conditioning phase.

Cocaine and nicotine, but not heroin, induced reinstatement of an extinguished operant behavior

The absence of cocaine- and nicotine-induced reinstatement in the *outside* groups is especially interesting because it suggests that rats are using the discriminative stimulus properties of cocaine and nicotine to guide their behavior. Again, these results are consistent with the cocaine study by Keiflin et al. (2008a). The present results from Experiment 1 showed that heroin injection did not result in resumption of lever pressing in any group. Three possible explanations can be raised: 1) The discriminative stimulus properties of heroin do not guide behavior, since cocaine induced reinstatement, and the discriminative stimulus effects of heroin and cocaine are mediated by different pharmacological mechanisms of action (Lamas et al. 1998). 2) The heroin rats needed a longer period of abstinence before reinstatement could be shown, since it has been demonstrated that drug-seeking behavior increases as the period of abstinence increases (Grigson and Twining 2002; Grimm et al. 2001; Shalev et al. 2001). This "incubation effect" might be specific to some drugs of abuse like heroin. In our experiment, the period of abstinence was of 15 days. 3) Reinstatement induced by heroin might be specific to the training and priming dose. In experiments in which the animals self-administered the drug, the priming dose is given by the experimenter, in a different dose than the one they received during the training. In our experiment the priming dose is the same dose given during the training. Furthermore, the effects of a drug after a period of abstinence may be very different from the effects of the same drug during chronic use (Stitzer and Cox 1996).

Lack of overlap between the discriminative stimulus properties of cocaine and heroin, and of cocaine and nicotine

We did not find any overlap between the discriminative stimulus properties of cocaine and heroin, which is in accordance with previous studies which have shown that a non-contingent injection of heroin fails to induce reinstatement in rats trained to self-administered cocaine (Comer et al. 1993; de Wit and Stewart 1981; 1983) and, in the same way, cocaine fails to induce reinstatement in rats trained to self-administered heroin (de Wit and Stewart 1983). This suggests that the interoceptive effects generated by cocaine are not similar to those generated by heroin, and that the reinstatement effect is pharmacologically specific (de Wit 1996).

Furthermore, it has been shown that drugs from the same pharmacological class, or those that affect the same neurotransmitter system as the drug received during the training, function most effectively as primes (Carroll and Comer 1996), suggesting that stimulation of mesolimbic DA activity is not the only mechanism mediating relapse.

In drug discrimination experiments, it has been shown that heroin dose-dependently substitutes in heroin-trained rats but not in cocaine-trained rats (Lamas et al. 1998) and in the same way, cocaine dose-dependently substitutes in cocaine-trained rats but not in most heroin-trained rats (Lamas et al. 1998), indicating that the discriminative stimulus effects of heroin and cocaine are mediated by different pharmacological mechanisms of action: the discriminative stimulus effects of heroin are mediated primarily at μ-opioid receptors (Bowen et al. 2002; Platt et al. 2001; Solecki et al. 2005) and those of cocaine are mediated by an action at dopaminergic systems (Platt et al. 2010). However, there are studies that have shown that heroin and cocaine, despite being pharmacologically distinct, can share interoceptive effects in cocaine-trained monkeys (Rowlett et al. 2004), suggesting that the extent to which heroin and cocaine share discriminative stimulus effects depends on the training drug, with heroin more likely to engender cocaine-like effects that the other way around. Although it is of scientific interest to study different orders of drug presentation and/or higher doses, we did not consider it practical. Nonetheless, we cannot rule out species (rats vs. monkeys) or procedural differences (routes of administration) that might underlie these different findings.

Regarding cocaine and nicotine, since both drugs are psychostimulant drugs, we asked if the discriminative stimulus properties of cocaine might be similar to those of nicotine. When we examined the interaction between cocaine and nicotine, we did not find any overlap between the discriminative

stimulus properties of cocaine and nicotine, suggesting that the discriminative properties of cocaine and nicotine are highly specific.

Non-contingent presentation of sucrose reinstated an extinguished operant behavior in all groups, independently of their drug history

When the reinforcer itself (sucrose) was non-contingently presented, all groups reinstated lever presses, independently of their drug history, indicating that reinstatement can be effectively induced in all groups.

Even if both cocaine and nicotine reinstate lever pressing through their respective discriminative stimulus properties, it is worth noting that sucrose, cocaine and nicotine induced different levels of reinstatement. These drugs increased lever presses on the active lever more than five times when compared to the control session, whereas sucrose increased responding about three times when compared to the control session. This difference could be explained by the discrete nature of sucrose solution delivery (1.2 mL of sucrose solution delivered non-contingently over the first 60 s) compared to the lasting psychostimulant effects of cocaine and nicotine. Regarding responding on the inactive lever, it is not clear whether the elevation in responding during reinstatement indicates an increase in general activity or a decrease in discrimination between active and inactive levers.

Incentive salience

The incentive sensitization theory posits that exposure to addictive drugs transforms sensory information about rewards and their cues into attractive incentives (Berridge and Robinson 1998; 2003; Robinson and Berridge 2003). According to this theory, the neural sensitization of the dopaminergic system by cocaine, heroin and nicotine should have modified the value of the reward (taste and smell of sucrose solution) and its cue (lever) into a "wanted motivational magnet". Our results do not support this view, since rats that experienced the drug state *outside* the operant cages did not reinstate lever pressing when challenged with the drug, suggesting that drug-treated rats did not simply attribute more incentive salience to stimuli predictive of reward (in this case, the lever). Another interpretation of this result is that the *outside* groups never associated the presence of the drug stimulus with the sucrose reinforced response; therefore, the challenge with the drug stimulus in the operant cage did not set the occasion for responding.

Interoception

According to an interoceptive conditioning hypothesis, in cocaine-experienced rats, the peripheral effects of cocaine acquire conditioned properties that can signal drug availability and evoke drug seeking (Wise and Kiyatkin 2011; Wise et al. 2008). Furthermore, these interoceptive cues elicited by cocaine can enter into association with other appetitive stimuli (sucrose). As a result of this cocaine-sucrose association, the acute re-experience of the conditioned interoceptive effects of cocaine would induce the representation of the sucrose that was obtained by lever pressing.

Under our experimental conditions, since the reinstatement tests are conducted several days after the incentive learning (conditioning) and in extinction conditions, the only cues that the rat can use to determine sucrose availability are the interoceptive cues, those associated with the peripheral and/or central actions of the drugs. Our results demonstrate that the rats are using the discriminative stimulus properties of cocaine and nicotine, but not heroin, to guide their behavior.

Conclusion

The present results indicate that: 1) The discriminative stimulus properties of cocaine and nicotine, but not heroin, play a significant role in driving reinstatement of an extinguished operant behavior not directed towards the drug itself but towards a natural reinforcer (sucrose solution), indicating that animals are using the discriminative stimulus properties of the drugs to guide their behavior. 2) Reinstatement can be effectively induced in all groups, because when the reinforcer itself (sucrose solution) is non-contingently presented, all groups reinstate lever pressing independently of their drug history. In conclusion, these results indicate that the role played by the discriminative stimulus properties of drugs in relapse can be critical for certain drugs of abuse, such as cocaine and

nicotine, but not for others such as heroin. This dissociation suggests that different neuropsychological processes might underlie the relapse process. A better understanding of how the discriminative stimulus properties of drugs of abuse lead to reinstatement will help to understand the relapse process and could provide new directions for relapse prevention therapies.

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Question 2:
Do the discriminative stimulus properties of cocaine and nicotine lead to habit-based behavior?

1. Experiment 3:

Nicotine-induced reinstatement for a food reward (sucrose solution): Goal-directed or habit-based behavior?

In the previous chapter it was shown that re-exposure to cocaine and nicotine reinstates an extinguished sucrose-seeking behavior trained under the influence of cocaine and nicotine, respectively. A further step is to understand the nature of this reinstatement: goal-directed (action-outcome behavior, sensitive to the current value of the outcome) or habit-based (stimulus-response behavior, independent of the current value of the outcome).

Preliminary data from our laboratory has shown, using food pellets, that re-exposure to *cocaine* reinstates an extinguished food-seeking behavior previously trained under the influence of cocaine, even after the food outcome has been made aversive by a devaluation procedure.

Thus, the aim of the third experiment in this thesis was to assess if re-exposure to *nicotine* reinstates an extinguished food-seeking behavior previously trained under the influence of nicotine, even when the food outcome (in this case 5% sucrose solution) has been made aversive by a devaluation procedure.

In order to test this hypothesis, and to extend the previously obtained preliminary data with cocaine and food pellets to nicotine and sucrose solution, we used an experimental procedure similar to the one previously used in Experiments 1 and 2, except that the food reinforcer (5% sucrose solution) was devalued for some rats (see Figure 25).

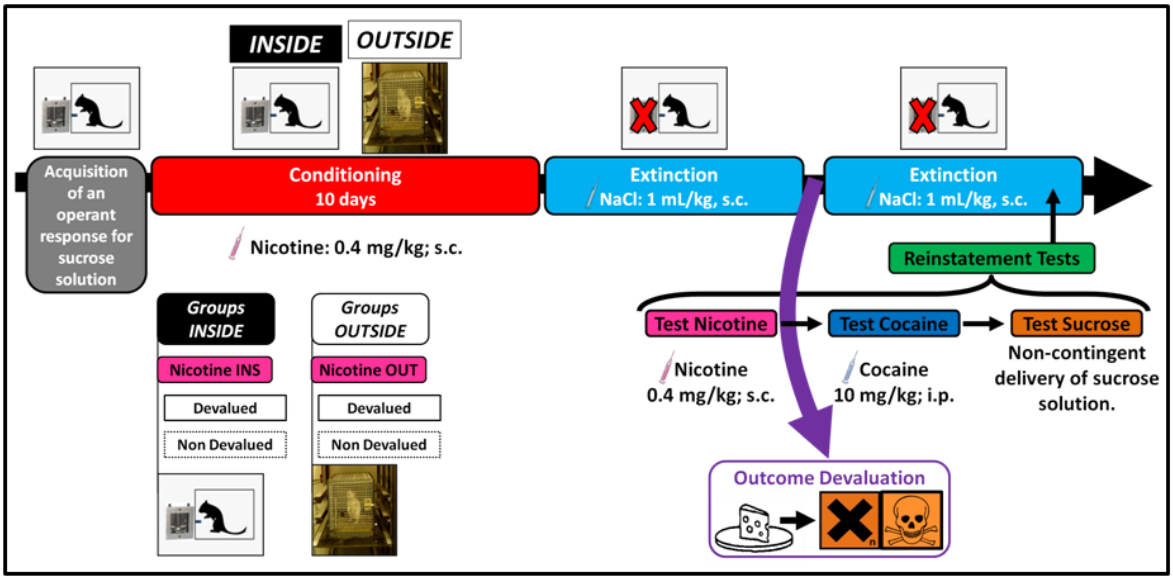

Figure 25 Experimental protocol for Experiment 3. Nicotine-induced reinstatement for a food reward: Goal-directed or habit-based behavior?

Materials and Methods

Subjects

32 male Wistar rats (175-200 g; Charles River Laboratories, France) were housed under the same conditions as in Experiments 1 and 2. Feeding conditions and time of experimental procedures were also identical to those in Experiments 1 and 2 (see Subjects, page 52).

Drugs

(-) Nicotine hydrogen tartrate and cocaine hydrochloride were administered at the same doses, routes of administration and injection volumes as in Experiment 2 (see Drugs, page 52).

Apparatus

The operant conditioning chambers and the standard individual chambers used in this experiment were the same as the ones used in Experiments 1 and 2 (see Apparatus, page 53).

Experimental Procedure

Operant conditioning and extinction. Exposure to environments and session timings were the same as those in Experiments 1 and 2 (see Operant conditioning and extinction, page 53).

Acquisition (Acquisition of an operant response for sucrose solution). The acquisition phase was exactly as described for Experiments 1 and 2 (see Acquisition, page 53).

Conditioning (Association between the discriminative stimulus properties of nicotine and the operant responses). After the second FR-5 session, rats were divided into two groups. The “inside” groups received a non-contingent peripheral injection of nicotine (0.4 mg/kg; s.c.) (n=18) immediately prior to the conditioning sessions in the operant chambers. In contrast, the “outside” groups received a peripheral injection of nicotine (0.4 mg/kg; s.c.) (n=14) immediately prior to the conditioning sessions in the standard individual chambers. To equate exposures to injections, the “inside” groups received saline injections in the standard individual chambers and the “outside” groups received saline injections in the operant chambers.

Extinction (Extinction of the operant responses in a non-drug state). The extinction phase was exactly as described for Experiments 1 and 2 (see Extinction, page 54).

Outcome devaluation (Association of sucrose solution consumption with gastric illness). After extinction of the operant responses, both groups ("inside" and "outside") were once again divided into two groups: devalued ("inside" n=9, "outside" n=7) and non-devalued ("inside" n=9, "outside" n=7). The outcome devaluation took place in individual feeding cages (which were completely different from the chambers previously used during the conditioning) made of clear plastic measuring 13 cm height × 30 cm length × 13 cm depth, with wire-mesh ceilings, which were fitted with plastic bottles containing either water or 5% sucrose solution. The bottles and their location in the wire mesh were similar to those used for providing water in the home cages. Rats were placed in these individual cages and allowed to drink sucrose solution freely from the plastic bottle for 20 min. Immediately after these 20 min had elapsed, the devalued rats received LiCl injections (0.3 M; 5 ml/kg; i.p.) to induce gastric illness, whereas the non-devalued rats received saline injections (5 ml/kg; i.p.). On alternate days, rats were placed in the same individual cages and allowed to drink tap water freely from the plastic bottle for 20 min. Immediately after these 20 min had elapsed, the devalued rats received saline injections (5 ml/kg; i.p.), whereas the non-devalued rats received LiCl injections (0.3 M; 5 ml/kg; i.p.). The amount of sucrose solution and water consumed was computed by weighing the bottles before and after the test. A total of four sucrose solution- and water-LiCl pairings were conducted, separated by 48 h. This procedure ensured that all rats received the same exposure to the sucrose solution and to the LiCl-induced gastric illness, but only the devalued group associated the sucrose solution consumption with gastric illness (see Figure 26).

Extinction (Extinction of the operant responses in a non-drug state). After outcome devaluation, rats were once again submitted to a period of extinction, during which lever presses no longer resulted in sucrose solution delivery. During this period, rats continued to be exposed to both environments. Three extinction sessions were needed to reach the level of 10 (or below) lever presses per session on the previously active lever. This "reminder" extinction period was performed in order to avoid spontaneous recovery from extinction (Rescorla 2004), since during the outcome devaluation procedure operant conditioning was not carried out.

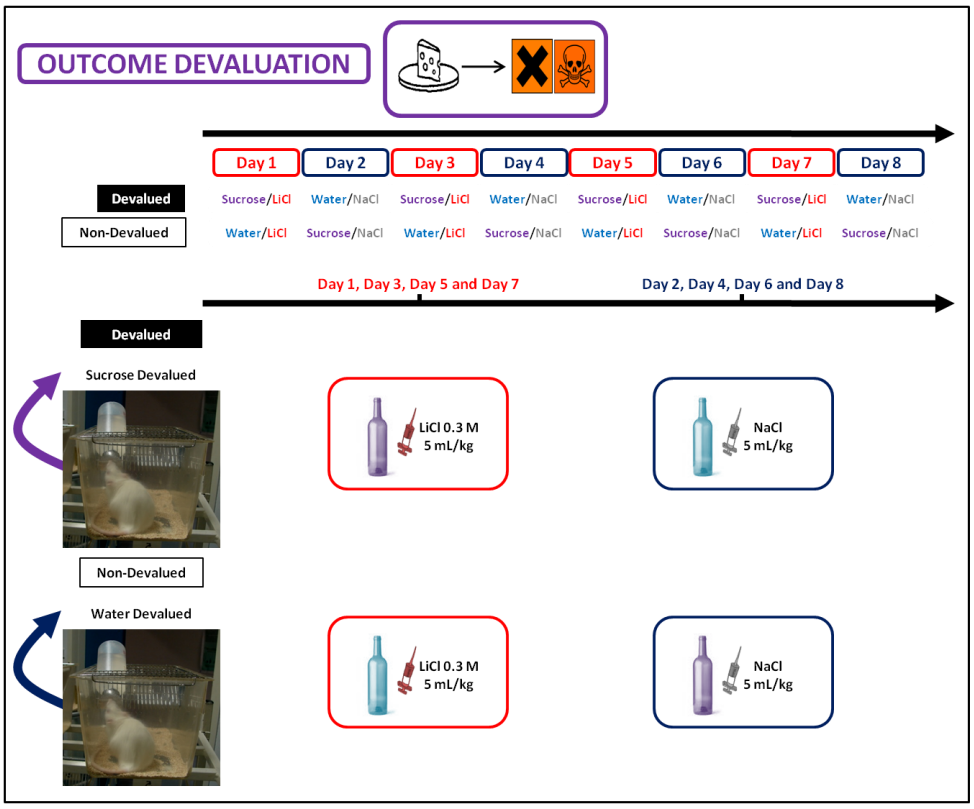

Figure 26 Outcome devaluation procedure. The outcome (5% sucrose solution) was devalued by pairing it (20 min free access in individual cages) with LiCl-induced illness (0.3 M; 5 ml/kg; 4 pairings). Animals for which the outcome was not devalued were exposed to LiCl-induced illness and received unpaired presentations of 5% sucrose solution (20 min free access in individual cages).

Reinstatement tests (Re-exposure to either the discriminative stimulus properties of nicotine, cocaine or the non-contingent presentation of the reinforcer itself in extinction conditions). Following the "reminder" extinction period, rats underwent three reinstatement tests: 1) nicotine-induced reinstatement, 2) cocaine-induced reinstatement and 3) sucrose-induced reinstatement. A minimum of 3 days of extinction was carried out between each test. Test sessions were conducted every third day (except when this fell on the first session of the week), providing that animals maintained a level of below 10 lever presses during the previous extinction sessions.

1) *Nicotine-induced reinstatement.* Reinstatement of instrumental sucrose-seeking behavior in extinction conditions was tested by a single, non-contingent injection of nicotine for both groups ("inside" and "outside") in both conditions ("devalued" and "non-devalued"). The nicotine-induced reinstatement test was conducted over two sessions: a saline session and a drug session, separated by 24 h. The first session included a single saline injection administered immediately prior to the test session whereas the second session included a single nicotine injection (0.4 mg/kg; s.c.) immediately prior to placing the rat in the chamber for a 1 h test session. During this 1 h test session, the house light was illuminated and lever presses had no programmed consequences. Operant responses and locomotor activity (crossovers) were recorded during both reinstatement sessions.
2) *Cocaine-induced reinstatement.* The reinstatement procedure was exactly the same as for nicotine-induced reinstatement, except cocaine (10 mg/kg; i.p.) replaced nicotine. This reinstatement test was performed in order to assess whether the discriminative stimulus properties of cocaine are similar to those generated by nicotine.

3) *Sucrose-induced reinstatement.* Finally, the effect of non-contingent sucrose solution delivery was assessed in both groups ("inside" and "outside") in both conditions ("devalued" and "non-devalued"). The sucrose-induced reinstatement test was again conducted over two sessions, as with the drug-induced reinstatement tests. The first session was a control extinction session, during which sucrose solution was not delivered. During the second session, 1.2 ml (volume needed to fill the dipper) of a 5% sucrose solution was delivered non-contingently over the first 60 s.

Licking test (Re-exposure to either water or sucrose solution). Following the sucrose-induced reinstatement test, rats underwent three "licking" trials. Rats were placed in new operant conditioning chambers, which were exactly the same as those described previously, except that they were equipped with liquid dippers capable of measuring the number of licks performed by the animals. On each of three consecutive days, rats were placed in these operant cages and received 37.5 µL free distribution of either water or sucrose solution. On day one, rats received water (habituation session). On day two, rats received water again (control session), whereas on day three they received sucrose solution (sucrose solution session). All rats had to lick the liquid dipper in order to receive more distributions. After 30 licks, the liquid dipper was refilled (150 µL). Each session lasted 20 min.

Statistical Analyses

Operant responses were analyzed using a repeated measures analysis of variance (ANOVA), with between-subjects factors of treatment conditioning environment (two levels: inside, outside) and devaluation condition (two levels: devalued, non-devalued), and within-subjects factors of lever (two levels: active, inactive), challenge (three levels: nicotine, cocaine, sucrose) and session. Locomotor activity data were analyzed using an ANOVA, with between-subjects factors of treatment conditioning environment (inside or outside) and devaluation condition (devalued or non-devalued) and within-subjects factor of challenge (nicotine, cocaine or sucrose) and session. Post hoc analyses were carried out using the Newman-Keuls (NK) test. The level of $p<0.05$ was the criterion for statistical significance. Group data are presented as the mean $\pm$ SEM in the text and figures.

Results

Conditioning and extinction of the operant behavior

Figure 1a and b show the level of operant responses across the conditioning sessions. During the conditioning phase, a four-factor ANOVA (treatment conditioning environment x devaluation condition x levers x session) revealed no significant interaction between treatment conditioning environment x devaluation condition x levers x sessions ($F(9,252)=0.63$, n.s.), indicating that operant responses did not differ significantly between groups. However, the global ANOVA also indicated a significant levers x session interaction ($F(9,252)=11.10$, $p<0.000$). Post hoc NK comparisons on this interaction revealed that nicotine had differential effects on operant responses. Rats' operant responses were directed towards the active lever (NK test, $p<0.000$ when comparing active and inactive lever presses throughout the conditioning sessions), independently of devaluation condition or whether the nicotine drug state was experienced *inside* or *outside* the operant cages.

During extinction sessions, a four-factor ANOVA (treatment conditioning environment x devaluation condition x levers x session) revealed no significant interaction between treatment conditioning

environment x devaluation condition x levers x session (F(16,448)=0.90, n.s.). In comparison to inactive lever responding, operant responses on the active lever progressively decreased until reaching the extinction criteria after 14 sessions, as indicated by a significant levers x session interaction (F(16,448)=72.35, p<0.000; Fig 1c,d). However, the treatment conditioning environment had an effect on extinction; rats that experienced the nicotine drug state *inside* the operant cages showed lower levels of operant responding only in the first two sessions, in comparison to rats that experienced the nicotine drug state *outside* the operant cages, as indicated by a significant levers x session x treatment conditioning environment interaction (F(16,448)=3.10, p<0.000; Fig 1c,d).

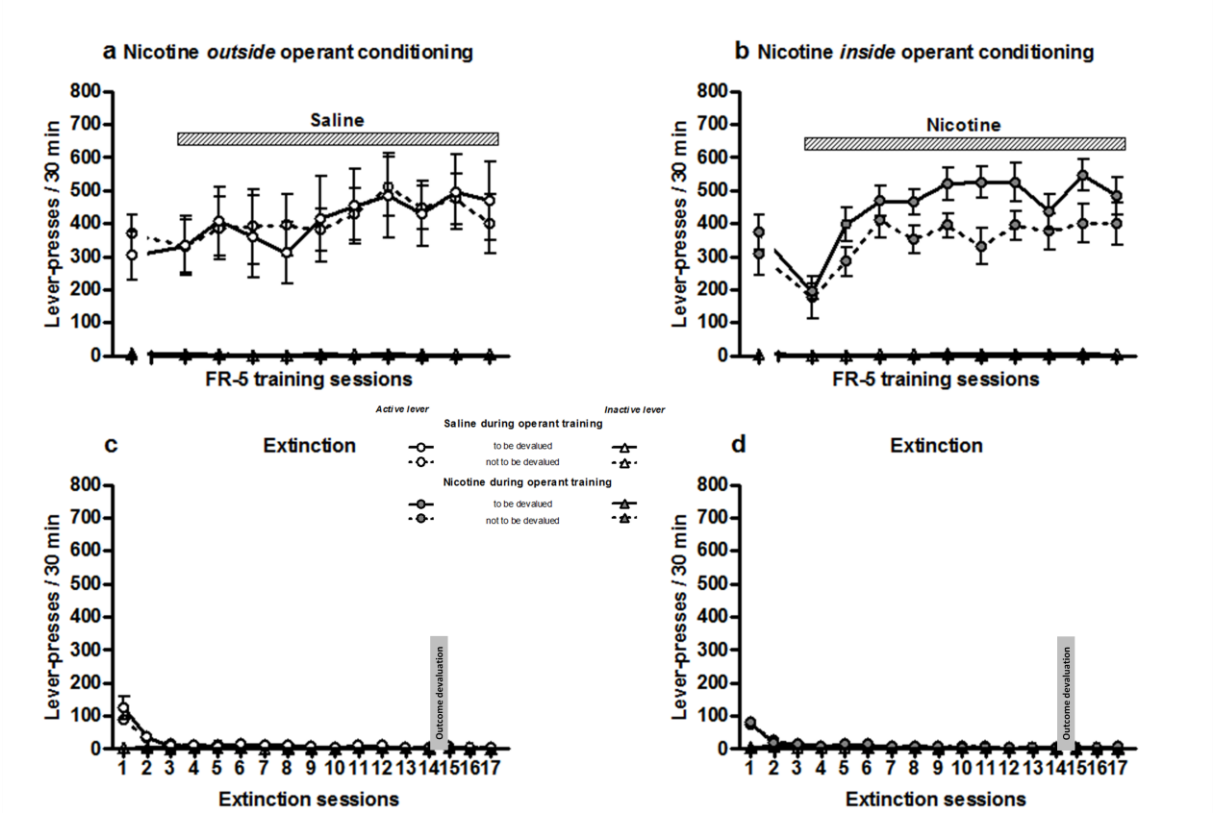

Fig. 1 Conditioning and extinction of an operant sucrose-reinforced behavior. Each point represents mean (± SEM) responses per session on the active or inactive lever. **a** and **b** show the acquisition of the operant behavior for rats that experienced the nicotine (0.4 mg/kg) drug state either *outside* **(a)** or *inside* **(b)** the operant cages. **c** and **d** show the extinction of the operant behavior for these same animals, previously exposed to the nicotine drug state *outside* **(c)** or *inside* **(d)** the operant cages.

Outcome devaluation

The results from the outcome devaluation are shown in Figure 2. Repeated associations of sucrose solution presentation with LiCl-induced gastric illness induced a progressive decrease in sucrose solution consumption in nicotine-treated rats, regardless of the environment ("inside" or "outside") where they experienced the nicotine effects. In contrast, when the sucrose solution was not associated with LiCl-induced gastric illness, rats always consumed as much sucrose solution as they were able to. A three-factor ANOVA (treatment conditioning environment x devaluation condition x trial) revealed a significant effect of devaluation condition (F(1,28)=707.46, p<0.000) and trial (F(2,56)=11.91, p<0.000), and a significant interaction between them (F(2,56)=42.001, p<0.000). Post hoc NK comparisons on this interaction confirmed that the devalued groups decreased sucrose solution consumption from the third trial and onward when compared to the second trial (NK test,

p<0.000 when compared devalued groups through the trials), independently of the environment where nicotine was experienced during the conditioning sessions ("inside" or "outside").

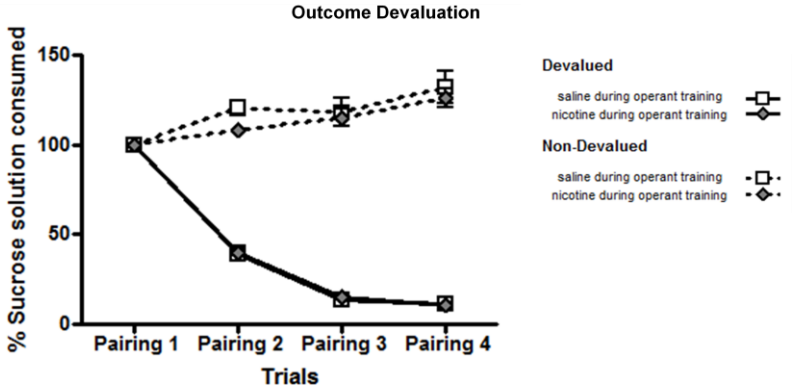

Fig. 2 Devaluation of sucrose solution. Repeated associations of sucrose solution presentation with LiCl-induced gastric illness induced a progressive decrease in sucrose solution consumption. In contrast, when sucrose solution consumption was not associated with LiCl-induced illness, rats always consumed as much sucrose solution as they were able to.

Nicotine reinstates lever pressing in rats that previously performed the operant responses under the effects of nicotine, even when the outcome (sucrose solution) has been devalued

Figure 3a shows the total number of lever presses on the active and inactive levers during the nicotine-induced reinstatement test. A four-factor ANOVA for the nicotine challenge (treatment conditioning environment x devaluation condition x levers x test session) revealed a significant effect of treatment conditioning environment ($F(1,28)=29.22$, $p<0.000$), levers ($F(1,28)=62.91$, $p<0.000$) and test session ($F(1,28)=33.64$, $p<0.000$). The global ANOVA also indicated a significant treatment conditioning environment x levers x test session interaction ($F(1,28)=34.38$, $p<0.000$). Post hoc NK comparisons on this interaction revealed that, only in the "inside" group, a priming injection of nicotine reinstated operant responding on the active lever alone, when compared to a control saline injection (NK test, $p<0.000$), independently of previous sucrose solution devaluation as revealed by a non-significant devaluation condition x levers x test session interaction ($F(1,23)=0.14$, n.s.). This result reveals the habitual nature of nicotine reinstatement.

Figure 3b shows the locomotor activity recorded during the saline and nicotine test sessions of the nicotine reinstatement phase. A three-factor ANOVA for the nicotine challenge (treatment conditioning environment x devaluation condition x test session) only revealed a significant effect of test session ($F(1,28)=108.25$, $p<0.000$), indicating that a priming injection of nicotine induced a motor hyperactivity in all groups of rats in comparison to their respective control saline injection, independently of the environment where nicotine was experienced during the conditioning sessions ("inside" or "outside") and independently of previous sucrose solution devaluation as revealed by a non-significant treatment conditioning environment x devaluation condition x test session interaction ($F(1,28)=2.81$, n.s.).

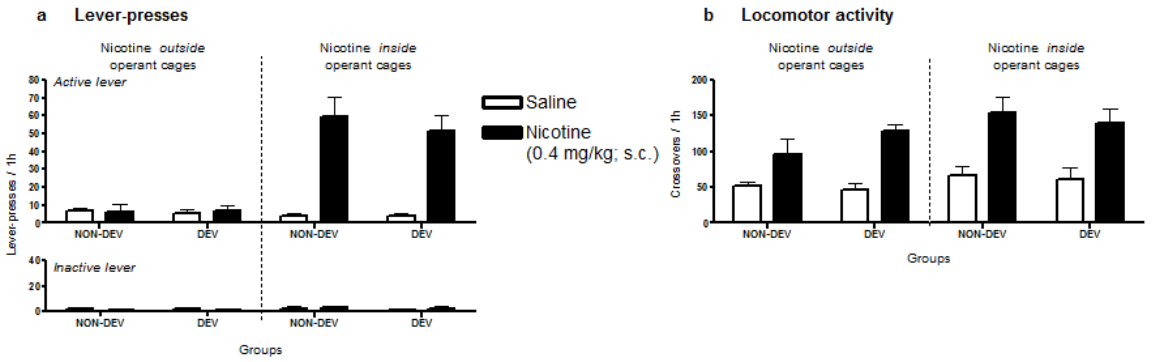

Fig. 3 Re-exposure to nicotine reinstates an extinguished sucrose-seeking behavior, following outcome devaluation. The effects of non-contingent nicotine 0.4 mg/kg were assessed for rats that previously experienced a nicotine (0.4 mg/kg) drug state either *outside* or *inside* the operant cages. **a** Histograms represent mean (± SEM) responses on the active or inactive lever. **b** Histograms represent mean (± SEM) crossovers recorded in the operant conditioning chambers during the test.

Cocaine reinstates lever pressing in rats that previously performed the operant responses under the effects of nicotine, even when the outcome (sucrose solution) has been devalued

Figure 4a shows the total number of lever presses on the active and inactive levers during a second reinstatement test, the cocaine-induced reinstatement test. A four-factor ANOVA for the cocaine challenge (treatment conditioning environment x devaluation condition x levers x test session) revealed a significant effect of treatment conditioning environment ($F(1,28)=5.80$, $p<0.022$), levers ($F(1,28)=11.29$, $p<0.002$) and test session ($F(1,28)=6.71$, $p<0.015$). The global ANOVA also indicated a significant treatment conditioning environment x levers x test session interaction ($F(1,28)=8.61$, $p<0.006$). Post hoc NK comparisons on this interaction revealed that, only in the “inside” group, a priming injection of cocaine reinstated operant responding just on the active lever when compared to control saline injection (NK test, $p<0.000$), independently of previous sucrose devaluation, as revealed by a non-significant devaluation condition x levers x test session interaction ($F(1,28)=0.21$, n.s.).

Figure 4b shows the locomotor activity recorded during the saline and cocaine test sessions of the cocaine reinstatement phase. A three-factor ANOVA for the cocaine challenge (treatment conditioning environment x devaluation condition x test session) only revealed a significant effect of test session ($F(1,28)=53.13$, $p<0.000$), indicating that a priming injection of cocaine induced a motor hyperactivity in all groups of rats in comparison to their respective control saline injection. This effect was independent of the environment where nicotine was experienced during the conditioning sessions (“inside” or “outside”) and of previous sucrose solution devaluation, as revealed by a non-significant treatment conditioning environment x devaluation condition x test session interaction ($F(1,28)=0.001$, n.s.).

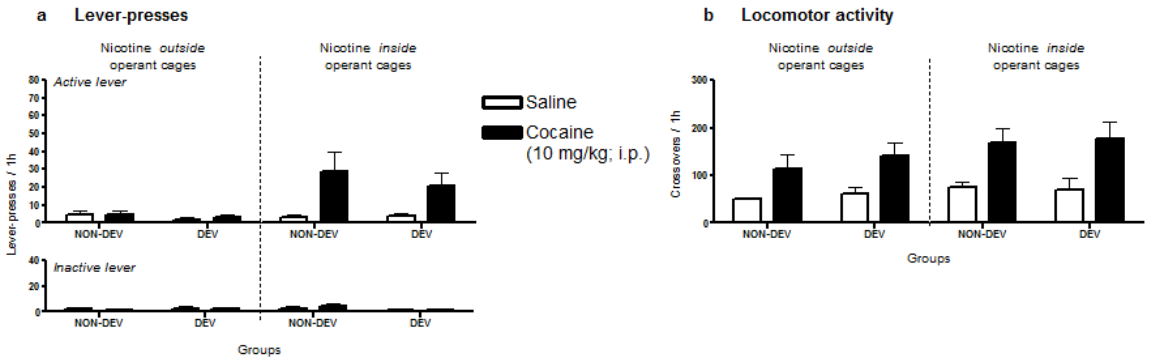

Fig. 4 Exposure to cocaine reinstates an extinguished sucrose-seeking behavior, following outcome devaluation. The effects of non-contingent cocaine 10 mg/kg were assessed for rats previously exposed to nicotine (0.4 mg/kg) either *outside* or *inside* the operant cages. **a** Histograms represent mean (± SEM) responses on the active or inactive lever. **b** Histograms represent mean (± SEM) crossovers recorded in the operant conditioning chambers during the test.

Non-contingent sucrose solution delivery reinstates lever pressing in rats that previously performed the operant responses under the effects of nicotine, even when the sucrose has been made aversive

Figure 5a shows the total number of lever presses on the active and inactive levers during the final, sucrose-induced, reinstatement test. A four-factor ANOVA for the sucrose solution challenge (treatment conditioning environment x devaluation condition x levers x test session) revealed a significant effect of levers ($F(1,28)=43.34$, $p<0.000$) and test session ($F(1,28)=36.69$, $p<0.000$), and a significant interaction between them ($F(1,28)=28.76$, $p<0.000$). Post hoc NK comparisons on this interaction revealed that, the non-contingent delivery of sucrose solution reinstated lever pressing only on the previously-active lever in all groups (NK test, $p<0.000$) when compared to the respective control (no non-contingent sucrose solution delivery), independently of previous sucrose solution devaluation and the environment where nicotine was experienced during the conditioning sessions (inside or outside the operant cages).

Figure 5b shows the locomotor activity recorded during the control (no non-contingent sucrose solution) and sucrose solution test sessions of the sucrose reinstatement phase. A three-factor ANOVA for the sucrose solution challenge (treatment conditioning environment x devaluation condition x test session) did not indicate a significant effect of treatment conditioning environment ($F(1,28)=1.81$, n.s.), devaluation condition ($F(1,28)=0.11$, n.s.) or test session ($F(1,28)=0.01$, n.s.), and no significant interaction between them ($F(1,28)=0.27$, n.s.), indicating that non-contingent delivery of sucrose solution had no effect on the locomotor activity of nicotine pretreated rats.

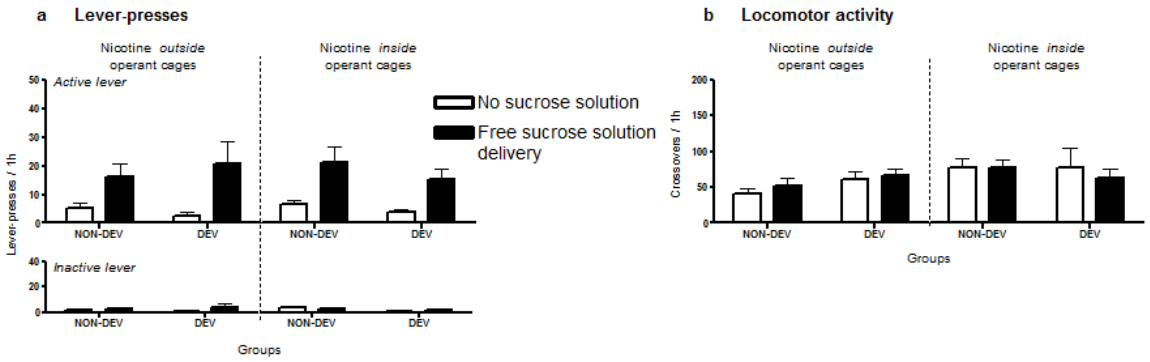

Fig. 5 Re-exposure to sucrose solution reinstates an extinguished sucrose-seeking behavior, following outcome devaluation. The effects of non-contingent sucrose solution delivery were assessed for rats previously exposed to nicotine (0.4 mg/kg) either *outside* or *inside* the operant cages. **a** Histograms represent mean (± SEM) responses on the active or inactive lever. **b** Histograms represent mean (± SEM) crossovers recorded in the operant conditioning chambers during the test.

Devalued nicotine pretreated rats perform fewer licks on the liquid dipper during a sucrose solution challenge than non-devalued nicotine pretreated rats

Figure 6 shows the total number of licks on the liquid dipper during a sucrose solution challenge. A three-factor ANOVA for the sucrose solution challenge (treatment conditioning environment x devaluation condition x liquid challenge) revealed a significant effect of devaluation condition ($F(1,28)=27.54$, $p<0.000$) and liquid challenge ($F(1,28)=198.03$, $p<0.000$), and a significant interaction between them ($F(1,28)=49.57$, $p<0.000$). Post hoc NK comparisons on this interaction revealed that the number of licks was higher during the sucrose solution session than during the control water session in both groups (NK test, when compared to its respective control water session in both groups: $p<0.000$ in the non-devalued and devalued groups), independently of the environment where nicotine was experienced during the conditioning sessions (inside or outside the operant chambers). Furthermore, post hoc NK comparisons on the same interaction also revealed that there was no difference in the number of licks during the water session between the non-devalued and the devalued groups, whereas the non-devalued group performed more licks of the liquid dipper during the sucrose solution session than the devalued group (NK test, when compared non-devalued to devalued group in both sessions: $p<0.86$ in the water session and $p<0.000$ in the sucrose solution session), independently of the environment where nicotine was experienced during the conditioning sessions (inside or outside the operant chambers).

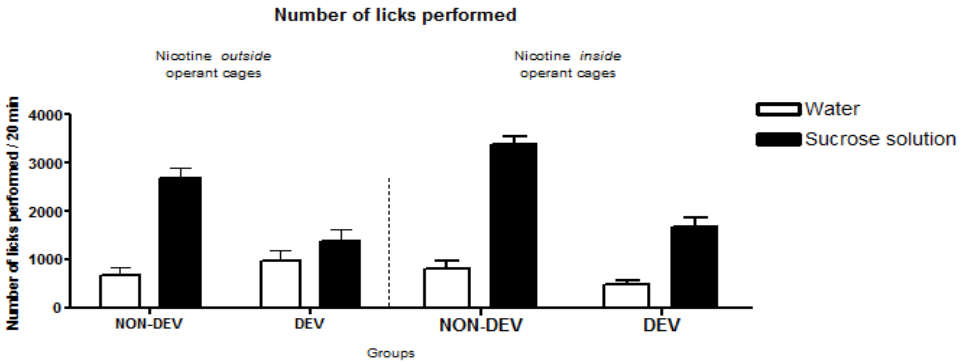

Fig. 6 Number of licks on a liquid dipper during a sucrose solution challenge, following outcome devaluation. The effects of water and sucrose solution delivery were assessed for rats previously exposed to nicotine (0.4 mg/kg) either *outside* or *inside* the operant cages. Histograms represent mean (± SEM) number of licks on the liquid dipper.

Devalued nicotine pretreated rats drink less sucrose solution from the dipper than non-devalued nicotine pretreated rats

Figure 7 shows the total amount of sucrose solution consumed from a liquid dipper during a sucrose solution challenge. A three-factor ANOVA for the sucrose solution challenge (treatment conditioning environment x devaluation condition x liquid challenge) revealed a significant effect of devaluation condition ($F(1,28)=44.16$, $p<0.000$) and liquid challenge ($F(1,28)=371.42$, $p<0.000$), and a significant interaction between treatment conditioning environment x devaluation condition x liquid challenge ($F(1,28)=5.60$, $p<0.025$). Post hoc NK comparisons revealed that the amount of liquid consumed was higher during the sucrose solution session than during the control water session in both groups (non-devalued and devalued) and in both conditioning environments (rats under the drug state of nicotine either *inside* or *outside* the operant chambers) (NK test for the "inside" groups: $p<0.000$ in the non-devalued and devalued groups, when compared to their respective control water sessions; NK test for the "outside" groups: $p<0.000$ in the non-devalued group and $p<0.002$ in the devalued group, when compared to their respective control water sessions). Furthermore, the post hoc NK test also revealed that there was no difference between the non-devalued and devalued groups, in either conditioning environment ("inside" or "outside") in the amount of water consumed during the control (water) session, whereas the non-devalued groups drank more sucrose solution during the test (sucrose) session than the devalued groups in either conditioning environments ("inside" and "outside") (NK test: $p<0.49$ in the "outside" group and $p<0.65$ in the "inside" group, when comparing the non-devalued with the devalued groups during the water session in both conditioning environments; $p<0.000$ in the "outside" group and $p<0.001$ in the "inside" group when comparing the non-devalued to devalued groups during the sucrose solution session in both conditioning environments).

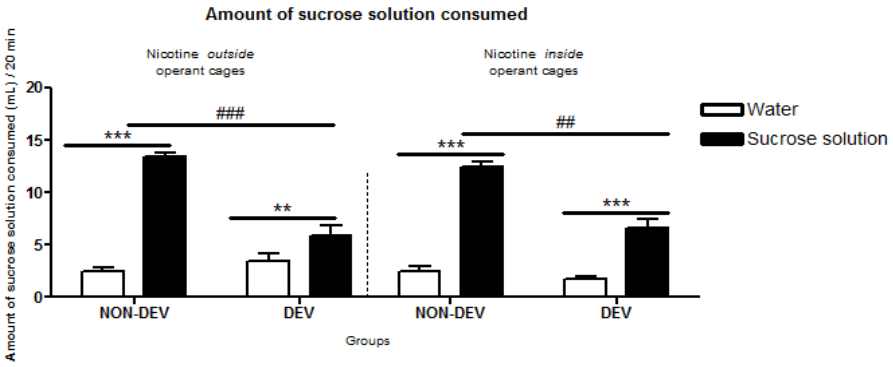

Fig. 7 Amount of sucrose solution consumed from a liquid dipper during a sucrose solution challenge, following outcome devaluation. The effects of a single water or sucrose solution delivery were assessed for rats previously exposed to nicotine (0.4 mg/kg) either *outside* or *inside* the operant cages. Histograms represent mean (± SEM) number of mL of sucrose solution consumed from the liquid dipper. * significantly different from control water session, # significantly different from non-devalued group in sucrose solution session (*/# p<0.05, **/## p<0.01, ***/### p<0.001).

Discussion

This study replicated the results obtained in Experiment 2, providing further evidence that the discriminative stimulus properties of nicotine are involved in the reinstatement of an extinguished food-seeking behavior trained under nicotine. More importantly, the present results indicate that reinstatement by nicotine is not goal-directed but independent of the representation of the reinforcer, since sucrose solution devaluation did not prevent nicotine-induced reinstatement. However, these results are difficult to interprete, since when the reinforcer itself was presented a habit-based reinstatement was observed.

No effect of nicotine on operant responding during conditioning

The group that experienced nicotine during operant conditioning (“inside” group) did not show different levels of instrumental responding when compared to the group that experienced saline during operant conditioning (“outside” group). However, during extinction sessions, the “inside” group showed lower levels of responding than the “outside” group during the first two sessions. This might be the result of the neuroadaptations induced by repeated nicotine treatment associated with the fact that the “inside” group experienced the nicotine effects in the operant cages. Because the “outside” group always experienced saline during conditioning in the operant cages, rats could have interpreted the first extinction sessions as regular conditioning sessions. These results replicated those obtained in Experiment 2.

Is there an overlap between the discriminative stimulus properties of nicotine and cocaine?

The fact that a priming injection of cocaine induced sucrose-seeking behavior in nicotine pretreated rats is in disagreement with our findings from Experiment 2, where a priming injection of cocaine did not induce sucrose-seeking behavior in nicotine pretreated rats (see page 60); however, there are some differences between Experiment 2 and this experiment. First of all, the sucrose solution was not devalued in Experiment 2. Furthermore, it is worth saying that the rats in this experiment drank more sucrose solution than the rats in Experiment 2 (during the outcome devaluation procedure). There are several studies that demonstrated that intake of palatable food and fluids can significantly alter the behavioral consequences of opioid agents and nicotine (D'Anci et al. 1997; Kanarek et al. 2000; Mandillo and Kanarek 2001; Rudski et al. 1997). Thus, we propose that chronic intake of the palatable sucrose solution enhanced the behavioral responses induced by cocaine (such as

locomotor activity), which was reflected in lever pressing reinstatement and can be confounded with a cross-sensitization phenomenon. The fact that only the nicotine "inside" rats reinstated lever pressing when challenged with cocaine might reflect the association drug effects (i.e. different effects than a saline state), operant cage, and lever presses experienced during the conditioning phase, since the nicotine "outside" rats performed the operant responses during the conditioning phase under a saline state. Interestingly, the level of reinstatement induced by a priming injection of nicotine (around 52 lever presses) was much higher than the one induced by a priming injection of cocaine (around 28 lever presses). Thus, we do not think that sucrose-seeking behavior induced by a priming injection of cocaine is due to the fact that the discriminative stimulus properties of nicotine and cocaine are similar.

Non-contingent delivery of sucrose solution reinstates an extinguished sucrose-seeking behavior in all groups, even when the reinforcer has been made aversive by a devaluation procedure

When the reinforcer itself (sucrose solution) was non-contingently delivered, all groups reinstated lever pressing, independently of the environment where they experienced the nicotine effects ("inside" or "outside" the operant cages) and, more importantly, independently of previous sucrose solution devaluation. The fact that the devalued groups reinstated lever pressing when challenged with the now aversive sucrose solution (i.e. they experienced and had access to the real value of the reinforcer) suggested that our devaluation procedure did not work properly. Thus, in order to test the efficacy of our devaluation procedure, we assessed the amount of sucrose solution consumption in operant cages equipped with a licking-operated dipper (licking test), in which rats were able to self-regulate their consumption, allowing us to measure their consummatory behavior. The results obtained from this test revealed that the devalued rats drank less sucrose solution (around 5 mL) in comparison to the non-devalued rats (around 13 mL), indicating that taste aversion was not context specific, since it transferred from the individual feeding cages, where it was established, to the operant cages. Importantly, it was not transferred to the instrumental training. It is worth noting that although the devalued rats drank less sucrose solution than the non-devalued rats, they still drank a considerable amount of sucrose (more than the 1.2 mL received during the sucrose-induced reinstatement test).

In an attempt to explain the results obtained during the sucrose-induced reinstatement test, we developed some hypotheses. Conventional conditioned taste aversion paradigms (such as one or two bottle tests), usually require a period of water deprivation before the test to incite the animal to drink (Bell et al. 1998). If our rats had been water deprived, they might have drunk larger amounts of sucrose solution during the outcome devaluation procedure and, as a consequence, a stronger conditioned taste aversion might have been induced. However, the use of water deprivation would have compromised our experiment, since it has been shown that water-deprived rats reduce consumption of dry lab chow, thus, over time, also become food-deprived (Dickinson and Balleine 1990), and our rats were already food deprived to maintain them at 90% of their free-feeding weight.

Another factor that might have had an influence in the results obtained is hunger. Hunger is one important way in which motivational behavior is controlled (e.g. food tastes pleasant when hungry) (Rolls 2007). Food provides two primary forms of reward value: nutritional (arising from metabolic and homeostatic signaling) and hedonic (arising from the sensory properties perceived as pleasurable) (Beeler et al. 2012). Thus, the reward value of the sucrose solution derives from nutrition and taste. It is probable that the non-contingent presentation of sucrose solution at the

beginning of the session (1.2 mL delivered over the first 60 s), signaled to the rats that the caloric load was proximal. As a consequence, the failure to suppress lever presses might be due to an enlarged perception of the caloric load imparted by the highly palatable sucrose solution (Gomez and Grigson 1999). Furthermore, it has been reported that withdrawal from nicotine in chronically nicotine-pretreated rats enhances sweet food consumption (Grunberg 1982; Jias and Ellison 1990), which might be caused by a hedonic shift in the palatability of sweet foods. We performed the sucrose reinstatement test approximately thirty-six days after the last nicotine injection during conditioning. Our results suggest that the rats' urge to satisfy their hunger overcame the now aversive sucrose solution by trying to satisfy their hunger (i.e. lever presses lead to sucrose solution during instrumental training). This hypothesis is supported by a recent study suggesting that the hedonic value associated with taste requires nutritional value associated with calories to organize appetitive behavior (Beeler et al. 2012).

Finally, it has been suggested that after an outcome devaluation procedure, a failure of integration (indicated by an inappropriate change in instrumental responding during an extinction test) may occur if animals experience a different amount of reinforcers in the conditioned taste aversion sessions than they did during the instrumental training sessions (Adams 1982). This seems like a possible explanation in the present case, since our rats had "free" access to the sucrose solution during the conditioned taste aversion sessions (20 min free access) and during the instrumental training (30 min session but without a limit on the number of reinforcers animals could earn), suggesting that sucrose solution consumption might have been different.

In summary, we concluded that there were several variables in our experimental protocol that could be improved. As a result, we decided to perform another experiment, this time with cocaine, which is described in the following section.

2. Experiment 4:

Cocaine-induced reinstatement for a food reward (food pellets): Goal-directed or habit-based behavior?

In the previous chapter it was shown that re-exposure to cocaine and nicotine reinstates an extinguished sucrose-seeking behavior trained under the influence of cocaine and nicotine, respectively. In order to understand the nature of this reinstatement (i.e. whether it is goal-directed or habit-based), we assessed in Experiment 3 if re-exposure to *nicotine* and *cocaine* reinstates an extinguished sucrose-seeking behavior previously trained under the influence of nicotine, even when the outcome (in this case 5% sucrose solution) has been made aversive by a devaluation procedure. Since we could not reach a conclusion with the results obtained from this experiment, we decided to perform another experiment.

As we previously mentioned, by using a devaluation procedure, preliminary data from our laboratory has shown that re-exposure to *cocaine* reinstates an extinguished food-seeking behavior previously trained under the influence of cocaine, even when the food outcome (food pellets) has been made aversive. This property seems to be unique to cocaine, since the reinstatement of food seeking induced by the food itself remained sensitive to the value of the outcome. Thus, the aim of the

following experiment was to replicate the preliminary data previously obtained by our laboratory, i.e. using food pellets (instead of sucrose solution as in the previous experiments in this thesis) and cocaine.

In order to replicate the preliminary experiment, we used the same experimental procedure as that used in Experiment 3, the only differences being that *cocaine* was tested instead of *nicotine*, and *food pellets* were used as a reinforcer instead of *5% sucrose solution.*

Methods summary

Rats were equally exposed to two different environments (operant cages and individual cages) for two daily 30 min sessions across the whole experiment. During the acquisition phase, rats were trained to press one of the two levers (the active lever) for a maximum of 70 food pellets, under a FR-5 schedule of reinforcement, until this maximum number of pellets had been earned.

Subsequently, during the conditioning phase, rats were divided into groups. The "inside" groups received a non-contingent peripheral injection of cocaine (10 mg/kg; i.p.) before being placed in the operant cages, whereas the "outside" groups received cocaine (10 mg/kg; i.p.) before being placed in the individual cages, where neither food pellets nor levers were present. As in the previous experiments, this method resulted in the association ("inside" group) or independence ("outside" group) of the discriminative stimulus properties of cocaine and the operant responses. To equate the exposures to injections, the "inside" groups received NaCl injections in the individual cages and the "outside" groups received NaCl injections in the operant cages.

After 10 days of cocaine treatment, rats were subjected to a period of extinction during which lever presses no longer resulted in food pellet distribution, and cocaine injections were replaced by NaCl injections. During this period, rats were still exposed to both environments. Extinction sessions continued until lever presses reached a level below 10 per session. After extinction of the operant behavior, both groups ("inside" and "outside") were each further divided in two groups: 1) devalued and 2) non-devalued. The food outcome was devalued by pairing the same food pellets received during the instrumental training (100 pellets in a bowl, one placed in each individual cage for 10 min) with LiCl injections which induce gastric illness (0.3 M; 5 ml/kg; i.p.) immediately after the 10 min exposure to the food pellets. On alternate days, animals for which the food outcome was not devalued received unpaired presentations of the same food pellets with LiCl injections. A total of four food- or no food-LiCl pairings were conducted, separated by 48 hours. This procedure ensured that all rats received the same exposure to food pellets and to LiCl-induced gastric illness, but that only the devalued group associated food pellet consumption with gastric illness. Reinstatement of instrumental food-seeking behavior under extinction conditions was tested by non-contingent injections of cocaine (10 mg/kg; i.p.) and nicotine (0.4 mg/kg; s.c.) for both groups ("inside" and "outside") in both conditions (devalued and non-devalued). Each drug-induced reinstatement test was conducted over two days: a saline day and a drug day. Finally, both groups ("inside" and "outside") in both conditions (devalued and non-devalued) were tested without food pellet distribution and with non-contingent food pellet distribution over two consecutive days (see Figure 27).

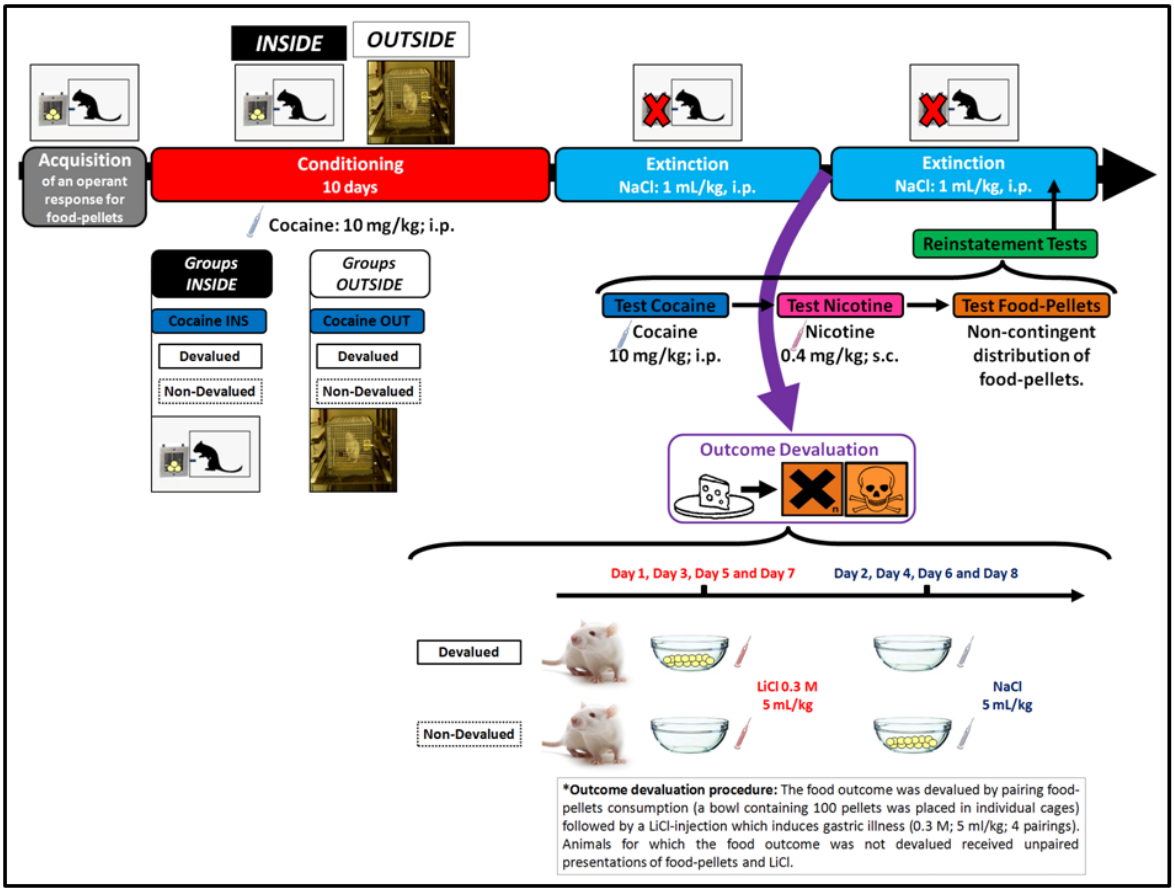

Figure 27 Experimental protocol for Experiment 4. Cocaine-induced reinstatement for a food reward: Goal-directed or habit-based behavior?

Results summary

This experiment is described in further detail in the full manuscript provided below. Briefly, the experiment demonstrated that:

1. The discriminative stimulus properties of cocaine play a significant role in driving reinstatement of an extinguished operant behavior not directed towards cocaine itself but towards a natural reinforcer (food pellets), indicating that the animals are using the discriminative stimulus properties of cocaine to guide their behavior (replication of preliminary data from our laboratory).

2. Reinstatement by cocaine is not goal-directed, i.e. does not depend on the representation of the reinforcer, since cocaine induced reinstatement despite outcome devaluation. Therefore, cocaine controls the expression of automatic, habit-based behaviors.

3. Non-contingent injections of nicotine do not reinstate lever pressing in cocaine pretreated rats.

4. When the reinforcer itself (food pellets) is non-contingently presented, a habit-based (devaluation-insensitive) food-seeking behavior is reinstated, since both groups (devalued and non-devalued) reinstated lever presses, independently of previous food devaluation

and the environment where they experienced the cocaine effects ("inside" or "outside" the operant chambers).

3. Experiment 5:

Nicotine-induced reinstatement for a food reward (food pellets): Goal-directed or habit-based behavior?

We have shown, by using a devaluation procedure, that re-exposure to *cocaine* reinstates an extinguished food-seeking behavior previously trained under the influence of cocaine, even when the food outcome (food pellets) has been made aversive. However, the results obtained from Experiment 4 raise the question whether cocaine is unique in comparison to other drugs of abuse, such as nicotine. Thus, the aim of this experiment was to assess if re-exposure to *nicotine* reinstates an extinguished food-seeking behavior previously trained under the influence of nicotine, even when the outcome (in this case food pellets) has been made aversive by a devaluation procedure.

Methods summary

The experimental procedure was exactly as described for Experiment 4, except nicotine (0.4 mg/kg; s.c.) was tested instead of cocaine (see Figure 28).

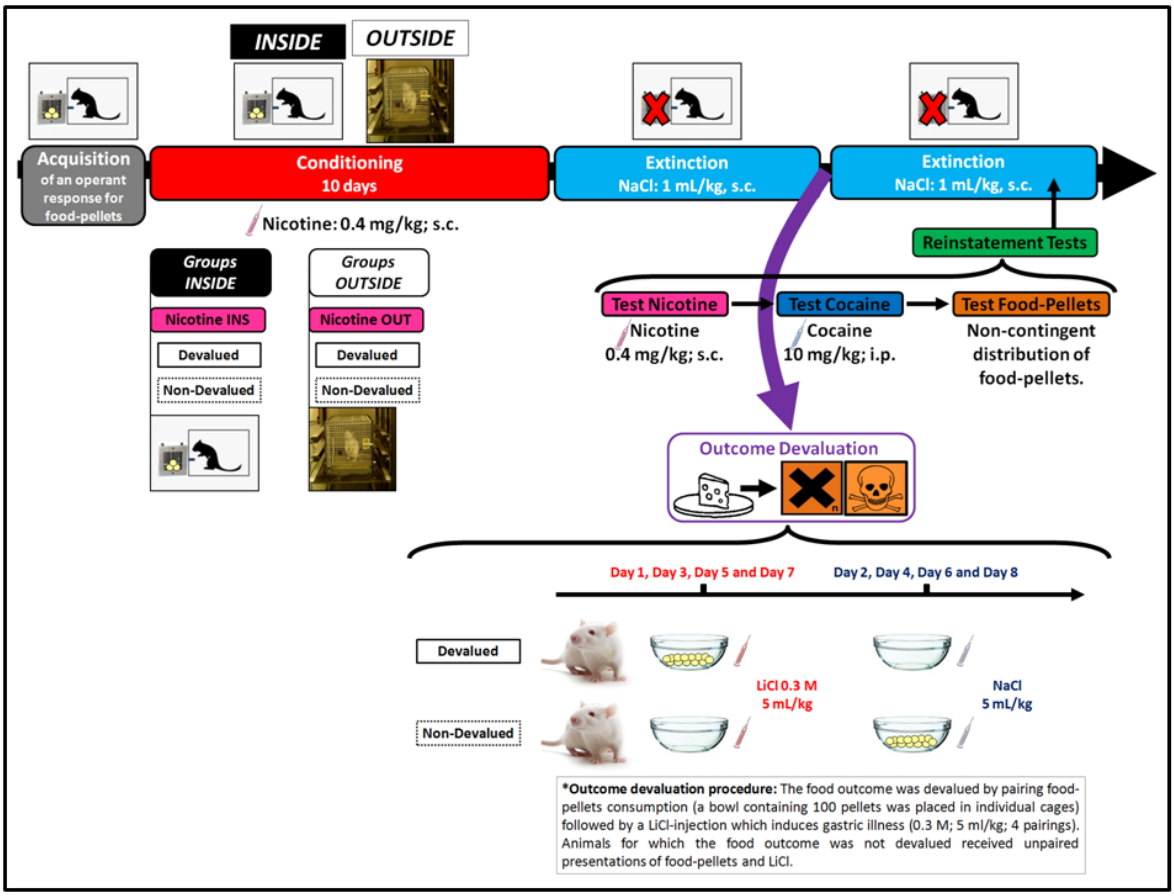

Figure 28 Experimental protocol for Experiment 5. Nicotine-induced reinstatement for a food reward: Goal-directed or habit-based behavior?

Results summary

This experiment is described in further detail in the full manuscript provided below. Briefly, the experiment demonstrated that:

1. The discriminative stimulus properties of nicotine play a significant role in driving reinstatement of an extinguished operant behavior not directed towards nicotine itself but towards a natural reinforcer (food pellets), indicating that the animals are using the discriminative stimulus properties of nicotine to guide their behavior.

2. Reinstatement by nicotine is not goal-directed, i.e. does not depend on the representation of the reinforcer, since nicotine induced reinstatement despite outcome devaluation. Therefore, nicotine controls the expression of automatic, habit-based behaviors.

3. Non-contingent injections of cocaine reinstate lever pressing in nicotine pretreated rats.

4. When the reinforcer itself (food pellets) is non-contingently presented, a goal-directed (devaluation sensitive) food-seeking behavior is reinstated, since only the non-devalued groups reinstated lever presses, independently of the environment where they experienced the nicotine effects ("inside" or "outside" the operant chambers), indicating that reinstatement can be effectively induced.

4. Experiment 6:

Context-induced reinstatement for a food reward (food pellets): Goal-directed or habit-based behavior?

We have shown, by using a devaluation procedure, that re-exposure to *cocaine* and *nicotine* reinstates an extinguished food-seeking behavior previously trained under the influence of cocaine and nicotine respectively, even when the food outcome (food pellets) has been made aversive. In order to determine whether this property is unique to drugs of abuse, analysis was carried out on data from a third experiment conducted previously in our laboratory, where the drug stimulus was replaced by a discriminative contextual stimulus (DC+) (texture + odor). Thus, the aim of this experiment was to assess if re-exposure to a DC+ reinstates an extinguished food-seeking behavior previously trained under the presentation of this DC+, even when the outcome (food pellets) has been made aversive by a devaluation procedure.

The experimental procedure was exactly as described for Experiment 5, except a tactile and olfactory DC+ was tested instead of nicotine.

Methods summary

Rats were equally exposed to two different environments (operant cages and individual cages) for two daily 30 min sessions across the whole experiment. During the acquisition phase, rats were

trained to press one of the two levers (the active lever) for a maximum of 70 food pellets, under a FR-5 schedule of reinforcement until this maximum number of pellets had been earned.

Subsequently, during the conditioning phase, rats were divided into different groups: “DC+” and “Ø” groups. For the “DC+” groups, the operant cages were adapted with a tactile and olfactory stimulus (which consisted of a semi-rough floor surface with some aniseed-scented sawdust scattered on it), whereas for the “Ø” groups, the animals experienced the same tactile and olfactory stimulus in the individual cages but not in the operant cages, thus ensuring that each group experienced equal exposure to the tactile and olfactory stimulus, either in the operant cages (“DC+” group) or in the individual cages (“Ø” group). Similarly to the previous experiments, this method resulted in the association (“DC+” group) or independence (“Ø” group) of the tactile and olfactory stimulus and operant responding for food pellets.

After 10 days of discriminative context training, rats were subjected to a period of extinction during which lever presses no longer resulted in food pellet distribution, and the tactile and olfactory stimulus was removed from both the operant and the individual cages. During this period, rats were still exposed to both environments. Extinction sessions continued until lever presses reached a level below 10 per session. After extinction of the operant behavior, both groups (“DC+” and “Ø”) were each further divided in two groups: 1) devalued and 2) non-devalued. The food outcome was devalued by pairing the same food pellets received during the instrumental training (100 pellets in a bowl, one placed in each individual cage for 10 min) with LiCl injections which induce gastric illness (0.3 M; 5 ml/kg; i.p.) immediately after the 10 min exposure to the food pellets. On alternate days, animals for which the food outcome was not devalued received unpaired presentations of the same food pellets with LiCl injections. A total of three food- or no food-LiCl pairings were conducted, separated by 48 hours. This procedure ensured that all rats received the same exposure to food pellets and to LiCl-induced gastric illness, but only the devalued group associated food pellet consumption with gastric illness. Reinstatement of instrumental food-seeking behavior under extinction conditions was tested by re-exposure to the DC+ for both groups (“DC+” and “Ø”) in both conditions (devalued and non-devalued). This context-induced reinstatement test was conducted over two days: a control day and a test day. Finally, both groups (“DC+” and “Ø”) in both conditions (devalued and non-devalued) were tested without food pellet distribution and with non-contingent food pellets distribution over two consecutive days (see Figure 29).

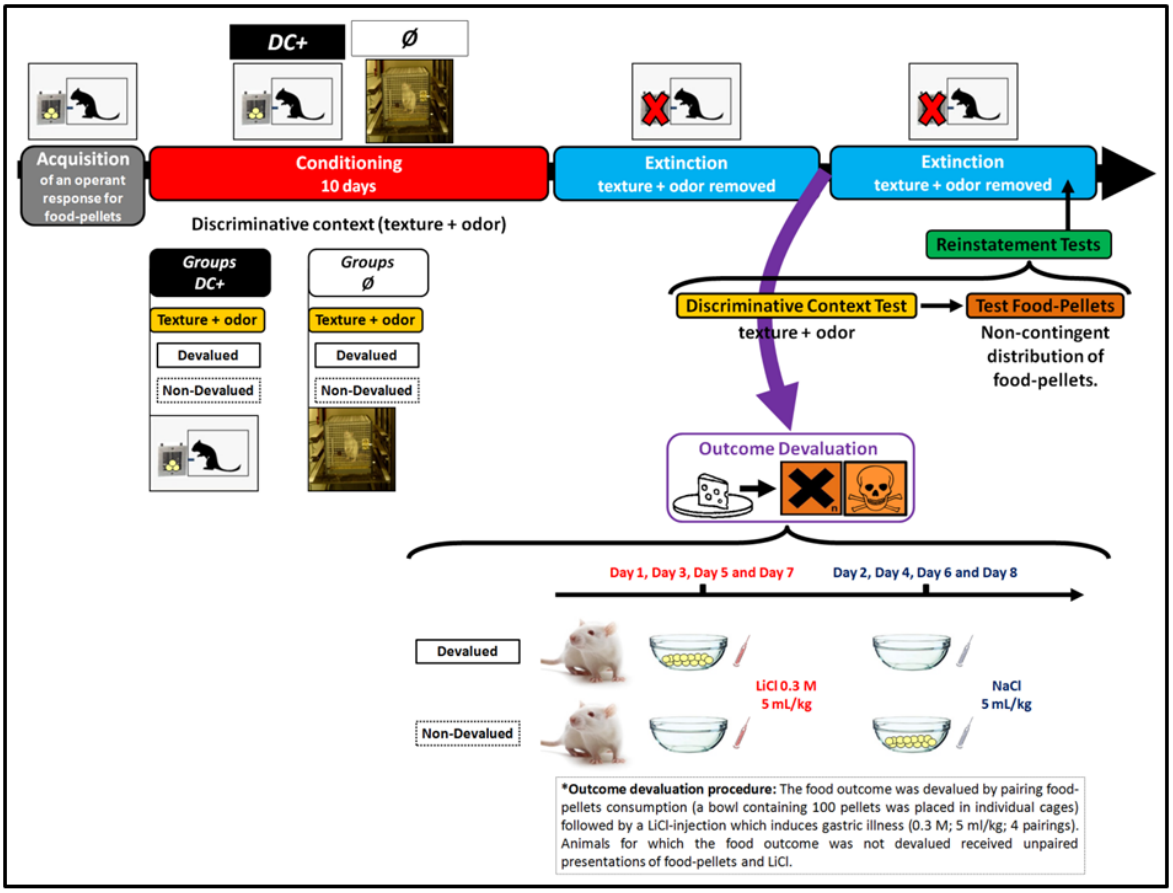

Figure 29 Experimental protocol for Experiment 6. Context-induced reinstatement for a food reward: Goal-directed or habit-based behavior?

Results summary

This experiment demonstrated that:

1. A discriminative tactile and olfactory stimulus can drive reinstatement of an extinguished operant behavior directed towards a natural reinforcer (food pellets), indicating that the animals are using the discriminative contextual cues to guide their behavior.

2. Reinstatement by a discriminative tactile and olfactory stimulus is goal-directed, i.e. depends on the representation of the reinforcer, since the discriminative tactile and olfactory stimulus induced reinstatement only in the non-devalued group.

3. When the reinforcer itself (food pellets) is non-contingently presented, a goal-directed (devaluation sensitive) food-seeking behavior is reinstated, since only the non-devalued groups reinstated lever presses, independently of the environment where they experienced the discriminative tactile and olfactory stimulus, indicating that reinstatement can be effectively induced in both groups ("DC+" and "Ø").

In conclusion, the results from Experiment 4, 5 and 6 indicate that re-exposure to cocaine and nicotine reinstates drug-related habitual behaviors, independent of the current value of the outcome, whereas re-exposure to a discriminative tactile and olfactory stimulus reinstates goal-directed behaviors, dependent on the current value of the outcome. These cocaine- nicotine-induced habits could explain the loss of control induced by re-exposure to cocaine and nicotine in former addicts and the rapid resumption of compulsive drug-taking, despite the explicit knowledge of the adverse consequences.

The work from Experiment 4, Experiment 5 and Experiment 6 resulted in the writing of a short communication (in preparation).

González-Marín, M.C.*, Keiflin, R.*, Vouillac, C., and Cador, M. (2012) The discriminative stimulus properties of cocaine and nicotine reinstate an automatic, habit-based food-seeking behavior. (*co-authors)

The discriminative stimulus properties of cocaine and nicotine reinstate an automatic, habit-based food-seeking behavior.

María del Carmen González Marín*, Ronald Keiflin*, Caroline Vouillac and Martine Cador

RATIONALE: Relapse remains the major problem for the treatment of addiction. Using an animal model of drug relapse, it has been shown that a rodent can reinstate drug-seeking behavior when re-exposed to the drug itself. We have shown that the discriminative stimulus properties of cocaine and nicotine, but not heroin, may play a crucial role in reinstatement since, when present as a stimulus during operant conditioning, cocaine and nicotine but not heroin, acquire conditioned properties which can then promote reinstatement of an extinguished operant behavior previously oriented towards a nutritional reinforcer (liquid sucrose or food pellets). **OBJECTIVE:** In order to evaluate the nature of the response expressed in cocaine and nicotine-induced reinstatement (goal-directed or habit-based) of food-seeking behavior, we aimed to assess whether re-exposure to cocaine and nicotine reinstates extinguished food-seeking behavior previously trained under the influence of cocaine and nicotine respectively, even when the outcome (food pellets) has been made aversive by a devaluation procedure. Furthermore, in order to demonstrate the specificity of drugs of abuse such as cocaine and nicotine, we also aimed to assess whether re-exposure to a discriminative context stimulus (DC+) reinstates extinguished food-seeking behavior previously trained under the presentation of this DC+, even when the outcome (food pellets) has been made aversive by a devaluation procedure. **METHODS:** In Experiment 1, male Wistar rats were trained to lever press for food pellets (FR-5; 10 sessions) while they were under the effects of a non-contingent peripheral injection of cocaine (10 mg/kg, i.p.). Additional control groups were trained to lever press for food pellets while under saline and received a peripheral injection of cocaine (10 mg/kg, i.p.) 5 hours later in individual cages, where neither food nor levers were present. Then, rats were subjected to a period of extinction (rats were placed in operant cages but food distribution and drug injections ceased) (10 sessions). After extinction of the operant responses, the food reward was made aversive for half of the rats in both groups, by pairing it with LiCl-induced gastric illness (0.3 M; 5 ml/kg; i.p.). Reinstatement of instrumental food-seeking behavior under extinction conditions was tested by non-contingent injections of cocaine (10 mg/kg; i.p.), nicotine (0.4 mg/kg; s.c.) and by non-contingent food pellet distribution. In two further experiments, the same protocol was followed, except nicotine (0.4 mg/kg; s.c.) was tested instead of cocaine (Experiment 2) and a discriminative context stimulus was tested instead of a drug stimulus (Experiment 3). **RESULTS:** Re-exposure to cocaine and nicotine reinstated lever pressing only in rats that previously performed the operant responses under the effects of cocaine and nicotine, respectively. Strikingly, this reinstatement of food seeking behavior promoted by the cocaine and nicotine states was observed even when the food outcome had been devalued, demonstrating the habit nature of the operant responses. A re-exposure to a non drug DC+ reinstated food seeking only in rats that previously performed the operant responses under the DC+. However, reinstatement of food seeking was totally abolished when the food reinforcer had been devalued, demonstrating the goal-directed nature of the reinstated operant responding. When access to the current value of the reinforcer itself was given via non-contingent delivery, lever-pressing was reinstated only in all non devalued groups, independently of previous experience with cocaine, nicotine or the DC+. **CONCLUSIONS:** This study shows that re-exposure to cocaine and nicotine reinstates drug-related habitual behaviors, independent of the current value of the outcome. These cocaine- and nicotine-induced habits could explain the loss of control induced by re-exposure to cocaine and nicotine in former addicts and the rapid resumption of compulsive drug-taking, despite the knowledge of the adverse consequences.

Introduction

The development of drug addiction involves a transition from recreational to compulsive drug-taking that can be difficult to reverse. Former drug addicts can eventually maintain protracted abstinence but can rarely return to recreational drug use (Marlatt and Gordon 1985). Indeed, when re-experiencing the drug effects, former addicts rapidly lose control and resume compulsive drug-seeking, despite their explicit knowledge of the adverse consequences. This loss of control suggests that once under the influence of the drug, behavior is no longer guided by the representation of the outcomes, but presumably by some stimulus-response (S-R) habits (Everitt and Robbins 2005; Tiffany 1990; Vanderschuren and Everitt 2005).

In opposition to goal-directed behaviors, habits are well trained behaviors that are no longer driven by the representation of their consequences but simply by discriminative stimuli in the environment (expression of S-R associations) (Dickinson 1985). As a consequence, habits are not sensitive to post-conditioning changes in the outcome value and thus are less flexible. Furthermore, in support of the habit theory of addiction, studies have shown that cocaine and amphetamine accelerates the formation of habits during behavioral training for a food reward (Nelson and Killcross 2006;

Nordquist et al. 2007; Schoenbaum and Setlow 2005), presumably through the neuroadaptative changes that these drugs induce within a neural system of habit formation.

In the context of relapse, the loss of control observed after re-exposure to the drug suggests that cocaine not only accelerates habit formation but also directly controls, through its discriminative/incentive stimulus properties, the resumption of drug-related habits. Using a preparation which allows to dissociate the discriminative from the incentive properties of reward in reinstatement, we demonstrated that the discriminative properties of cocaine and nicotine (but not heroin) can reinstate on their own an instrumental responding directed towards a non drug reward such as a food pellet or a sucrose solution (Keiflin et al. 2008a) (González Marín et al. in preparation).

Since cocaine and nicotine share the mutual effect of increasing the concentration of dopamine (DA) (Kalivas et al. 2009; Koob 2009; Luscher and Ungless 2006; Wise 2009; Wolf et al. 2004), we proposed to test the habit hypothesis by assessing in rodents whether re-exposure to the discriminative stimulus properties of cocaine and nicotine can reinstate operant responding towards food, even when the food has been made aversive by a devaluation procedure.

Specifically, cocaine and nicotine were not used as behavioral reinforcers but simply as discriminative stimuli. Rats were trained to self administer a non-drug reward (food pellets) while under the influence of the drug. The use of food pellets as an outcome allows us to easily devalue it by pairing its consumption with experimentally-induced gastric illness.

In Experiment 1, one group of rats was trained to lever press for food pellets while they were under the effects of cocaine ("cocaine *inside*" group) whereas an additional control group was trained in the same operant task but under saline ("cocaine *outside*" group) and experienced the same amount of cocaine 5 hours later in individual cages (dissociated from the operant training sessions), where neither food pellets nor levers were present **(supplementary Fig 1A)**. Cocaine injections were then stopped for both groups ("inside" and "outside") and lever presses were extinguished by withholding food pellets. After extinction of the lever pressing behavior **(supplementary Fig 1B)**, the value of the food pellets was changed (devalued) for half of the rats in each group ("inside" and "outside") by pairing food pellet consumption with a gastric illness induced by lithium chloride **(supplementary Fig 4)**. After food outcome devaluation, all rats were re-exposed to cocaine in a reinstatement test. The test was conducted under extinction conditions, meaning that lever pressing did not result in food pellet distribution. This procedure ensured that performance during the test was not controlled by the appetitive or aversive effects or the food pellets themselves, but reflected the representation of the value of the food pellets (Dickinson and Balleine 2004).

For the "outside" groups (the rats that experienced cocaine dissociated from the operant training), re-exposure to cocaine had no effect on lever pressing. In contrast, for the "inside" groups (the rats that performed the operant task under the effects of cocaine), re-exposure to cocaine dramatically reinstated lever pressing, as previously described (Keiflin et al. 2008a). Thus, it appears that cocaine does not reinstate any extinguished behavior but acts as a discriminative stimulus that specifically reinstates drug-related behaviors (i.e. behaviors previously performed under the effects of cocaine). More importantly, lever pressing resumption was totally insensitive to previous devaluation of food pellets **(Fig 1A)**. This reinstatement of lever pressing did not result from a general increase in behavioral activity, since lever pressing was selectively directed towards the previously active lever and responding on the inactive lever was negligible **(supplementary Fig 7A)**. Furthermore, lever pressing resumption did not result from an increased sensitivity to cocaine psychomotor effects as all groups increased their locomotor activity in response to cocaine to a similar degree **(supplementary Fig 10A)**. The ability of cocaine to reinstate the extinguished food-seeking behavior despite devaluation of the food outcome indicates that the reinstated behavior is not guided by the current value of the outcome but by some stimulus-response (S-R) associations. After cocaine-induced reinstatement test, we conducted a nicotine-induced reinstatement. The reinstatement procedure was the same as for cocaine-induced reinstatement except nicotine was tested instead of cocaine. Nicotine did not reinstate neither active nor inactive lever pressing in cocaine pretreated rats **(Fig 1B and supplementary Fig 7B respectively)** and did not result in increased locomotor activity **(supplementary Fig 10B)**, suggesting that the interoceptive effects generated by cocaine are not similar to the ones generated by nicotine.

Finally, to ensure that the devaluation procedure effectively modified the perceived value of the food pellets, a third reinstatement test was conducted. In this reinstatement test, all rats were re-

exposed to the food pellets (seven food pellets freely distributed at the start of the session) but lever pressing did not result in further food pellets distribution. This food-induced reinstatement test revealed that when the food pellets had not been devalued, the distribution of these same food pellets induced a modest lever pressing reinstatement. However, lever pressing reinstatement was much lower in the group that experienced the cocaine effects during operant training than in the group that did not experience the cocaine effects during operant training. This result suggests that lever pressing reinstatement is dependent on previous conditions of cocaine exposure. Cocaine during operant training modified the appetitive value of the food pellets. One factor that is relevant for interpreting these results is the response rate during instrumental training. Only the rats that experienced the cocaine effects during operant training ("cocaine *inside*" group) showed lower levels of responding across the 10 days of conditioning, whereas the levels of responding of the groups that experienced saline during operant training ("cocaine *outside*" group) remained stable, suggesting that this difference in response rate during instrumental training lead to different response rates during the food reinstatement test. Interestingly, previous work from our laboratory has previously shown that cocaine pretreated rats reinstate operant responding in a food reinstatement test despite the fact that cocaine treatment dose-dependently (5, 10 or 15 mg/kg) decreased instrumental responding during the conditioning phase (Keiflin et al. 2008a). The food-induced reinstatement test also revealed that when the food pellets had been devalued, lever pressing resumption was not prevented by previous food pellets devaluation **(Fig 1C)**. Responding on the inactive lever and behavioral locomotor activity was negligible during this food-induced reinstatement test **(supplementary Fig 7C and Fig 10C respectively)**. Palatable food has unconditioned incentive motivational properties which lead to approach and eating responses (Nair et al. 2009); additionally, it has been shown that incentive sensitization by previous amphetamine exposure enhances the pursuit of natural rewards (Wyvell and Berridge 2001). One hypothesis is that the non-contingent distribution of food pellets reinstated operant responding in the devalued groups (even when the food pellets are now aversive) by enhancing the incentive salience of the lever (i.e. lever pressing lead to highly palatable food pellets during instrumental training). Furthermore, the ability of the food pellets to induce operant responding was much lower in the devalued than in the non-devalued groups, suggesting that rats required experiencing the current value of the food pellets to stop their temporary operant responding. Our hypothesis is in agreement with a study where it was shown that amphetamine microinjections into the nucleus accumbens shell enhanced the ability of a reward cue to increased operant responding for sucrose pellets (in the absence of reinforcement) without increasing sucrose "liking" (Wyvell and Berridge 2000). However, these results can alternatively be explained by increased stimulus-response associations (Robbins and Everitt 1999), suggesting that after cocaine treatment conditioned reinforcement is increased.

Taken together, these results confirm the hypothesis that once under the influence of cocaine, rats fail to adjust their behavior to the current value of the outcome, but reinstate habitual behaviors previously performed while under the effects of cocaine.

To determine whether this property is unique to cocaine, a second experiment was conducted. In Experiment 2, the same protocol was followed, except nicotine was tested instead of cocaine **(see supplementary Fig 2 for operant training and extinction and supplementary Fig 5 for outcome devaluation)**. After food outcome devaluation, all rats were re-exposed to nicotine in a reinstatement test. For the groups "outside" (the rats that experienced nicotine dissociated from the operant training), re-exposure to nicotine had no effect on lever pressing. In contrast, for the groups "inside" (the rats that performed the operant task under the effects of nicotine), re-exposure to nicotine dramatically reinstated lever pressing. Thus, it appears that nicotine (like cocaine) does not reinstate any extinguished behavior but acts as a discriminative stimulus that specifically reinstates drug-related behaviors. More importantly, lever pressing resumption was totally insensitive to previous food pellets devaluation **(Fig 2A)**. This lever pressing resumption did not result from a general increase in behavioral activity, since lever pressing was selectively directed towards the previously active lever and responding on the inactive lever was negligible **(supplementary Fig 8A)**. Although only the non-devalued groups increased their locomotor activity, we do not think it is likely that lever pressing resumption resulted from an increased sensitivity to nicotine psychomotor effects **(supplementary Fig 11A)**.

The ability of nicotine to reinstate the extinguished food-seeking behavior despite devaluation of the food outcome indicates that the reinstated behavior is not guided by the current value of the outcome but by some stimulus-response (S-R) associations. After nicotine-induced reinstatement test, we conducted a cocaine-induced reinstatement. The reinstatement procedure was the same as for nicotine-induced reinstatement except cocaine was tested instead of nicotine. Cocaine reinstated lever pressing on the previously active lever in nicotine pretreated rats **(Fig 2B and supplementary Fig 8B respectively)** and also induced a motor hyperactivity in most of the rats **(supplementary Fig 11B)**. The fact that acute exposure to cocaine induced motor hyperactivity in most of nicotine pretreated rats can be explained by the fact that nicotine pretreatment enhanced the behavioral effects of cocaine. Accordingly, Collins and Izenwasser (2004) suggest that cross-sensitization to the locomotor activating effects of cocaine in nicotine pretreated rats is due to changes in the nicotinic acetylcholinergic system or to distinct effects on the dopamine system than the ones produced by repeated cocaine treatment, which results in increased sensitivity to the locomotor activating effects of cocaine. We think that the motor hyperactivity induced by cocaine was reflected in lever pressing reinstatement. Interestingly, the only group that did not reinstate lever pressing ("nicotine *outside* non-devalued" group) was the group with the highest locomotor activity, suggesting that this group was more sensitive to the locomotor activating effects of cocaine (e.g. exploratory behavior like sniffing, jumping and rearing) which overshadowed lever pressing reinstatement. On the other hand, the only group that did not show motor hyperactivity ("nicotine *outside* devalued" group) reinstated operant responding. This can be due to the way we measured locomotor activity since it has been shown that cocaine induces stereotypy (Bhattacharyya and Pradhan 1979). Specifically, crossovers were measured by counting the number of times a rat broke light beams; however, repetitive actions (e.g. head bobbing) have to be measured by counting repeated breaks of the same light beam. Thus, the "lack" of motor hyperactivity observed in the "nicotine *outside* devalued" group, might be due to the increased stereotypy not measured.

Finally, to ensure that the devaluation procedure effectively modified the perceived value of the food pellets, a third reinstatement test was conducted. In this reinstatement test, all rats were re-exposed to the food pellets (seven food pellets freely distributed at the start of the session) but lever pressing did not result in further food pellets distribution. This food-induced reinstatement test revealed that when the food pellets had not been devalued, the distribution of these same food pellets induced a modest lever pressing reinstatement. This lever pressing reinstatement was independent of the previous conditions of nicotine exposure, which shows that experiencing the effects of nicotine during operant training did not modify the appetitive value of the food pellets. However, in contrast to what was observed during nicotine-induced reinstatement test, the food-induced reinstatement test revealed that when the food pellets had been devalued, lever pressing resumption was totally prevented by previous food pellets devaluation **(Fig 2C)** and most of the rats did not even consume the free food pellets available in the food dispenser, which shows that lever pressing was guided by the representation of the actual value of the food pellets. Responding on the inactive lever and behavioral locomotor activity was negligible during this food-induced reinstatement test **(supplementary Fig 8C and Fig 11C respectively)**. This finding confirms that the outcome devaluation procedure was efficient and induced a long-lasting avoidance of the food pellets.

Taken together, these results confirm the hypothesis that once under the influence of nicotine, rats fail to adjust their behavior to the current value of the outcome, but reinstate habitual behaviors previously performed while under the effects of nicotine. Importantly, this inability to adjust the behavior to the current value of the outcome was specific to the nicotine-induced reinstatement test as it was not observed during the food-induced reinstatement test.

To determine whether this property is unique to drugs of abuse, a third experiment was conducted. In Experiment 3, the same protocol was followed, except a discriminative context stimulus (texture + odor) was tested instead of a drug stimulus **(see supplementary Fig 3 for operant training and extinction and supplementary Fig 6 for outcome devaluation)**. After food outcome devaluation, all rats were re-exposed to the presentation of the discriminative context stimulus (DC+) in a reinstatement test. Re-exposure to the DC+ reinstated lever pressing in the "*DC+* non-devalued" group (the rats that performed the operant task under the presentation of the DC+) and in the "*Ø* devalued" group (the rats that experienced the DC+ dissociated from the operant training) **(Fig 3A)**. However, the reinstatement observed in the "*Ø* devalued" group appears to be non-specific.

Specifically, rats in "*Ø*" groups experienced for the first time the DC+ in the operant cages, which resulted in an increase of operant responding in both, the active and the inactive levers **(supplementary Fig 9A)**, suggesting that this increase of responding observed during re-exposure to the DC+ was the result of a novelty effect (reflected in response generalization) which was significant by chance. Furthermore, re-exposure to the DC+ in the "*DC+*" groups induced higher levels of lever pressing in the non-devalued group than in the devalued group, which reveals the goal-directed nature of the DC+ reinstatement (sensitive to previous food pellets devaluation). Thus, it appears that a discriminative context does not reinstate any extinguished behavior but acts as a discriminative stimulus that specifically reinstates goal-directed behaviors (unlike cocaine and nicotine). Furthermore, lever pressing resumption did not result from an increased sensitivity to the texture and odor of the DC+, since all groups decreased their locomotor activity in response to the DC+ **(supplementary Fig 12A)**. The ability of the discriminative context stimulus to reinstate the extinguished food-seeking behavior in a goal-directed manner indicates that the reinstated behavior is guided by the current value of the outcome. These results are in agreement with a previous study which showed that context plays an important role in regulating extinguished instrumental responding for a sucrose reward (Hamlin et al. 2006).

Finally, to ensure that the devaluation procedure effectively modified the perceived value of the food pellets, a third reinstatement test was conducted. In this reinstatement test, all rats were re-exposed to the food pellets (seven food pellets freely distributed at the start of the session) but lever pressing did not result in further food pellet distribution. This food-induced reinstatement test revealed that when the food pellets had not been devalued, the distribution of these same food pellets induced lever pressing reinstatement. This lever pressing reinstatement was independent of the previous conditions of exposure to the discriminative context, which shows that experiencing the discriminative context stimulus during operant training did not modify the appetitive value of the food pellets. Furthermore, in accordance to with what was observed during the discriminative context-induced reinstatement test, the food-induced reinstatement test revealed that when the food pellets had been devalued, lever pressing reinstatement was totally prevented by previous food pellet devaluation **(Fig 3B)** and most of the rats did not even consume the free food pellets available in the food dispenser, which shows that lever pressing was guided by the representation of the actual value of the food pellets. Responding on the inactive lever was negligible during this food-induced reinstatement test **(supplementary Fig 9B)**. Regarding behavioral locomotor activity, the non-contingent distribution of food pellets induced an increase in locomotor activity only in the non-devalued groups **(supplementary Fig 12B)**. Furthermore, this increase in locomotor activity was independent of the previous conditions of exposure to the discriminative context, which suggests that the non-contingent distribution of the highly palatable food pellets promoted exploratory behavior in order to pursue the food pellets. These findings confirm that the outcome devaluation procedure was efficient and induced a long-lasting avoidance of the food pellets.

Taken together, these results confirm the hypothesis that only under the influence of drugs of abuse, rats fail to adjust their behavior to the current value of the outcome by reinstating habitual behaviors previously performed while under the effects of the drug. Importantly, rats that did not experience the effects of drugs of abuse are able to adjust their behavior to the current value of the outcome (as observed in the context and in the food reinstatement tests).

The results from Experiment 1, 2 and 3 indicate that both drugs of abuse and a discriminative context can reinstate an extinguished food-seeking behavior, even when the food outcome has been made aversive by a devaluation procedure. However, our results indicate that they act on different aspects of reinstatement. Specifically, the results from Experiments 1 and 2 showed that the discriminative stimulus properties of cocaine and nicotine reinstated the extinguished food-seeking behavior even when the food outcome was made aversive, indicating that neither cocaine nor nicotine act as an environmental discriminative stimulus used to predict the availability of the food outcome, but rather the cocaine and nicotine stimuli are integrated within a S-R association in a manner that remains to be determined. Importantly, our data suggests that these S-R associations remained latent during abstinence periods, as attested by the rats' ability to adjust their behavior when faced with the aversive outcome, but recur when they re-experienced the cocaine and the nicotine effects. On the other hand, and unlike the results obtained with drugs of abuse, the results from Experiment 3 showed

that a discriminative context reinstated the extinguished food-seeking behavior in a goal-directed manner (sensitive to food outcome devaluation), indicating that the discriminative context acts as a reliable predictor of the availability of the food outcome during instrumental training and is integrated within an action-outcome (A-O) association. Importantly, our data showed that this A-O association was present all the time, as attested by the rats' ability to adjust their behavior when re-exposed to the presentation of the discriminative context and when faced with the aversive outcome.

Conclusion

In conclusion, this study shows for the first time that re-exposure to cocaine and nicotine reinstates drug-related habitual behaviors independently of the current value of the outcome. As seen in our study, the expression of habit formation was the result of the neuroadaptations induced by chronic exposure to cocaine and nicotine since the behavior of animals that did not receive drug treatment remained goal-directed. It is worth noting that even by using a fixed-ratio schedule of reinforcement, operant responding in cocaine and nicotine-treated rats did not remain A-O driven, suggesting that cocaine and nicotine accelerate the formation of habits. However, future studies are necessary to analyze if the formation of habits by chronic drug exposure is stronger by using a schedule of reinforcement which promotes habit-based behavior. Nevertheless, these cocaine and nicotine-induced habits could explain the loss of control induced by re-exposure to cocaine and nicotine in former addicts, and the rapid resumption of compulsive drug-taking despite the explicit knowledge of the adverse consequences.

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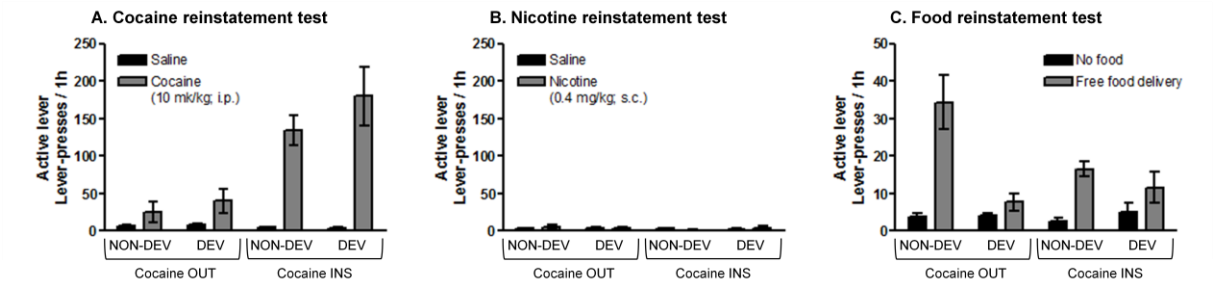


Figure 1 (Experiment 1). Reinstatement of an extinguished food-seeking behavior, following food outcome devaluation. **(A)** In a first reinstatement test, rats were re-exposed to cocaine. Only rats previously injected with cocaine during operant training (cocaine "inside" group) significantly reinstated operant responding when re-exposed to cocaine. A four-factor ANOVA for the cocaine challenge (treatment conditioning environment x devaluation condition x levers x test session) revealed a significant effect of treatment conditioning environment ($F(1,40)=26.19$, $p<0.000$), levers ($F(1,40)=57.61$, $p<0.000$) and test session ($F(1,40)=51.43$, $p<0.000$). The global ANOVA also indicated a significant treatment conditioning environment x levers x test session interaction ($F(1,40)=24.99$, $p<0.000$). Post hoc NK comparisons on this interaction revealed that, only in the "inside" group, a priming injection of cocaine reinstated operant responding just on the active lever when compared to a control saline injection (NK test, $p<0.000$), independently of previous food pellet devaluation as revealed by a non-significant devaluation condition x levers x test session interaction ($F(1,40)=1.65$, n.s.). These results reveal the habitual nature of cocaine reinstatement. **(B)** In a second reinstatement test, rats were exposed to nicotine. A four-factor ANOVA for the nicotine challenge revealed no significant interaction between treatment conditioning environment x devaluation condition x levers x test session ($F(1,40)=3.94$, n.s.). When compared to a control saline injection, a priming injection of nicotine did not reinstate lever pressing in any group. The global ANOVA indicated only a significant effect of levers ($F(1,40)=15.48$, $p<0.000$). **(C)** Finally, in the third reinstatement test, seven food pellets were freely distributed at the start of the session. A four-factor ANOVA for the food challenge (treatment conditioning environment x devaluation condition x levers x test session) revealed a significant effect of devaluation condition ($F(1,40)=10.77$, $p<0.002$), levers ($F(1,40)=49.20$, $p<0.000$) and test session ($F(1,40)=36.49$, $p<0.000$), and a significant interaction between them ($F(1,40)=11.47$, $p<0.001$). Post hoc NK comparisons on this interaction revealed that, when compared to a control no-food session, the non-contingent distribution of food pellets reinstated lever pressing just on the previously active lever and in all groups (NK test, $p<0.000$ in the non-devalued groups and $p<0.031$ in the devalued groups), independently of previous food pellet devaluation and of the environment where cocaine was experienced during the conditioning sessions (inside or outside the operant cages). Histograms represent mean (± SEM) responses on the active lever.

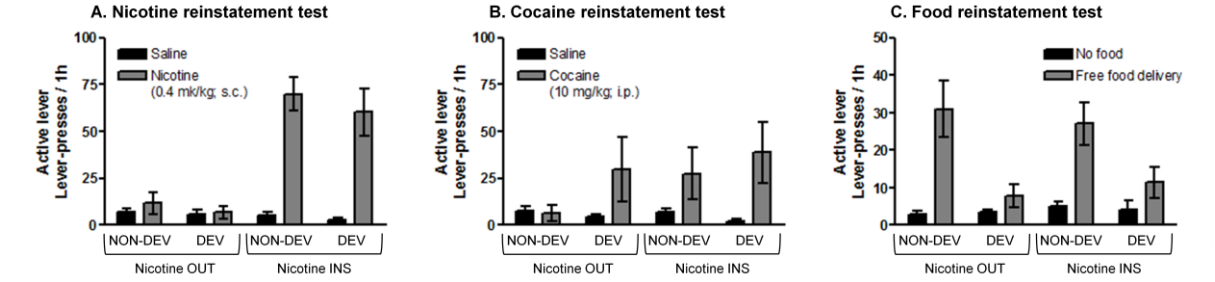


Figure 2 (Experiment 2). Reinstatement of an extinguished food-seeking behavior, following food outcome devaluation. **(A)** In a first reinstatement test, rats were re-exposed to nicotine. Only rats previously injected with nicotine during operant training (nicotine "inside" group) significantly reinstated operant responding when re-exposed to nicotine. A four-factor ANOVA for the nicotine challenge (treatment conditioning environment x devaluation condition x levers x test session) revealed a significant effect of treatment conditioning environment ($F(1,23)=31.90$, $p<0.000$), levers ($F(1,23)=68.75$, $p<0.000$) and test session ($F(1,23)=52.80$, $p<0.000$). The global ANOVA also indicated a significant treatment conditioning environment x levers x test session interaction ($F(1,23)=44.16$, $p<0.000$). Post hoc NK comparisons on this interaction revealed that, only in the "inside" group, a priming injection of nicotine reinstated operant responding just on the active lever when compared to a control saline injection (NK test, $p<0.000$), independently of previous food pellets devaluation as revealed by a non-significant devaluation condition x levers x test session interaction ($F(1,23)=0.28$, n.s.), which reveals the habitual nature of nicotine reinstatement. **(B)** In a second reinstatement test, rats were exposed to cocaine. A four-factor ANOVA for the cocaine challenge indicated no significant interaction between treatment conditioning environment x devaluation condition x levers x test session ($F(1,23)=0.13$, n.s.). However, the global ANOVA also indicated a significant levers effect ($F(1,23)=14.46$, $p<0.000$) and test session ($F(1,23)=6.66$, $p<0.016$), and a significant interaction between them ($F(1,23)=8.76$, $p<0.007$). Post hoc NK comparisons on this interaction revealed that, when compared to a control saline injection, a priming injection of cocaine reinstated lever pressing just on the active lever (NK test, $p<0.000$) independently of previous food pellet devaluation and the environment where nicotine was experienced (inside or outside the operant cages). **(C)** Finally, in the third reinstatement test, seven food pellets were freely distributed at the start of the session. A four-factor ANOVA for the food challenge (treatment conditioning environment x devaluation condition x levers x test session) revealed a significant effect of devaluation condition ($F(1,23)=10.91$, $p<0.003$), levers ($F(1,23)=38.79$, $p<0.000$) and test session ($F(1,23)=18.81$, $p<0.000$), and a significant interaction between them ($F(1,23)=15.91$, $p<0.000$). Post hoc NK comparisons on this interaction revealed that, when compared to a control no-food session, the non-contingent distribution of food pellets reinstated lever pressing just on the previously active lever and only in the non-devalued groups (NK test, $p<0.000$), independently of the environment where nicotine was experienced during the conditioning sessions (inside or outside the operant cages). This result confirms the efficiency of the devaluation procedure. Histograms represent mean (± SEM) responses on the active lever.

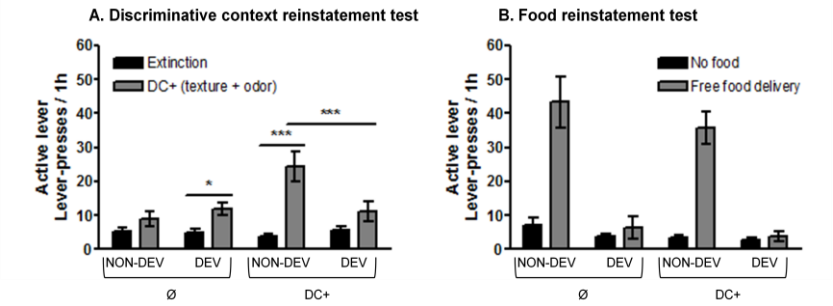
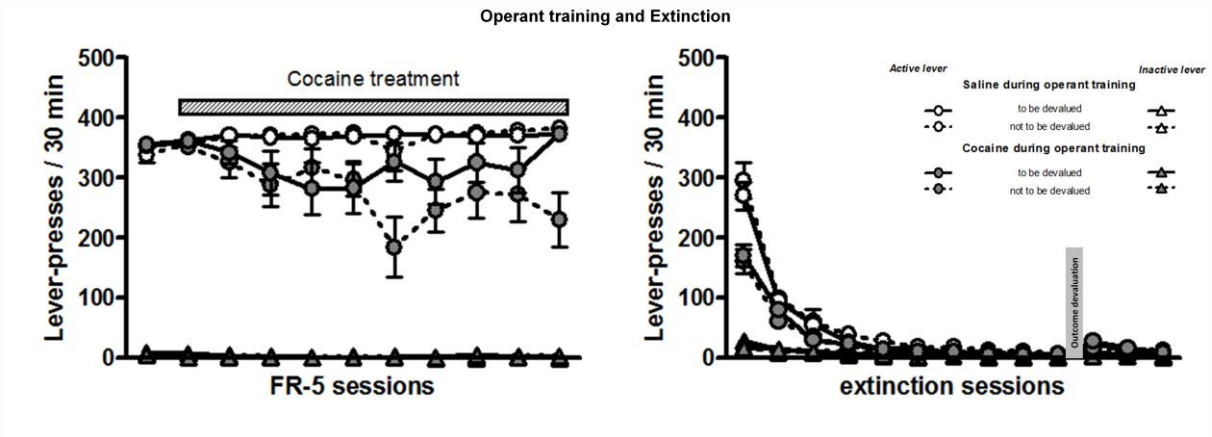
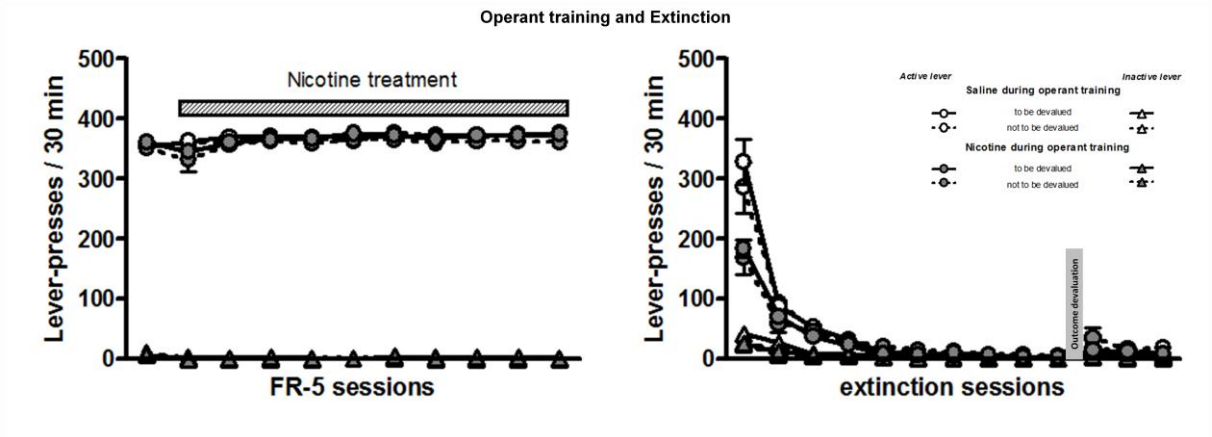


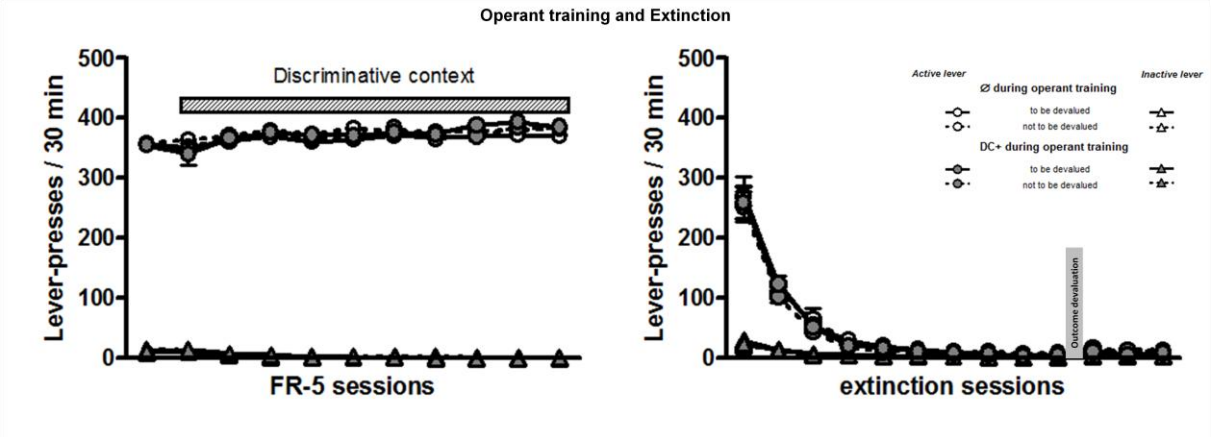
Figure 3 (Experiment 3). Reinstatement of an extinguished food-seeking behavior, following food outcome devaluation. **(A)** In a first reinstatement test, rats were re-exposed to the presentation of the discriminative context (DC+). Re-exposure to the DC+ differently affected operant responding between groups. A four-factor ANOVA for the DC+ challenge (DC+ conditioning environment x devaluation condition x levers x test session) revealed a significant effect of levers ($F(1,44)=30.99$, $p<0.000$) and test session ($F(1,44)=46.15$, $p<0.000$), and a significant DC+ conditioning environment x devaluation condition x levers x test session interaction ($F(1,44)=11.19$, $p<0.001$). Post hoc NK comparisons on this interaction revealed that re-exposure to the DC+ reinstated operant responding on the active lever in the non-devalued rats that experienced the DC+ during operant training (NK test, $p<0.000$ when compared to its respective control extinction session) and in the devalued rats that did not experience the DC+ during operant training (NK test, $p<0.041$ when compared to its respective control extinction session). However, the increase of operant responding observed in the devalued rats appears to be non-specific. Specifically, rats in the Ø group experienced for the first time the DC+ in the operant cages, which resulted in an increase of operant responding on both the active and the inactive levers, suggesting that the reinstatement observed during the DC+ challenge was the result of a novelty effect. Furthermore, during the DC+ challenge and in the DC+ group (the group of rats that experienced the DC+ during operant training), operant responding on the active lever in the non-devalued rats was significantly different from that in the devalued rats (NK test, $p<0.000$), which reveals the goal-directed nature of the DC+ reinstatement. **(B)** Finally, in the second reinstatement test, seven food pellets were freely distributed at the start of the session. A four-factor ANOVA for the food challenge (DC+ conditioning environment x devaluation condition x levers x test session) revealed a significant effect of devaluation condition ($F(1,44)=39.24$, $p<0.000$), levers ($F(1,44)=78.06$, $p<0.000$) and test session ($F(1,44)=44.00$, $p<0.000$), and a significant interaction between them ($F(1,44)=39.91$, $p<0.000$). Post hoc NK comparisons on this interaction revealed that, when compared to a control no-food session, the non-contingent distribution of food pellets reinstated lever pressing just on the previously active lever and only in the non-devalued groups (NK test, $p<0.000$), independently of the environment where the discriminative context was experienced during the conditioning sessions (operant cages or individual cages). This result confirms the efficiency of the devaluation procedure. Histograms represent mean (± SEM) responses on the active lever. * significantly different from control extinction session (* $p<0.05$, ** $p<0.01$, *** $p<0.001$).



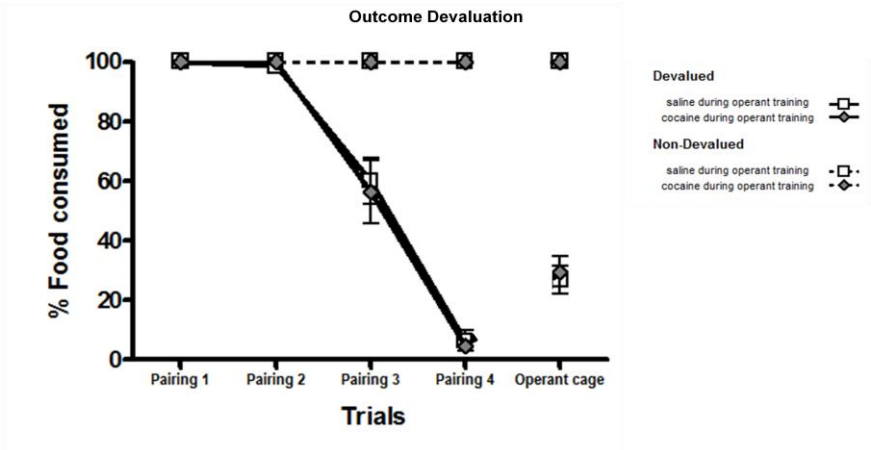
Supplementary Figure 1 (Experiment 1). Training and extinction of the operant behavior. A maximum of 70 food pellets could be earned during each 30 min training session. This cutoff corresponds approximately to the number of food pellets that rats trained under the influence of cocaine could earn in a session, considering the rate-decreasing effects of cocaine on operant behavior (Keiflin et al. 2008a). **(Left)** During the conditioning phase, a four-factor ANOVA (treatment conditioning environment x devaluation condition x levers x session) revealed no significant interaction between treatment conditioning environment x devaluation condition x levers x session ($F(9,360)=1.76$, n.s.), indicating that operant responses did not differ significantly between groups. However, the global ANOVA also indicated a significant treatment conditioning environment x levers x session interaction ($F(9,360)=2.48$, $p<0.009$). Post hoc NK comparisons on this interaction revealed that cocaine had differential effects on operant responses on the active lever depending on whether the animals were in the drug state inside or outside the operant cages. Only rats injected with cocaine prior to being placed in the operant cages showed lower levels of responding when compared to the first conditioning session (NK test, $p<0.000$), independently of devaluation condition. **(Right)** During extinction sessions, a four-factor ANOVA (treatment conditioning environment x devaluation condition x levers x session) revealed no significant interaction between treatment conditioning environment x devaluation condition x levers x session ($F(12,480)=0.61$, n.s.). In comparison to inactive lever responding, operant responses on the active lever progressively decreased until reaching the extinction criteria after 10 sessions, as indicated by a significant levers x session interaction ($F(12,480)=202.77$, $p<0.000$). However, the treatment conditioning environment had an effect on extinction; rats under the drug effect of cocaine inside the operant cages showed lower levels of operant responding only in the first two sessions in comparison to rats under the effect of cocaine outside the operant cages, as indicated by a significant levers x session x treatment conditioning environment interaction ($F(12,480)=15.42$, $p<0.000$). Each point represents mean (± SEM) responses per session on the active or inactive lever.



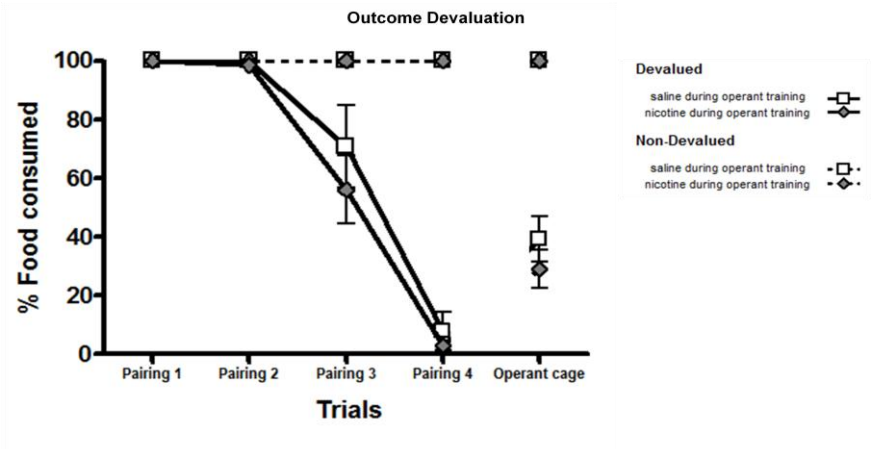
Supplementary Figure 2 (Experiment 2). Training and extinction of the operant behavior. A maximum of 70 food pellets could be earned during each 30 min training session. **(Left)** During the conditioning phase, a four-factor ANOVA (treatment conditioning environment x devaluation condition x levers x session) revealed no significant interaction between treatment conditioning environment x devaluation condition x levers x session ($F(9,207)=0.32$, n.s.), indicating that operant responses did not differ significantly between groups. However, the global ANOVA also indicated a significant levers x session interaction ($F(9,207)=5.89$, $p<0.000$). Post hoc NK comparisons on this interaction revealed that nicotine had differential effects on operant responses. Rats' operant responses were directed towards the active lever (NK test, $p<0.000$ when comparing active and inactive lever presses throughout the conditioning sessions), independently of devaluation condition and on whether the rats were under the drug state of nicotine inside or outside the operant cages. **(Right)** During extinction sessions, a four-factor ANOVA (treatment conditioning environment x devaluation condition x levers x session) revealed no significant interaction between treatment conditioning environment x devaluation condition x levers x session ($F(12,276)=0.26$, n.s.). In comparison to inactive lever responding, operant responses on the active lever progressively decreased until reaching the extinction criteria after 10 sessions, as indicated by a significant levers x session interaction ($F(12,276)=123.61$, $p<0.000$). However, the treatment conditioning environment had an effect on extinction; rats under the effect of nicotine inside the operant cages showed lower levels of operant responding only in the first two sessions in comparison to those injected with nicotine before being placed in the individual cages, as indicated by a significant levers x session x treatment conditioning environment interaction ($F(12,276)=10.60$, $p<0.000$). Each point represents mean (± SEM) responses per session on the active or inactive lever.



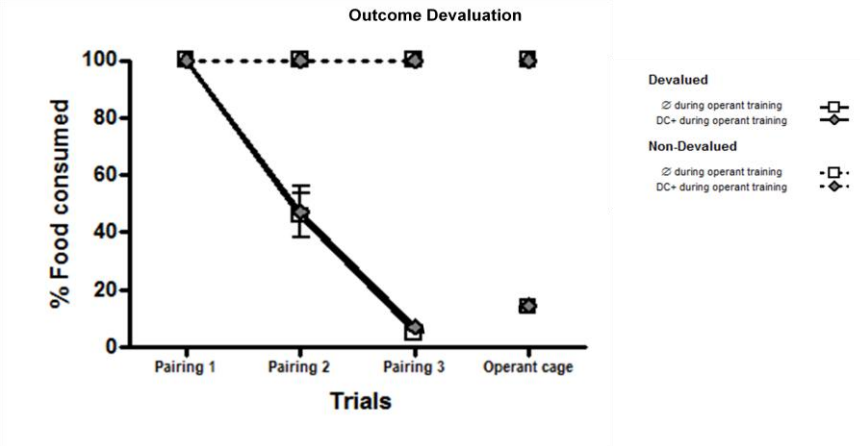
Supplementary Figure 3 (Experiment 3). Training and extinction of the operant behavior. A maximum of 70 food pellets could be earned during each 30 min training session. **(Left)** During the conditioning phase, a four-factor ANOVA (DC+ conditioning environment x devaluation condition x levers x session) revealed no significant interaction between DC+ conditioning environment x devaluation condition x levers x session ($F(9,396)=0.55$, n.s.), indicating that operant responses did not differ significantly between groups. However, the global ANOVA also revealed a significant levers x session interaction ($F(9,396)=17.18$, $p<0.000$). Post hoc NK comparisons on this interaction revealed that rats' operant responses were directed towards the active lever (NK test, $p<0.000$ when comparing active and inactive lever presses throughout the conditioning sessions), independently of devaluation condition and whether the DC+ was present in the operant or in the individual cages. **(Right)** During extinction sessions, a four-factor ANOVA (DC+ conditioning environment x devaluation condition x levers x session) revealed no significant interaction between DC+ conditioning environment x devaluation condition x levers x session ($F(12,528)=0.14$, n.s.). In comparison to inactive lever responding, operant responses on the active lever progressively decreased until reaching the extinction criteria after 10 sessions, as indicated by a significant levers x session interaction ($F(12,528)=265.59$, $p<0.000$), independently of devaluation condition and whether the DC+ was present in the operant or in the individual cages during the conditioning sessions. Each point represents mean (± SEM) responses per session on the active or inactive lever.



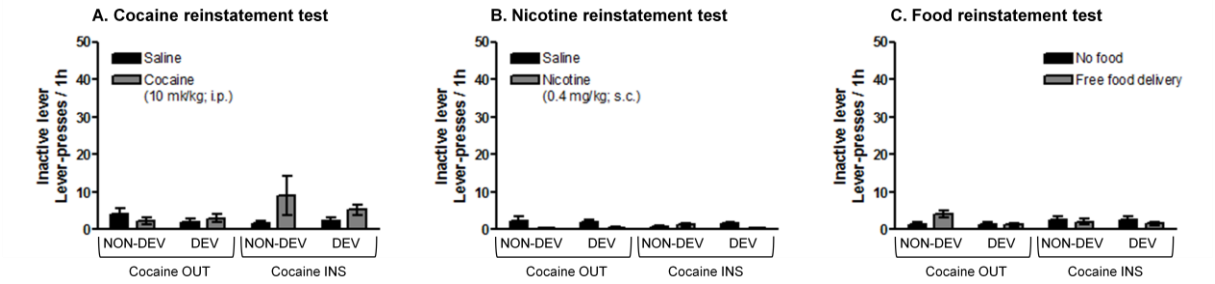
Supplementary Figure 4 (Experiment 1). Devaluation of the food pellets in cocaine-treated rats. Repeated associations of food pellets with LiCl-induced gastric illness induced a progressive decrease in food pellet consumption in cocaine-treated rats, regardless of the environment where they experienced the cocaine effects (inside or outside the operant cages). In contrast, when the food pellets were not associated with LiCl-induced gastric illness, rats always consumed all the food pellets available. A three-factor ANOVA (treatment conditioning environment x devaluation condition x trial) revealed a significant effect of devaluation condition ($F(1,40)=416.29$, $p<0.000$) and trial ($F(3,120)=150.24$, $p<0.000$), and a significant interaction between them ($F(3,120)=150.24$, $p<0.000$). Post hoc NK comparisons on this interaction confirmed that the devalued groups decreased food pellet consumption from the third trial and onward when compared to the second trial (NK test, $p<0.000$), independently of the environment where cocaine was experienced during the conditioning sessions (inside or outside the operant cages). The food pellet devaluation induced by LiCl was long-lasting and context-independent, as confirmed by a final food pellet consumption test conducted in the operant cages at the end of the experiment. Each point represents mean (± SEM) percentage of food pellets consumed per trial.



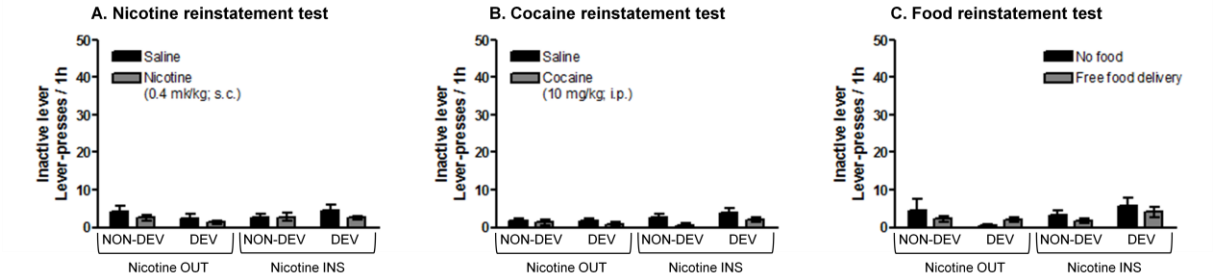
Supplementary Figure 5 (Experiment 2). Devaluation of the food pellets in nicotine-treated rats. Repeated associations of food pellets with LiCl-induced gastric illness induced a progressive decrease in food pellet consumption in nicotine-treated rats, regardless of the environment where they experienced the nicotine effects (inside or outside the operant cages). In contrast, when the food pellets were not associated with LiCl-induced gastric illness, rats always consumed all the food pellets available. A three-factor ANOVA (treatment conditioning environment x devaluation condition x trial) revealed a significant effect of devaluation condition ($F(1,23)=240.76$, $p<0.000$) and trial ($F(3,69)=52.64$, $p<0.000$), and a significant interaction between them ($F(3,69)=52.64$, $p<0.000$). Post hoc NK comparisons on this interaction confirmed that the devalued groups decreased food pellets consumption from the third trial and onward when compared to the second trial (NK test, $p<0.000$), independently of the environment where nicotine was experienced during the conditioning sessions (inside or outside the operant cages). The food pellet devaluation induced by LiCl was long-lasting and context-independent, as confirmed by a final food pellet consumption test conducted in the operant cages at the end of the experiment. Each point represents mean (± SEM) percentage of food pellets consumed per trial.



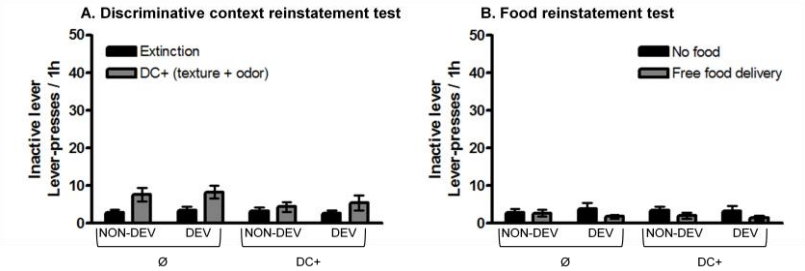
Supplementary Figure 6 (Experiment 3). Devaluation of the food pellets during the discriminative context (DC+) experiment. Repeated associations of food pellets with LiCl-induced gastric illness induced a progressive decrease in food pellet consumption in rats, regardless of the environment where they experienced the DC+ (operant cages or individual cages). In contrast, when the food pellets were not associated with LiCl-induced gastric illness, rats always consumed all the food pellets available. A three-factor ANOVA (DC+ conditioning environment x devaluation condition x trial) revealed a significant effect of devaluation condition ($F(1,44)=1354.40$, $p<0.000$) and trial ($F(2,88)=36.76$, $p<0.000$), and a significant interaction between them ($F(2,88)=36.76$, $p<0.000$). Post hoc NK comparisons on this interaction confirmed that the devalued groups decreased food pellets consumption from the third trial and onward when compared to the second trial (NK test, $p<0.000$), independently of the environment where the DC+ was experienced during the conditioning sessions (operant or individual cages). The food pellet devaluation induced by LiCl was long-lasting and context-independent, as confirmed by a final food pellet consumption test conducted in the operant cages at the end of the experiment. Each point represents mean (± SEM) percentage of food pellets consumed per trial.



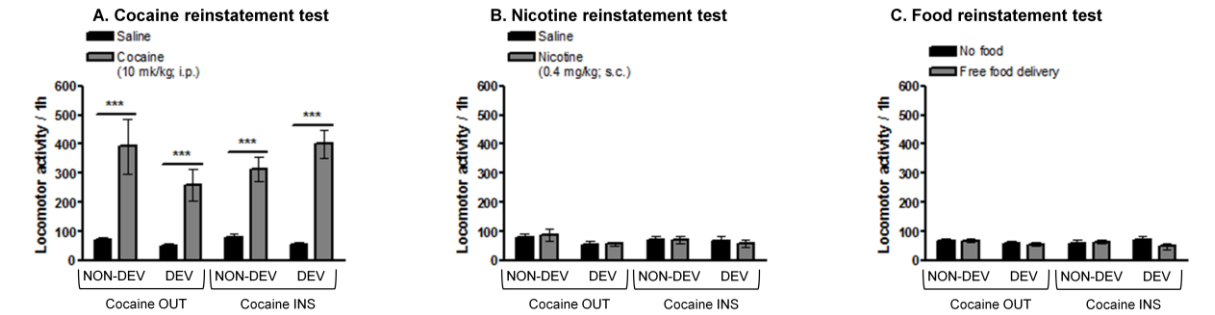
Supplementary Figure 7 (Experiment 1). Responding on the inactive lever during the different reinstatement tests of an extinguished food-seeking behavior, following outcome devaluation. Operant responses of cocaine pre-treated rats on the inactive lever were negligible compared to operant responses on the active lever, which shows that lever pressing reinstatement on the active lever was not a consequence of a general, non-specific, behavioral activation. Histograms represent mean (± SEM) responses on the inactive lever.



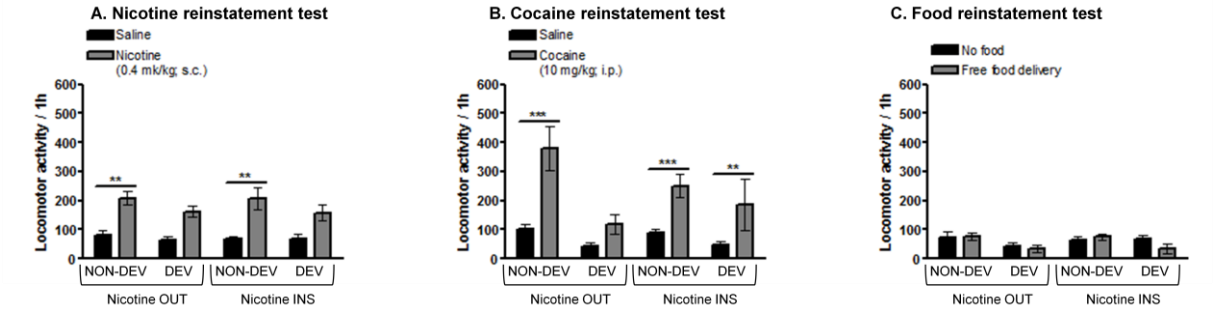
Supplementary Figure 8 (Experiment 2). Responding on the inactive lever during the different reinstatement tests of an extinguished food-seeking behavior, following outcome devaluation. Operant responses of nicotine pre-treated rats on the inactive lever were negligible compared to operant responses on the active lever, which shows that lever pressing reinstatement on the active lever was not a consequence of a general, non-specific, behavioral activation. Histograms represent mean (± SEM) responses on the inactive lever.



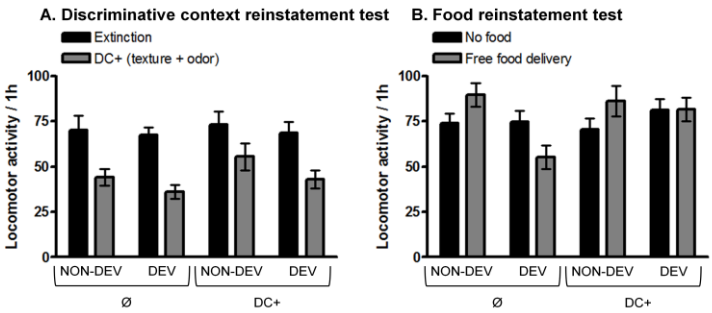
Supplementary Figure 9 (Experiment 3). Responding on the inactive lever during the different reinstatement tests of an extinguished food-seeking behavior, following outcome devaluation. Operant responses of DC+ and Ø rats on the inactive lever were negligible compared to operant responses on the active lever, which shows that lever pressing reinstatement on the active lever was not a consequence of a general, non-specific, behavioral activation. Histograms represent mean (± SEM) responses on the inactive lever.



Supplementary Figure 10 (Experiment 1). Locomotor activity of cocaine pretreated rats recorded during the different reinstatement tests. A four-factor ANOVA for the cocaine, nicotine and food challenges (treatment conditioning environment x devaluation condition x challenge x test session) revealed a significant effect of challenge ($F(2,64)=93.30$, $p<0.000$) and test session ($F(1,32)=92.60$, $p<0.000$), and a significant interaction between treatment conditioning environment x devaluation condition x challenge x test session ($F(2,64)=3.79$, $p<0.027$). Post hoc NK comparisons indicated that a priming injection of cocaine induced a motor hyperactivity in all groups of rats in comparison to their respective control saline injection (NK test, $p<0.000$), independently of the environment where cocaine was experienced during the conditioning sessions (inside or outside the operant cages) and independently of previous food pellet devaluation **(A)**. A priming injection of nicotine **(B)** and non-contingent distribution of food pellets **(C)** had no differential effects on cocaine pretreated rats' locomotor activity. Histograms represent mean (± SEM) crossovers recorded in the operant conditioning chambers during the test. * significantly different from control saline injection (* $p<0.05$, ** $p<0.01$, *** $p<0.001$).



Supplementary Figure 11 (Experiment 2). Locomotor activity of nicotine pretreated rats recorded during the different reinstatement tests. A four-factor ANOVA for the nicotine, cocaine and food challenges (treatment conditioning environment x devaluation condition x challenge x test session) revealed a significant effect of devaluation condition ($F(1,23)=8.60$, $p<0.007$), challenge ($F(2,46)=23.90$, $p<0.000$) and test session ($F(1,23)=48.90$, $p<0.000$), and a significant interaction between treatment conditioning environment x devaluation condition x challenge x test session ($F(2,46)=3.70$, $p<0.032$). Post hoc NK comparisons indicated that a priming injection of nicotine induced a motor hyperactivity only in the non-devalued groups in comparison to their respective control saline injection (NK test, non-devalued groups: $p<0.004$ in the nicotine outside group and $p<0.003$ in the nicotine inside group), independently of the environment where nicotine was experienced during the conditioning sessions (inside or outside the operant cages) **(A)**. A priming injection of cocaine induced a motor hyperactivity in most rats in comparison to their respective control saline injection (NK test, nicotine "outside" group: $p<0.000$ in the non-devalued group; NK test, nicotine "inside" groups: $p<0.000$ in the non-devalued group and $p<0.005$ in the devalued group), independently of the environment where nicotine was experienced during the conditioning sessions **(B)**. Finally, non-contingent distribution of food pellets had no effect on nicotine pretreated rats' locomotor activity **(C)**. Histograms represent mean (± SEM) crossovers recorded in the operant conditioning chambers during the test. * significantly different from control saline injection (* $p<0.05$, ** $p<0.01$, *** $p<0.001$).



Supplementary Figure 12 (Experiment 3). Locomotor activity of DC+ and Ø rats recorded during the different reinstatement tests. **(A)** A three-factor ANOVA for the discriminative context challenge (DC+ conditioning environment x devaluation condition x test session) only revealed a significant effect of test session ($F(1,44)=77.92$, $p<0.000$). Post hoc NK comparisons on this main effect revealed that re-exposure to the discriminative context induced a decrease in motor activity in comparison to their respective extinction control session (NK test, $p<0.000$), independently of whether the DC+ was present in the operant or in the individual cages during the conditioning sessions and independently of previous food pellet devaluation. **(B)** A three-factor ANOVA for the food challenge (DC+ conditioning environment x devaluation condition x test session) only revealed a significant devaluation condition x test session interaction ($F(1,44)=16.02$, $p<0.000$). Post hoc NK comparisons on this interaction revealed that non-contingent distribution of food pellets had differential effects on rats' locomotor activity. Specifically, the non-contingent distribution of food pellets induced an increase in motor activity in comparison to their respective no-food control session only in the non-devalued groups and independently of whether the DC+ was present in the operant or in the individual cages during the conditioning sessions (NK test, $p<0.002$). Histograms represent mean (± SEM) crossovers recorded in the operant conditioning chambers during the test.

Supplementary Material and Methods

Subjects

119 male Wistar rats (175-225 g; Charles River Laboratories, France) were housed in groups of four per cage on a 12 h reverse light/dark cycle (lights on from 20:00 h to 08:00 h) in a temperature (22°C) and humidity (60%) controlled room. Rats were acclimated to handling and allowed to adapt for a minimum of four days prior to the start of the experiment. Experiments were conducted during the dark phase of the light/dark cycle. Water was available ad libitum and food was restricted to 18 g chow per day to maintain rats at 90% of their free-feeding weight. Food was distributed in the home cages everyday at 19:00 h. All experimental procedures were performed in accordance with the European directive (86/609/EEC) and with the approval of the Centre National de la Recherche Scientifique.

Drugs

Cocaine hydrochloride (Cooperative Pharmaceutique Française, France) and (-) Nicotine hydrogen tartrate (Sigma, St. Louis, MO, USA) were dissolved in sterile physiological saline (0.9%). Cocaine dose is expressed as the weight of the salt, whereas nicotine dose is expressed as the base and pH was adjusted to approximately 7.0 before use. Cocaine (10 mg/kg) was administered intraperitoneally and nicotine (0.4 mg/kg) was administered subcutaneously. Injection volumes were 1 ml/kg. Injection routes and doses were selected from previous reports in the literature (Keiflin et al. 2008b; Rogers et al. 2008; Shoaib 2006).

Apparatus

Operant conditioning chambers. Operant conditioning for food pellet reinforcement was conducted in twelve standard operant conditioning chambers (30 cm height × 40 cm length × 36 cm depth, Imetronic, Pessac, France) located in an experimental room equipped with white noise generators. Each experimental chamber was individually housed in wood noise attenuation boxes fitted with ventilation fans and had two clear Plexiglass walls on the front and back sides and two opaque panels on the left and right sides. The floor consisted of 6 mm diameter steel bars spaced 15 mm apart, center-to-center. Two retractable levers (0.03 N force required for activation) were located on either side of the left panel (7 cm above the floor) counterbalanced as active or inactive across left and right. Illumination of a soft house light bulb (2W) signaled the start of the session. Each response on the active lever activated a food pellet dispenser located outside the operant chamber allowing the immediate delivery of the food pellets [45 mg Rodent Tablet, Test Diet (5TUL)] into a food magazine located midway between the two levers. Responses on the inactive lever were recorded but were without consequence. In order to record the locomotor activity of the rats from one side of the cage to the other (crossovers), each experimental chamber was also equipped with two pairs of infrared beams. Number of lever presses, food pellets delivered, and crossovers were automatically recorded (software Imetronic, Pessac, France).

Standard individual chambers. In addition to the operant conditioning chambers, rats were also exposed to standard individual chambers which were very different from the operant chambers (35 cm height × 25 cm length × 25 cm depth, Imetronic, Pessac, France) located in a dark experimental room equipped with white noise generators, where neither food pellets nor levers were present. The door, floor, and ceiling of each experimental cage consisted of wire mesh and the side walls were made of 10 mm thick clear Plexiglass.

Experimental Procedure

Experiment 1: Cocaine-induced reinstatement for a food reward: Goal-directed or habit-based behavior?

Operant conditioning and extinction. Rats were equally exposed to two different environments in two daily 30 min sessions. The first environment was the operant conditioning chamber and the second environment was the standard individual chamber as described above. Sessions were run at the same time of the day, 5-6 days per week.

Acquisition

Rats were randomly assigned to one operant conditioning chamber and were trained to press one of the two levers (the active lever) for food pellet distribution. Rats were initially trained under a fixed ratio 1 (FR-1) schedule of reinforcement until they earned the maximum number of food pellets (100 food pellets) per session; afterwards, the response requirement was raised to FR-5 (70 food pellets maximum) for two days before the start of the conditioning sessions.

Conditioning

After the second FR-5 session, rats were divided into two groups. The "inside" group received a non-contingent peripheral injection of cocaine (10 mg/kg; i.p.) (n=22) immediately prior to the conditioning sessions in the operant chambers ("cocaine *inside*" group). In contrast, the "outside" group received a peripheral injection of cocaine (10 mg/kg; i.p.) (n=22) immediately prior to the conditioning sessions in the standard individual chambers ("cocaine *outside*" group). To equate the environment exposures, the "inside" group received saline injections in the standard individual chambers and the "outside" group received saline injections in the operant chambers. Cocaine has unconditioned disruptive effects on operant behavior that could potentially induce differences between groups during conditioning (in terms of lever pressing). In order to avoid such a difference and ensure that all rats had similar experience with the operant behavior, a maximum number of 70 food pellets could be earned during each session. When this maximum number of food pellets had been earned, the levers retracted and remained like that for the rest of the session. This maximum number of food pellets corresponds approximately to that which rats trained under the influence of cocaine could earn during a 30 min operant session. Rats trained under normal physiological conditions take slightly less than 30 min to earn these 70 food pellets during a 30 min operant session (Keiflin et al. 2008a).

Extinction

After 10 days of cocaine treatment, rats were subjected to a period of extinction during which lever presses no longer resulted in food pellet distribution. During this period, rats continued to be exposed to both environments but cocaine injections were replaced by saline injections. Extinction sessions continued for a minimum of 10 days and until the lever pressing rate fell below 10 per session for 3 consecutive days on the previously active lever.

Food outcome devaluation

After extinction of the operant responses, both groups ("cocaine *inside*" and "cocaine *outside*") were once again divided in two groups: 1) devalued and 2) non-devalued (n=11 per group). The outcome devaluation took place in individual feeding cages (which were completely different from the chambers previously used during the conditioning) made of clear plastic measuring 13 cm height x 30 cm length x 13 cm depth, with wire-mesh ceilings. Rats were placed in these individual cages and allowed to consume 100 food pellets from a bowl placed in the individual cage for 10 min. Immediately after these 10 min had elapsed, the devalued rats received LiCl-induced gastric illness injections (0.3 M; 5 ml/kg; i.p.), whereas the non-devalued rats received saline injections (5 ml/kg; i.p.). On alternate days, rats were placed in the same individual cages with an empty bowl placed in the individual cage for 10 min. Immediately after these 10 min had elapsed, the devalued rats received saline injections (5 ml/kg; i.p.), whereas the non-devalued rats received LiCl-induced gastric illness injections (0.3 M; 5 ml/kg; i.p.). The number of food pellets consumed was counted before and after the test. A total of four food- or no food-LiCl pairings were conducted, separated by 48 h. This

procedure ensured that all rats received the same exposure to the food pellets and to LiCl-induced gastric illness, but only the devalued group associated food pellet consumption with gastric illness.

Extinction

After food pellet devaluation, rats were once again submitted to a period of extinction during which lever presses no longer resulted in food pellet distribution. During this period, rats continued to be exposed to both environments. Three extinction sessions were needed to reach the level of 10 (or below) lever presses per session on the previously active lever. This "reminder" extinction period was performed in order to avoid spontaneous recovery from extinction (Rescorla 2004) because operant conditioning was not carried out during the food outcome devaluation procedure.

Reinstatement tests

Following the "reminder" extinction period, rats underwent three reinstatement tests with a minimum of 3 days of extinction between each test. Test sessions were conducted every third day (except when this fell on the first session of the week), providing that subjects maintained the below 10 lever press response criteria during the previous extinction sessions.

Cocaine-induced reinstatement

Reinstatement of instrumental food-seeking behavior in extinction conditions was tested by a single, non-contingent injection of cocaine for both groups ("inside" and "outside") in both conditions (devalued and non-devalued). The cocaine-induced reinstatement test was conducted over two sessions: a saline session and a drug session, separated by 24 h. The first session included a single saline injection administered immediately prior to the test session, whereas the second session included a single cocaine injection (10 mg/kg; i.p.) immediately prior to placing the rat in the chamber for a 1 h test session. During this 1 h test session, the house light was illuminated and lever presses had no programmed consequences. Operant responses and locomotor activity (crossovers) were recorded during both reinstatement sessions.

Nicotine-induced reinstatement

The reinstatement procedure was exactly the same as for cocaine-induced reinstatement except nicotine (0.4 mg/kg; s.c.) replaced cocaine.

Food-induced reinstatement

Finally, the effect of non-contingent food pellet distribution was assessed in both groups ("inside" and "outside") in both conditions (devalued and non-devalued). The food-induced reinstatement test was again conducted over two sessions, as in the drug-induced reinstatement tests. The first session was a control extinction session during which food pellets were not distributed. During the second session, 3 food pellets were placed in the food magazine and 4 additional food pellets were distributed non-contingently over the first 60 s. The sessions lasted 1 h and were separated by 24 h.

Experiment 2: Nicotine-induced reinstatement for a food reward: Goal-directed or habit-based behavior?

The experimental procedure was exactly as described for Experiment 1, except nicotine (0.4 mg/kg; s.c.) was tested instead of cocaine.

Conditioning

After the second FR-5 session, rats were divided into two groups. The "inside" group received a non-contingent peripheral injection of nicotine (0.4 mg/kg; s.c.) (n=14) immediately prior to the conditioning sessions in the operant chambers (group "nicotine *inside*"). In contrast, the "outside" group received a peripheral injection of nicotine (0.4 mg/kg; s.c.) (n=13) immediately prior to the conditioning sessions in the standard individual chambers ("nicotine *outside*" group). To equate the environment exposures, the "inside" group received saline injections in the standard individual chambers and the "outside" group received saline injections in the operant chambers. As in Experiment 1, a maximum number of 70 food pellets could be earned during each session. When this

maximum number of food pellets had been earned, the levers retracted and remained retracted for the rest of the session.

The experiment then continued to progress exactly as outlined in Experiment 1.

Food outcome devaluation

After extinction of the operant responses, both groups ("nicotine *inside*" and "nicotine *outside*") were once again divided in two groups: 1) devalued and 2) non-devalued (n=7 per group, except for "nicotine *outside* non-devalued", which was n=6). The outcome devaluation procedure was exactly the same as described for Experiment 1.

The experiment then continued to progress exactly as outlined in Experiment 1.

Nicotine-induced reinstatement

Reinstatement of instrumental food-seeking behavior in extinction conditions was tested by a single, non-contingent injection of nicotine for both groups ("inside" and "outside") in both conditions (devalued and non-devalued). The nicotine-induced reinstatement test was conducted over two sessions: a saline session and a drug session, separated by 24 h. The first session included a single saline injection administered immediately prior to the test session whereas the second session included a single nicotine injection (0.4 mg/kg; s.c.) immediately prior to placing the rat in the chamber for a 1 h test session. During this 1 h test session, the house light was illuminated and lever presses had no programmed consequences. Operant responses and locomotor activity (crossovers) were recorded during both reinstatement sessions.

Cocaine-induced reinstatement

The reinstatement procedure was exactly the same as for nicotine-induced reinstatement except cocaine (10 mg/kg; i.p.) replaced nicotine.

Food-induced reinstatement

The procedure used for food-induced reinstatement was identical to that described in Experiment 1.

Experiment 3: Context-induced reinstatement for a food reward: Goal-directed or habit-based behavior?

The experimental procedure was exactly as described for Experiments 1 and 2, except a tactile and olfactory discriminative contextual stimulus (DC+) was tested instead of a drug stimulus.

Conditioning

After the second FR-5 session, rats were divided into two groups. The "*DC+*" group (n=24) experienced a tactile and olfactory stimulus (which consisted of a semi-rough floor surface with some aniseed-scented sawdust scattered on it) during conditioning sessions in the operant chambers. In contrast, the "*Ø*" group (n=24) experienced the same tactile and olfactory stimulus during conditioning sessions in the standard individual chambers. To equate the environment exposures, the "*DC+*" group did not experience the tactile and olfactory stimulus in the standard individual chambers and the "*Ø*" group did not experience the tactile and olfactory stimulus in the operant chambers. As in Experiments 1 and 2, a maximum number of 70 food pellets could be earned during each session. When this maximum number of food pellets had been earned, the levers retracted, and remained retracted for the rest of the session.

Food outcome devaluation

After extinction of the operant responses, both groups ("*DC+*" and "*Ø*") were once again divided in two groups: 1) devalued and 2) non-devalued (n=12 per group). The outcome devaluation procedure was exactly the same as described for Experiments 1 and 2.

Reinstatement tests

Following the "reminder" extinction period, rats underwent two reinstatement tests with a minimum of 3 days of extinction between each test. Test sessions were conducted every third day (except when this fell on the first session of the week), providing that subjects maintained the below 10 lever press response criteria during the previous extinction sessions.

Context-induced reinstatement

Reinstatement of instrumental food-seeking behavior in extinction conditions was tested by re-exposure to the tactile and olfactory stimulus for both groups ("*DC+*" and "*Ø*") in both conditions (devalued and non-devalued). Context-induced reinstatement test was conducted over two 1 h sessions: a control session and a test session, separated by 24 h. The first session was a control extinction session whereas the second session included the re-exposure to the tactile and olfactory stimulus. During this 1 h test session, the house light was illuminated and lever presses had no programmed consequences. Operant responses and locomotor activity (crossovers) were recorded during both reinstatement sessions.

Food-induced reinstatement

The procedure used for food-induced reinstatement was identical to that described in Experiments 1 and 2.

Statistical Analyses

Operant responses were analyzed with repeated measures analysis of variance (ANOVA). Data from the three experiments were analyzed on separate four way ANOVAs with between-subjects factors of treatment conditioning environment (two levels: inside, outside; or DC+, Ø) and devaluation condition (two levels: devalued, non-devalued) and within-subjects factors of lever (two levels: active, inactive), challenge (either two levels: context, food; or three levels: cocaine, nicotine, food) and session. Locomotor activity data from the three experiments was also analyzed on separate four way ANOVAs with between-subjects factors of treatment conditioning environment (two levels: inside, outside; or DC+, Ø) and devaluation condition (two levels: devalued, non-devalued) and within-subjects factors of challenge (either two levels: context, food; or three levels: cocaine, nicotine, food) and test session. Finally, food outcome devaluation from both experiments was also analyzed on separate three way ANOVAs with between-subjects factors of treatment conditioning environment (two levels: inside, outside; or DC+, Ø) and devaluation condition (two levels: devalued, non-devalued) and trial (from pairing 1 to test in operant cage) as the within-subject factor. Post hoc analyses were carried out using the Newman-Keuls test. The level of $p<0.05$ was the criterion for statistical significance. Group data are presented as the mean ± SEM in the text and figures.

Question 3:
Does pretreatment with different drugs of abuse lead to an acceleration of habit-based behavior formation?

1. Experiment 7:

Does cocaine pretreatment accelerate the formation of habits?

In the previous chapter we have shown, by using a devaluation procedure, that reinstatement by cocaine and nicotine of an instrumental responding previously reinforced by food pellets, is not goal-directed (does not depend on the representation of the reinforcer). Indeed, cocaine and nicotine induced reinstatement despite the fact that the outcome (food pellets) had been devalued, demonstrating the habit nature of this reinstatement. On the other hand, a discriminative tactile and olfactory stimulus reinstates instrumental responding in a goal-directed manner (dependent on the representation of the reinforcer). These results suggest that cocaine and nicotine control the expression of automatic, habit-based behaviors. However, these results also raise the question of how cocaine and nicotine induce habit-based behaviors.

Some studies (Nelson and Killcross 2006; Nordquist et al. 2007; Schoenbaum and Setlow 2005) have suggested that the neuroadaptations that take place across the prolonged use of drugs of abuse underlies the progression from a goal-directed behavior (under the control of the representation of the outcome) to a habit-mode (not controlled by the representation of the consequences of the action). Such transition would explain why drug addicts lose control over their actions and maintain drug use despite the detrimental consequences.

Specifically, Schoenbaum and Setlow (2005) gave rats daily cocaine (30 mg/kg; i.p.) or saline injections for two weeks and, after each injection, placed them into the training chambers. At the end of this two weeks treatment, rats were left in their home cages for a three weeks withdrawal period. After this three weeks withdrawal period and before training began, rats were food deprived to 85% of their baseline free-feeding weight. Rats received light-food conditioning (45 mg Noyes food pellets) in eight sessions and, at the end of this light-food conditioning, rats in each group were assigned to “devalued” and “non-devalued” groups for conditioned taste aversion, which took place in the animal’s home cages over four days. Rats in the devalued groups received 10 min access to a ceramic bowl containing 100 Noyes food pellets and immediately after received an injection of 0.3 M LiCl solution (5 ml/kg; i.p.); whereas rats in the non-devalued groups did not receive an injection. After conditioned taste aversion training, all rats (“devalued” and “non-devalued”) were presented with the cue again in an extinction session. When comparing conditioned responding in “devalued” and “non-devalued” rats to determine whether cocaine treatment altered the spontaneous decrease

in responding to the cue normally caused by reinforcer devaluation, these authors found that cocaine-exposed rats were unable to modify conditioned responding as a result of devaluation and concluded that cocaine exposure can cause maladaptive behavior due to a longlasting and selective impairment in the ability to encode and/or use information about outcome value to guide behavior.

In another study, Nelson and Killcross (2006) examined the effects of amphetamine sensitization on the development of habitual instrumental responding for a natural reward by comparing the performance of amphetamine-exposed rats with vehicle control rats. Furthermore, in order to assess whether the amphetamine effects were mediated by learning or performance of the instrumental response, these autors exposed rats to amphetamine before training (Experiment 1) or between training and testing (Experiment 2). These autors gave rats daily amphetamine (2 mg/kg; i.p.) or vehicle (sterile PBS) injections for seven days and, after each injection, placed them into their home cages. At the end of this seven days treatment, rats were left in their home cages for a seven days injection-free period and were reduced to 80% of their ad libitum weight before undergoing training. After sensitization treatment, rats in Experiment 1 were trained instrumentally on a random interval schedule over five days in either the morning or the afternoon (at the other time of the day, an alternative reinforcer was presented non-contingently); whereas rats in Experiment 2 received the training before the sensitization treatment. The reinforcers used were maltodextrin solution with a cherry flavor and sucrose solution with a grape flavor. All animals from Experiment 1 received one session of devaluation by specific satiety in feeding cages [1 h ad libitum access to either the instrumental reinforcer ("devalued" group) or the alternative reinforcer ("non-devalued" group)], followed by an extinction test. Thereafter, all animals from Experiment 1 and Experiment 2 received, in the operant chambers, non-contingently access (40 free presentations on an RT-30s schedule) either to the instrumental reinforcer ("devalued" group) or to the alternative reinforcer ("non-devalued" group) for three days, followed by 0.15 M LiCl-injections (10 ml/kg; i.p.) for the devalued groups and saline-injections (10 ml/kg; i.p.) for the non-devalued groups. Finally, an extinction test was conducted. When comparing instrumental responding in "devalued" and "non-devalued" rats to determine whether amphetamine pretreatment altered the spontaneous decrease in lever pressing responding normally caused by reinforcer devaluation, these authors found that, after reinforcer devaluation by either specific satiety or LiCl, vehicle-injected control animals showed a decrease in lever pressing (goal-directed), whereas amphetamine-injected animals showed rates of lever pressing similar to those of the non-devalued groups (habit-based). Furthermore, an effect of post-training was observed, since animals exposed to amphetamine after training showed a normal goal-directed behavior. Overall, the results from this study indicate that amphetamine sensitization accelerates the progression from goal-directed to habit-based responding but does not affect the performance of established goal-directed actions.

In a final study, Nordquist and colleagues (2007) employed different schedules of reinforcement in operant conditioning in order to examine if amphetamine sensitization is associated with augmented incentive motivational value as well as stimulus-response learning processes. These authors gave rats daily amphetamine (2.5 mg/kg; i.p.) or saline injections for five days and, after each injection, placed them into their home cages. At the end of this five days treatment, rats were left in their home cages for a two weeks abstinence period. Rats were trained to press a lever to obtain a sucrose pellet reward (Noyes Formula F sucrose pellets) under a random ratio schedule of reinforcement. Then, rats were placed in individual cages and allowed to consume sucrose pellets ad libitum for 45 min. These authours found that, when tested in extinction conditions, sated animals showed decreased

lever pressing responding in comparison to non-sated animals, indicating that a random ratio schedule of reinforcement does not lead to stimulus-response learning. These authors performed another experiment, with the difference of using a random interval instead of a random ratio schedule of reinforcement. The reinforcers used were Noyes Formula P and Noyes Formula F sucrose pellets. Training began on the random interval schedule of reinforcement with one session per day (12 sessions in total) with half of the animals from each treatment trained to press a lever for Noyes Formula P pellets, and the other half trained to press a lever for Noyes Formula F sucrose pellets. Two sets of satiety devaluations [45 min ad libitum access to either the instrumental reinforcer ("devalued" group) or the alternative reinforcer ("non-devalued" group)] were performed in separate cages, with the first one performed between the sixth and the seventh session and the second one performed after the twelfth session. These authours found that, when tested in extinction conditions, the saline-treated animals showed a goal-directed behavior during the first test, but a habit-based behavior during the second test (after further training); whereas the amphetamine-treated rats showed a habit-based behavior since the first test, indicating that the transition from goal-directed to habit-based behaviors occurs much earlier in the amphetamine-treated rats. Overall, the results from this study indicate that amphetamine sensitization increases the motivational value of food reward and accelerates stimulus-response learning.

Thus, the studies previously described demonstrated that: 1) cocaine exposure can cause habit-based behaviors (in *pavlovian* conditioning setting) and, 2) amphetamine sensitization accelerates the progression from goal-directed to habit-based behaviors (in *instrumental* conditioning setting). Importantly, these studies employed different outcome devaluation procedures (either satiety devaluation or LiCl-induced gastric illness) and different schedules of reinforcement (either random ratio or random interval) to elucidate goal-directed or habit-based responding. However, in order to actually assign a key role to behavioral automatisms in drug addiction, it is necessary to verify if these two psychostimulants (cocaine and amphetamine) act in the same way on an *instrumental* task, as a result of the neuroadaptations they induce. Despite their different modes of action in the nervous system, cocaine and amphetamine are drugs of abuse that increase dopaminergic neurotransmission, therefore, they could have a common mechanisms in the process of behavioral automatisms. Thus, in order to determine if sensitization produced by cocaine causes habit-based behaviors and/or accelerates (or not) the transformation from goal-directed behavior to habit-based behavior, we created our own protocol based on the studies described above and examined the effects of cocaine pretreatment on rats' ability to perform an instrumental response to obtain a food pellet reward. Importantly, in this experiment animals did not perform the operant responses while under the drug effects (like the *inside* groups in our previous experiments) nor perform any instrumental learning while drug sensitized in another environment (like the *outside* groups in our previous experiments). In this experiment we assessed if *merely* cocaine sensitization can produce automatic, habit-based food-seeking behavior after an outcome devaluation procedure. Briefly, rats were pretreated with different doses of cocaine before the start of a limited instrumental training, then, following food outcome devaluation with LiCl, we compared their instrumental performance with saline pretreated rats (control group) on their ability to perform a goal-directed behavior (see Figure 30). If cocaine sensitization causes and/or accelerates the formation of habits, the cocaine pretreated rats will show a habit-based lever pressing responding (insensitive to the current value of the outcome) in comparison to saline pretreated rats that will show a goal-directed lever pressing responding (sensitive to the current value of the outcome).

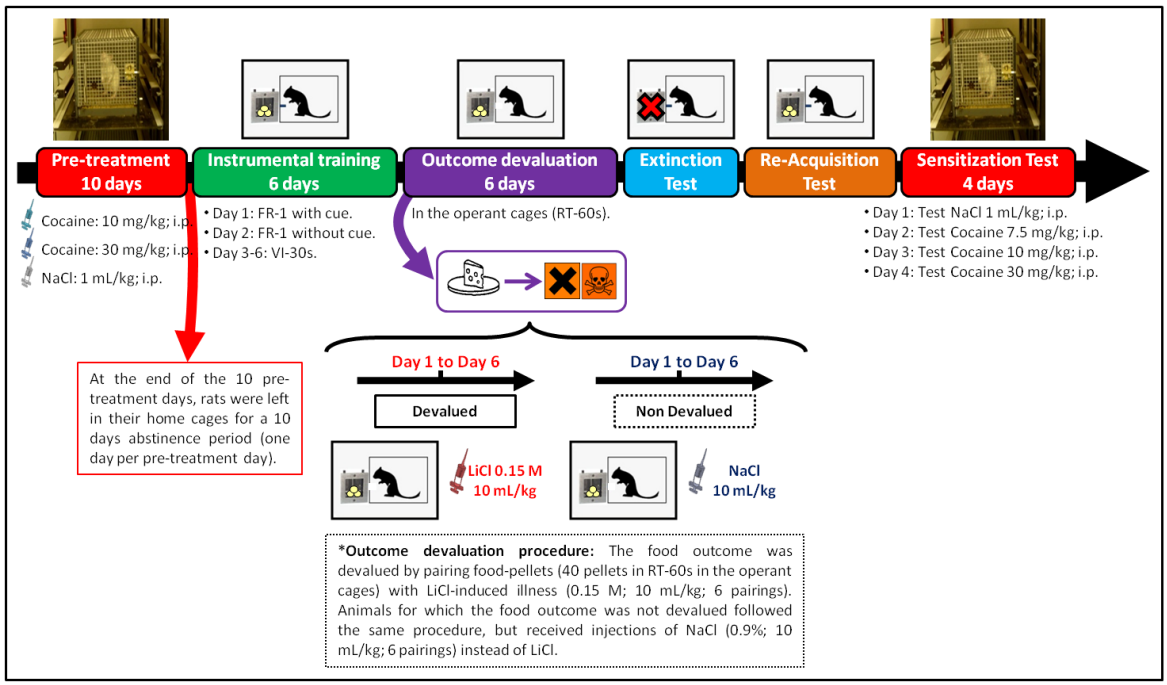


Figure 30 Experimental protocol for Experiment 7. Does cocaine pretreatment accelerate the formation of habits?

Material and Methods

Subjects

52 male Wistar rats (200-225 g; Charles River Laboratories, France) were housed in groups of four per cage on a 12 h reverse light/dark cycle (lights on from 21:30 h to 09:30 h) in a temperature (22°C) and humidity (60%) controlled room. Rats were acclimated to handling and allowed to adapt to the environment for a minimum of four days prior to the start of the experiment. Experiments were conducted during the dark phase of the light/dark cycle. Water was available ad libitum and food was restricted two days before instrumental learning and until the end of the experiment to 18 g chow per day to maintain rats at 90% of their free-feeding weight. Food was distributed in the home cages everyday at 19:00 h. All experimental procedures were performed in accordance with the European directive (86/609/EEC) and with the approval of the Centre National de la Recherche Scientifique.

Drugs

Cocaine hydrochloride (Cooperative Pharmaceutique Française, France) was dissolved in sterile physiological saline (0.9%). Doses are expressed as the weight of the salt. Cocaine (7.5, 10 and 30 mg/kg) was administered intraperitoneally. Injection volumes were 1 ml per kg body weight. Control treatment consisted of an equivalent volume of saline. Injection route and doses were selected from previous reports in the literature (Keiflin et al. 2008a; Schoenbaum and Setlow 2005).

Apparatus

Locomotor activity cages. Cocaine pretreatment was conducted in twenty-six locomotor activity cages (35 cm height × 25 cm length × 25 cm depth, Imetronic, Pessac, France) located in a dark experimental room equipped with white noise generators. The door, floor, and ceiling of each experimental cage consisted of wire mesh and the side walls were made of 10 mm thick clear

Plexiglass. Infrared beams automatically recorded (Imetronic software, Pessac, France) the locomotor activity of the animals.

Operant conditioning chambers. The operant conditioning chambers used in this experiment were the same ones used in Experiment 4, 5 and 6 (see Apparatus, page 105), with the only difference that only one lever was used, like Nelson and Killcross (Nelson and Killcross 2006).

Experimental Procedure

Pretreatment. Animals were divided into three homogenous groups: NaCl (n=17), cocaine-10 (n=18) and cocaine-30 (n=17), on the basis of their locomotor activity measured during a 2 h habituation session. During 10 days, and after 1 h habituation in the activity cages, animals received daily i.p. injections of either NaCl, cocaine 10 mg/kg or cocaine 30 mg/kg. Immediately after the injections, rats were placed back in the activity cages and locomotor activity was measured for another 2 h.

Instrumental learning. Instrumental learning started ten days after the last day of sensitization (one day of abstinence per day of pretreatment). Before the beginning of instrumental learning, and in order to avoid neophobia to the food reinforcer [45 mg Rodent Tablet, Test Diet (5TUL)], animals were habituated to the food pellets in their home cages. Each animal was randomly assigned to one of twelve operant cages in which they were always trained. Illumination of the house light signaled the start of the session and dimness of the houselight and lever retraction signaled the end of the session. In the first two 15 min sessions, animals were habituated to collect the food pellets from the food magazine (15 food pellets previously placed into the food magazine). Then, they were trained on a 30 min magazine-training session, where the lever was not available and a food pellet was distributed every sixty seconds under a fixed time 60 (FT-60 sec). Rats were initially trained to press a lever for a food reward under a fixed ratio 1 (FR-1) schedule of reinforcement until they earned the maximum number of food pellets (25 food pellets) per session (2 sessions); afterwards, the response requirement was changed to a variable interval 30-sec (VI-30 sec) schedule of reinforcement until they earned the maximum number of food pellets (40 food pellets) or after 40 min had elapsed (4 sessions). In interval schedules of reinforcement, during which a maximum number of reinforcers can be earned (in a set period of time) irrespective of response rate (low instrumental contingency between lever press and reward rate), responding following *extended* training can be transformed from goal-directed to habit-based behavior (Dickinson 1985). Thus, in order to maintain a goal-directed behavior, animals were subjected to a *limited* training, four sessions on VI-30 sec.

Food outcome devaluation. After instrumental learning, and based on the number of lever presses performed in the last two instrumental learning sessions, all groups (NaCl, cocaine-10 and cocaine-30) were once again divided in two groups: devalued (NaCl n=8, cocaine-10 n=9, cocaine-30 n=9) and non-devalued (NaCl n=8, cocaine-10 n=9, cocaine-30 n=8). Food outcome devaluation did not have an effect in one rat of the NaCl devalued group, therefore, it was removed from the experiment. The outcome devaluation took place in the operant cages (Nelson and Killcross 2006). Rats were placed in the operant cages and received six devaluation sessions which proceeded as a magazine-training session, where a food pellet was distributed every sixty seconds under a random time 60 (RT-60 sec) until the distribution of 40 food-pellets. Immediately after the 40 min session had elapsed, the devalued rats received LiCl-induced gastric illness injections (0.15 M; 10 ml/kg; i.p.) (Nelson and Killcross 2006; Tran-Tu-Yen et al. 2009) whereas the non-devalued rats received saline injections (10

ml/kg; i.p.) before being replaced in their homecages. The number of food pellets consumed was counted after each session.

Effect of food outcome devaluation on instrumental responding in extinction conditions. The day after the last food outcome devaluation session, rats were submitted to a 30 min extinction test during which lever presses no longer resulted in food pellets delivery. If lever pressing responding is controlled by the representation of the outcome (goal-directed), we should see a decrease of the number of lever presses only in the devalued groups; whereas if lever pressing responding is independent of the current value of the outcome (habit-based), we should not see a decrease of the number of lever presses in any group (devalued and non-devalued).

Re-acquisition test. In order to verify that conditioned taste aversion to the food pellets was effectively induced, rats were subjected to a re-acquisition test (lever presses are reinforced). This re-acquisition test was conducted in the same way that the last four learning sessions (VI-30 sec, 40 food pellets, 40 min. session). If the food outcome devaluation was effectively induced, the devalued groups, whether in goal-directed or habit-based mode, should lever press less than the non-devalued groups once re-experiencing the real value (now aversive) of the food pellets.

Sensitization test. In order to see the expression of sensitization to the locomotor activating effects of cocaine in cocaine pretreated rats, animals received cocaine injections at different doses. In the first test, and after 1 h habituation in the activity cages, animals received i.p. injections of NaCl, and then in the second, third and fourth tests, animals received i.p. injections of cocaine 7.5, 10 and 30 mg/kg respectively. Immediately after the injection, rats were placed back in the activity cages and locomotor activity was measured for another 2 h. If rats are sensitized to the locomotor activating effects of cocaine, locomotor activity should be higher in the cocaine pretreated rats in comparison to the control rats.

Statistical Analyses

Operant responses were analyzed with repeated measures analysis of variance (ANOVA), with between-subjects factors of treatment (three levels: NaCl, cocaine-10, cocaine-30) and devaluation condition (two levels: devalued, non-devalued), and within-subjects factors of lever presses, session, time and food pellet consumption. Locomotor activity data were analyzed using an ANOVA, with between-subjects factors of treatment (three levels: NaCl, cocaine-10, cocaine-30) and devaluation condition (devalued or non-devalued) and within-subjects factor of session and dose (four levels: 0, 7.5, 10, 30). Post hoc analyses were carried out using the Newman-Keuls (NK) test. The level of $p<0.05$ was the criterion for statistical significance. Group data are presented as the mean $\pm$ SEM in the text and figures.

Results

Cocaine pretreatment

Animals received 10 daily NaCl, cocaine-10 or cocaine-30 mg/kg injections. A two factor ANOVA (treatment x day) revealed a significant effect of treatment ($F(2,48)=86.27$, $p<0.000$) and day ($F(9,432)=2.85$, $p<0.002$), and a significant interaction between them ($F(18,432)=2.68$, $p<0.000$). Post hoc NK comparisons revealed that cocaine injections produced a dose-dependent increase in locomotor activity in comparison to NaCl injections through the 10 days of injections. However, the cocaine-30 group showed a decreased in locomotor activity through the days (when compared to day one in the cocaine-30 group, NK test: $p<0.039$ in day four, $p<0.005$ in day five and, $p<0.007$ in day six), which might have been due to appearance of stereotypies induced by the daily injections of a high dose of cocaine (see Figure 1). These classical results have been reported in the literature (Aliane et al. 2009; Robinson and Berridge 2001).

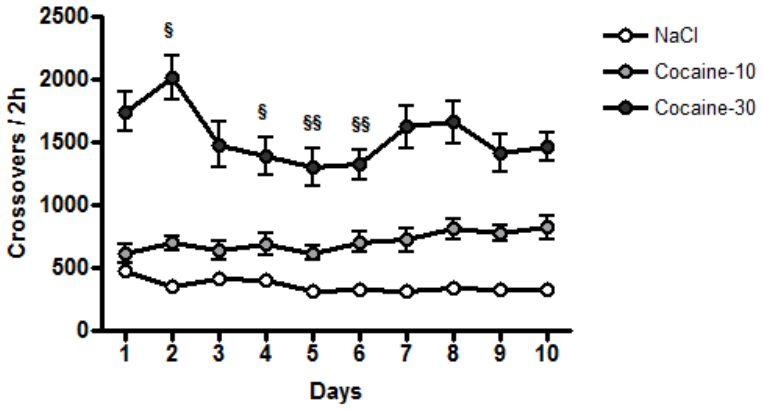


Figure 1 Cocaine pretreatment. Each point represents mean (± SEM) crossovers recorded in the locomotor activity cages during the 2 h session. Data is represented according to the three different pretreatments (NaCl, cocaine-10 or cocaine-30 mg/kg). § significantly different from day 1 in the cocaine-30 mg/kg group (§ $p<0.05$, §§ $p<0.01$, §§§ $p<0.001$).

Acquisition of the instrumental response

The total number of lever presses from the four learning sessions (VI-30 sec) is expressed by minute. A two factor ANOVA (treatment x session) revealed only a significant effect of session ($F(3,144)=146.13$, $p<0.000$), indicating that the instrumental response was easily acquired in all animals independently of previous cocaine treatment. In fact, post hoc NK comparisons on this session effect revealed that the number of lever presses started to increase in session three in a similar manner across all groups (when compared session three to session one and session two, NK test: $p<0.000$; when compared session four to session one, two and three, NK: $p<0.000$), independently of previous pretreatment (NaCl, cocaine-10 and cocaine-30 mg/kg) (see Figure 2).

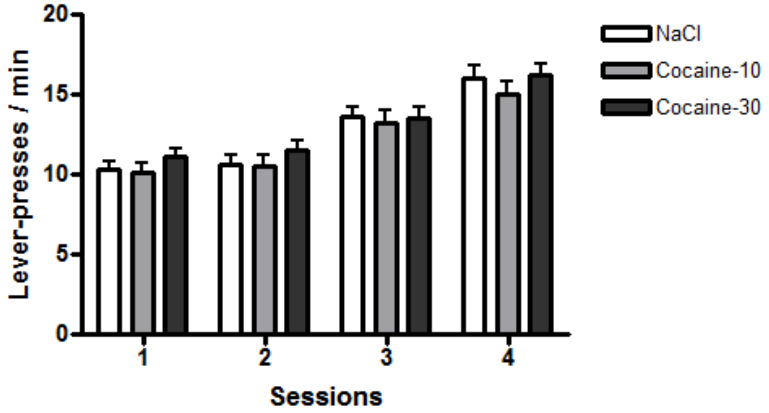


Figure 2 Acquisition of an operant food-reinforced behavior. Histograms represent mean (± SEM) lever presses responses per group (NaCl, cocaine-10 and cocaine-30 mg/kg). The total number of lever presses is expressed by minute and in function of learning sessions (four VI-30 sec sessions).

Outcome devaluation by LiCl

The results from food outcome devaluation are shown in Figure 3. Repeated associations of food pellets presentation with LiCl-induced gastric illness induced a progressive decrease in food pellet consumption in all rats (NaCl, cocaine-10 and cocaine-30 mg/kg). In contrast, when food pellets were not associated with LiCl-induced gastric illness, rats always consumed all the food pellets available. A three-factor ANOVA (treatment x devaluation condition x day) revealed a significant effect of devaluation condition ($F(1,45)=324.04$, $p<0.000$) and day ($F(3,135)=170.31$, $p<0.000$), and a significant interaction between them ($F(3,135)=168.84$, $p<0.000$). Post hoc NK comparisons on this interaction confirmed that the devalued groups decreased food pellet consumption from day four onward when compared to day three (when compared devalued groups through the trials, NK test: $p<0.000$), independently of the pretreatment (NaCl, cocaine-10 and cocaine-30 mg/kg).

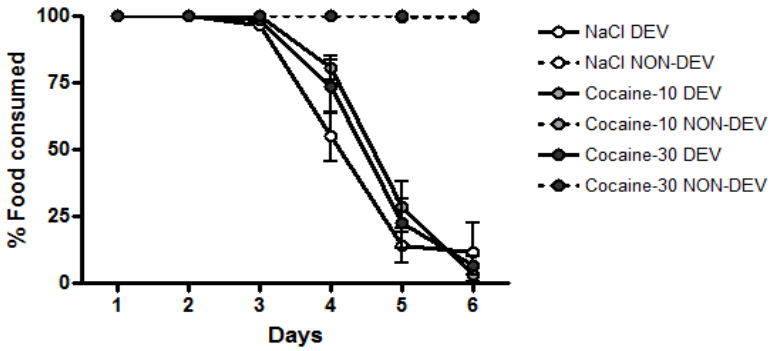


Figure 3 Devaluation of food pellets. Repeated associations of food pellets presentation with LiCl-induced gastric illness induced a progressive decrease in food pellets consumption in the devalued groups. In contrast, when food pellets presentation was not associated with LiCl-induced gastric illness, the non-devalued groups consumed all the food pellets available. Food pellet consumption is expressed in consumption percentage of the maximum 40 food pellets available per session (RT-60 sec) (± SEM).

Effect of cocaine pretreatment on instrumental responses in extinction conditions

The following day after the last devaluation session by LiCl, animals were subjected to a 30 min extinction test in which the lever was available but no reward was obtained. The results obtained are expressed by 3 minutes blocks and for each block the total number of lever presses is expressed by

minute and percentage of the baseline (Nelson and Killcross 2006), that is to say, the mean of lever presses per minute from the last two instrumental learning sessions (VI-30 sec).

A three factor ANOVA (treatment x devaluation condition x time block) revealed a significant effect of devaluation condition ($F(1,45)=91.33$, $p<0.000$), time block ($F(9,405)=110.43$, $p<0.000$) and a significant interaction between treatment x devaluation condition x time block ($F(18,405)=1.63$, $p<0.048$). Post hoc NK comparisons revealed that outcome devaluation by LiCl induced lower levels of lever pressing in the devalued groups in comparison to the non devalued groups across the entire 30 min extinction test and independently of drug pretreatment. Specifically, the NaCl devalued group showed lower levels of responding in comparison to the NaCl non-devalued group during the first nine minutes of the test [when compared devalued to non-devalued groups, NK test: $p<0.000$ in time block 1 (from minute 1 to 3), $p<0.023$ in time block 2 (from minute 4 to 6), $p<0.000$ in time block 3 (from minute 7 to 9)]. The cocaine-10 devalued group also showed lower levels of responding in comparison to the cocaine-10 non-devalued group, but with the difference that this effect was observed during the first twelve minutes of the test [when compared devalued to non-devalued groups, NK test: $p<0.000$ in time block 1 (from minute 1 to 3), $p<0.004$ in time block 2 (from minute 4 to 6), $p<0.003$ in time block 3 (from minute 7 to 9), $p<0.000$ in time block 4 (from minute 10 to 12)]. Finally, in the case of the cocaine-30 group, this difference emerged until time block 2 and lasted until time block 5 [when compared devalued to non-devalued groups, NK test: $p<0.000$ in time block 2 (from minute 4 to 6), $p<0.000$ in time block 3 (from minute 7 to 9), $p<0.004$ in time block 4 (from minute 10 to 12), $p<0.000$ in time block 5 (from minute 13 to 15)]. Thus, groups NaCl and cocaine-10 showed a goal-directed behavior (sensitive to the current value of the outcome), whereas group cocaine-30 showed a habit-based behavior (insensitive to the current value of the outcome) only during the first three minutes of the test (see Figure 4).

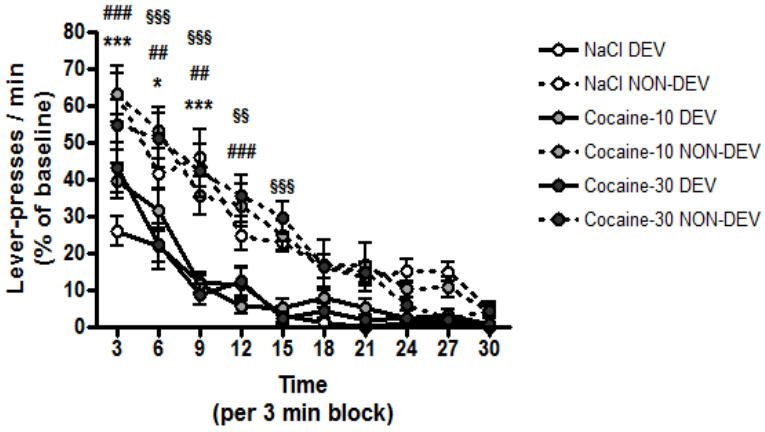


Figure 4 Effect of cocaine pretreatment on lever pressing after devaluation by LiCl in extinction conditions. Each point represents mean (± SEM) lever presses during the 30 min extinction test. The number of lever presses is expressed per minute as a proportion of baseline over time (mean of lever presses per minute from the last two instrumental learning sessions) (± SEM). * significant differences between NaCl devalued and NaCl non-devalued groups, # significant differences between cocaine-10 devalued and cocaine-10 non-devalued groups, § significant differences between cocaine-30 devalued and cocaine-30 non-devalued groups (*/#/§ $p<0.05$, **/##/§§ $p<0.01$, ***/###/§§§ $p<0.001$).

Verification of conditioned taste aversion during a re-acquisition test

A three factor ANOVA (treatment x devaluation condition x time block) revealed a significant effect of devaluation condition ($F(1,45)=449.93$, $p<0.000$) and time block[•] ($F(5,225)=31.64$, $p<0.000$), and a significant interaction between them ($F(5,225)=60.82$, $p<0.000$). Post hoc NK comparisons on this interaction revealed that during the re-acquisition test, the non-devalued groups showed a gradual increase on the number of lever presses, whereas the opposite effect was observed on the devalued groups [when compared non-devalued groups through the 18 min, NK test: $p<0.000$; when compared time block 5 (from minute 13 to 15) to time block 1 (from minute 1 to 3) in the devalued groups, NK test: $p<0.004$; when compared time block 6 (from minute 16 to 18) to time block 1 (from minute 1 to 3) in the devalued groups, NK test: $p<0.000$], independently of the pretreatment (NaCl, cocaine-10 and cocaine-30 mg/kg). In fact, since the beginning of the test, the number of lever presses was higher in the non-devalued groups than in the devalued groups [when compared non-devalued groups to devalued groups in time block 1 (from minute 1 to 3), NK test: $p<0.000$], independently of the pretreatment (NaCl, cocaine-10 and cocaine-30 mg/kg), which confirms the efficiency of the outcome devaluation (see Figure 5).

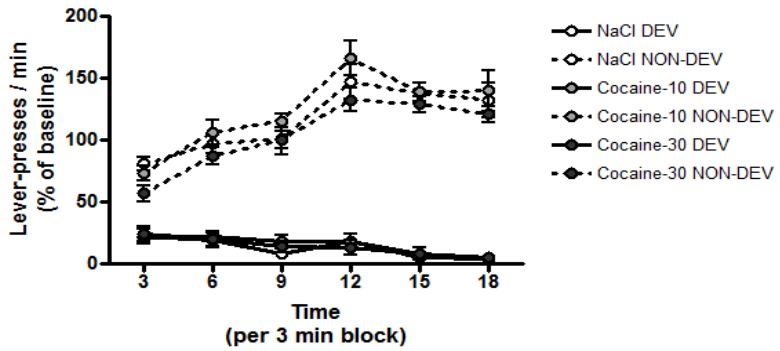


Figure 5 Instrumental responding during a re-acquisition test. Each point represents mean (± SEM) lever presses during the re-acquisition test. The first 18 min of the re-acquisition test are shown (per 3 min block). This test was conducted in the same way than the four learning sessions (VI-30 sec). The number of lever presses is expressed per minute as a proportion of baseline over time (mean of lever presses per minute from the last two instrumental learning sessions) (± SEM).

Verification of sensitization on cocaine pretreated rats

Cocaine injections led to an increase in locomotor activity in all animals in a dose-dependent manner. A three factor ANOVA (treatment x devaluation condition x dose) revealed a significant effect of dose ($F(3,135)=98.26$, $p<0.000$) and a significant interaction between treatment and dose ($F(6,135)=2.85$, $p<0.012$). Post hoc NK comparisons on this interaction revealed that locomotor activity evolved differently depending on the pretreatment received at the beginning of the experiment (NaCl, cocaine-10 and cocaine-30 mg/kg). Specifically, NaCl pretreated rats only showed a higher level of locomotor activity when challenged with the highest dose of cocaine (30 mg/kg) in comparison to a control saline challenge (NK test, $p<0.000$), independently of previous food outcome devaluation by LiCl. Cocaine pretreated rats showed higher levels of locomotor activity when challenged with each dose of cocaine (7.5, 10 and 30 mg/kg) in comparison to a control saline challenge (NK test in the cocaine-10 group: $p<0.006$ in dose 7.5, $p<0.000$ in dose 10 and dose 30; NK test in the cocaine-30 group: $p<0.004$ in dose 7.5, $p<0.000$ in dose 10 and dose 30), independently of previous food outcome devaluation by LiCl. However, when making comparisons between pretreatments (NaCl vs. cocaine-10 vs. cocaine-30) in each cocaine challenge (7.5, 10 and 30 mg/kg) no significant differences

[•] The ANOVA was performed only for the first 18 min of the session since the devalued groups stop pressing the lever at this period.

were revealed, indicating that the cocaine pretreated rats were not behaviorally sensitized. Nonetheless, this "lack" of behavioral sensitization suggests the appearance of stereotypies (which compete with motor activation) induced by a high sensitivity to cocaine (see Figure 6).

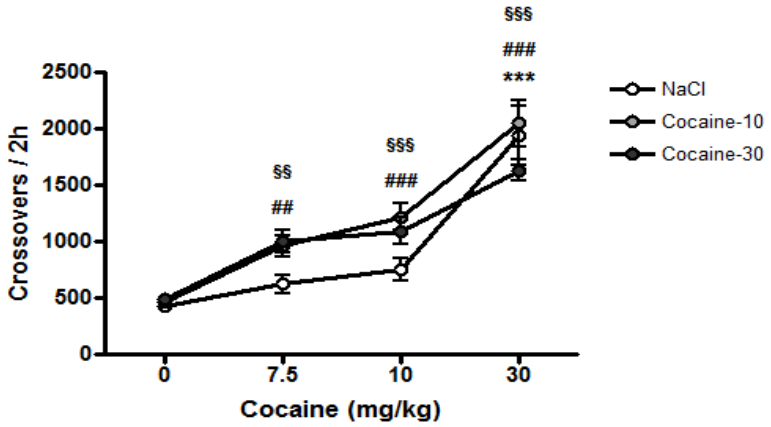


Figure 6 Cocaine sensitization test. Each point represents mean (± SEM) crossovers recorded in the locomotor activity cages during the 2 h session. Data is represented according to the three different pretreatments (NaCl, cocaine-10 or cocaine-30 mg/kg) and to the dose of cocaine injected (0, 7.5, 10 and 30 mg/kg). * significantly different from dose 0 mg/kg in the NaCl group, # significantly different from dose 0 mg/kg in the cocaine-10 group, § significantly different from dose 0 mg/kg in the cocaine-30 group (*/#/§ $p<0.05$, **/##/§§ $p<0.01$, ***/###/§§§ $p<0.001$).

Discussion

This study showed that, globally, cocaine pretreatment does not accelerate the formation of habits. Concretely, the extinction test revealed a goal-directed behavior in NaCl and cocaine-10 mg/kg pretreated animals; whereas the cocaine-30 mg/kg animals showed a habit-based behavior only during the first three minutes of the test, which should be carefully interpreted.

No different effects of cocaine pretreatment in acquisition of operant responding

During acquisition of an instrumental response for food pellets, the groups pretreated with cocaine (10 and 30 mg/kg) did not show different levels of instrumental responding when compared to the group pretreated with saline. Furthermore, no differences were observed between animals pretreated with cocaine 10 mg/kg and animals pretreated with cocaine 30 mg/kg. These results extend previous findings by Schoenbaum and Setlow (2005) in a pavlovian conditioning setting (cocaine 30 mg/kg) to an instrumental conditioning setting.

Outcome devaluation by LiCl

The results from the outcome devaluation procedure demonstrated that the devalued groups showed a decrease in food pellet consumption, whereas the non-devalued groups did not show a reduction in food pellet consumption. Furthermore, this result was independent of previous pretreatment (NaCl, cocaine-10 and cocaine-30 mg/kg). It is worth mentioning that, in this experiment, six LiCl-food pellets pairings were needed, whereas in our previous experiment – regarding cocaine treated rats-, four LiCl-food pellets pairings were needed (Experiment 4, see page 100). We think this can be explained by the fact that this outcome devaluation procedure was performed in a different way. It was performed in the operant chambers, with a different dose and a different volume injected of LiCl (0.15 M, 10 ml/kg) and experiencing fewer food pellets (40 pellets). We performed this protocol based on the work by Nelson and Killcross (2006) and, although their animals received three days of devaluation with LiCl, they used sucrose solution as reinforcers and

their rats were amphetamine sensitized. Whether these differences can explain our results, the final result was the same: the devalued groups decrease food pellet consumption during outcome devaluation by LiCl independently of previous drug pretreatments and, importantly, the fact that all devalued animals acquired an aversion to the food pellets was further confirmed during the re-acquisition test.

Cocaine 30 mg/kg pretreatment induces habit-based lever-pressing responding during an extinction test

The results from the extinction test revealed that the devalued saline and cocaine-10 pretreated rats showed lower levels of lever pressing than the non-devalued saline and cocaine-10 pretreated rats, whereas this difference was not present in the cocaine-30 pretreated rats, indicating that lever pressing responding during the extinction test was goal-directed in the saline and cocaine-10 pretreated rats but habit-based in the cocaine-30 pretreated rats (but only for the first three minutes). Our results are in accordance with those found by Schoenbaum and Setlow (2005) to a certain degree. These authors found that cocaine (30 mg/kg) pretreated rats responded in a habit-mode to a light cue during an extinction test performed after outcome devaluation, whereas saline pretreated rats responding remained goal-directed. Importantly, in this study, this difference remained during the 64 min session. In our experiment, habit-based responding was observed only in the cocaine-30 pretreated rats but just during the first three minutes of the 30 min extinction test, which make a big difference. Therefore, the effect is so small in our experiment that it is difficult to give a definitive conclusion. Nonetheless, it is clear that our effect is much smaller than the one in Schoenbaum and Setlow's experiment.

However, there are differences between our protocol and the one used by Schoenbaum and Setlow which might explain the results observed during the extinction tests. Firstly, our study was performed in an instrumental task, whereas theirs was performed in a pavlovian task. Secondly, our cocaine pretreatment lasted 10 days and was performed outside the operant cages, whereas their cocaine pretreatment lasted 14 days and was performed inside the operant cages and, finally, we started operant training after 10 days of abstinence period, whereas they started pavlovian conditioning after 21 days of abstinence period. We hypothesize that the fact that Schoenbaum and Setlow were able to observe the difference between the devalued and the non-devalued rats during their 64 min session is due to the fact that they measured the percent of time spent in the food cup during the presentation of the cue light, whereas we measured the number of lever pressing responding during a 30 min extinction test. Specifically, in pavlovian conditioning the animal's responses are more simple (e.g. the animal learns that the reinforcer comes after a specific stimulus), whereas in instrumental conditioning the animal's responses are more complex (e.g. the animal learns that the reinforcer comes after a specific response). Another hypothesis it that the effect on lever pressing responding would be observed after longer periods of abstinence (21 days off in Schoenbaum and Setlow's experiment).

In summary, we concluded that, globally, cocaine pretreatment does not accelerate the formation of habits. However, the habit-based responding effect observed in the cocaine-30 pretreated rats during the first three minutes of the extinction test, raise the question of whether a stronger effect could be observed after an extended period of training (where the behavior is habit-based) instead of the limited period of training (where the behavior is goal-directed) used in this experiment. As a result, we decided to perform another experiment, this time adding a "control" amphetamine group and testing rats' ability to perform a goal-directed behavior following an outcome devaluation procedure after a *limited* and an *extended* period of training, which is described in the following section.

2. Experiment 8:

Do amphetamine and/or cocaine pretreatment accelerate the formation of habits after limited and/or extended instrumental training?

In Experiment 7 we hypothesized that cocaine pretreatment accelerates the formation of habits. In order to test this hypothesis, we studied the effect of cocaine pretreatment on operant behavior after a *limited* instrumental training and following outcome devaluation with LiCl. Our results showed that cocaine pretreatment accelerates the formation of habits only with a 30 mg/kg dose. Concretely, the extinction test revealed a goal-directed behavior in NaCl and cocaine-10 mg/kg pretreated animals since animals for which the food had been devalued lever pressed less than animals for which the food had not been devalued.

In normal conditions and early during acquisition, instrumental responses are mediated by action-outcome associations (goal-directed behavior); however, as training proceeds, instrumental performance is no longer driven by action-outcome but by stimulus-response associations (habit-based behavior), which allows the individual to economize its cognitive resources (Adams 1982; Adams and Dickinson 1981; Dickinson 1985; Dickinson and Balleine 1994; Dickinson et al. 1995).

In the context of drug addiction, one study performed by Zapata and colleagues (2010) has shown that cocaine-seeking behavior, initially a goal-directed behavior at the beginning of cocaine access, is transformed into a habit-based behavior (insensitive to the devaluation of the instrumental response through an extinction process) after several weeks of cocaine access, whereas the behavior remains goal-directed when cocaine access is of short duration. However, this study raises the question whether this transformation from goal-directed to habit-based behavior is due to: 1) the different neuroadaptations induced by several weeks of cocaine access or, 2) the transformation from goal-directed to habit-based behavior is due to the length of the training.

Thus, in order to determine whether the length of instrumental training is a crucial factor in the *acceleration* of the formation of habits in drug sensitized rats, we created our own protocol based on the studies previously described (Nelson and Killcross 2006; Nordquist et al. 2007; Schoenbaum and Setlow 2005). Specifically: 1) since the studies performed in an instrumental setting are those by Nelson and Killcross and Nordquist and colleagues (amphetamine pretreatment), we decided to add an amphetamine group to be able to compare our results with theirs and, 2) our previous results

showed that cocaine pretreatment "accelerates" the formation of habits only with a 30 mg/kg dose (during the first three minutes of the test and in comparison to saline pretreated rats after a *limited* period of training); however, in order to affirm that drug (either amphetamine or cocaine) pretreatment accelerates the formation of habits, we need to show that saline pretreated rats will actually transform their goal-directed behavior (behavior observed after a *limited* period of training) to a habit-based mode, after an *extended* period of training, whereas drug pretreated rats would show this habit-based behavior earlier (i.e. after a *limited* period of training) or, in the specific case of cocaine-30 mg/kg pretreated rats, this habit-based behavior will be stronger after an *extended* period of training. Summarizing, we studied the effect of amphetamine and cocaine pretreatment on rats' ability to perform an instrumental response to obtain a food-pellet reward at different stages of instrumental learning: 1) after a *limited* period of training and, 2) after an *extended* period of training; then, following outcome devaluation, we compared their instrumental performance with saline pretreated rats (control group) on their ability to perform a goal-directed behavior. In order to be able to perform the food outcome devaluation at these different stages of instrumental training, the devaluation procedure was carried out by specific satiety, allowing its repetition (which is not the case when LiCl is used) (see Figure 31). If amphetamine and cocaine pretreatment accelerates the formation of habits, only the drug pretreated rats will show a habit-based lever-pressing responding (insensitive to the current value of the outcome) after *limited* training, whereas both saline and drug pretreated rats will show a habit-based lever pressing responding after *extended* training.

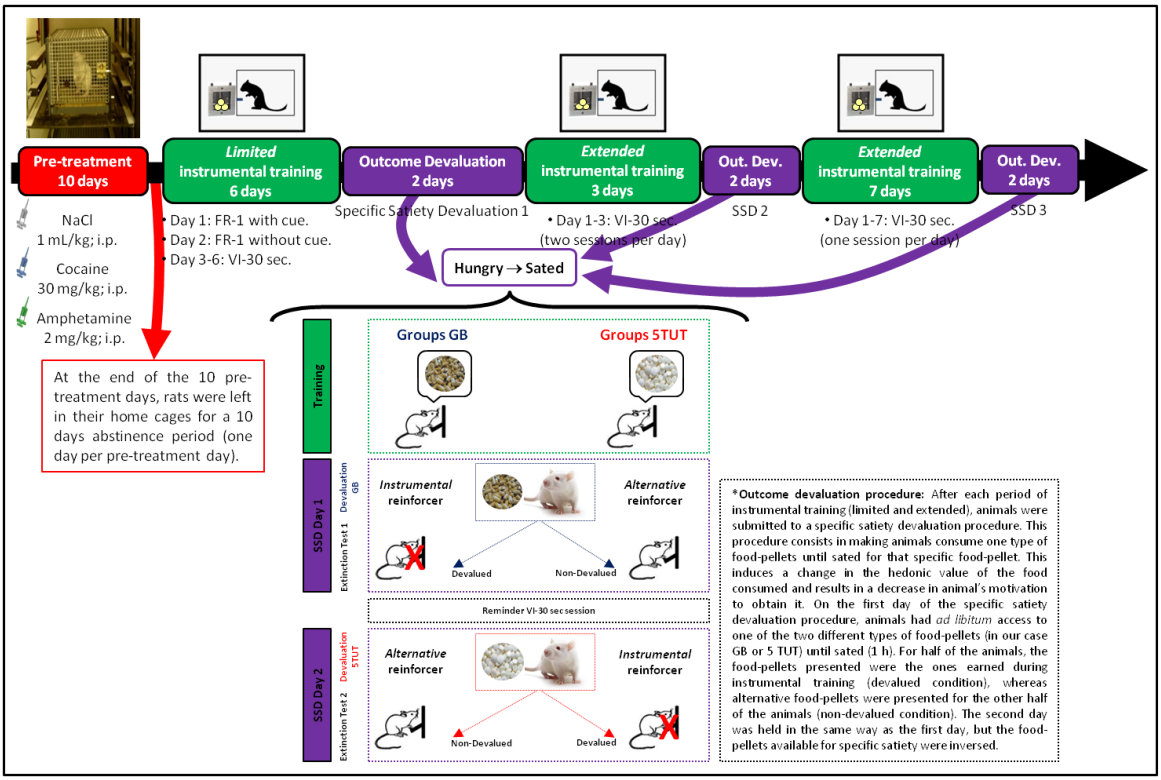


Figure 31 Experimental protocol for Experiment 8. Do amphetamine and/or cocaine pretreatment accelerate the formation of habits after limited and/or extended instrumental training?

Material and Methods

Subjects

36 male Wistar rats (200-225 g; Charles River Laboratories, France) were housed under the same conditions as in Experiment 7. Feeding conditions and time of experimental procedures were also identical to those in Experiment 7 (see Subjects, page 114).

Drugs

Cocaine hydrochloride and amphetamine (Cooperative Pharmaceutique Française, France) were dissolved in sterile physiological saline (0.9%). Doses are expressed as the weight of the salt. Cocaine (30 mg/kg[•]) and amphetamine (2 mg/kg) were administered intraperitoneally. Injection volumes were 1 ml per kg body weight. Control treatment consisted of an equivalent volume of saline. Injection route and dose of amphetamine was selected from a previous report in the literature (Nelson and Killcross 2006).

Apparatus

The locomotor activity cages and the operant conditioning chambers used in this experiment were the same as the ones used in Experiment 7 (see Apparatus, pages 114 and 115).

Experimental Procedure

Pretreatment. Animals were divided into three homogenous groups: NaCl (n=16), amphetamine (n=10) and cocaine (n=10), on the basis of their locomotor activity measured during a 2 h habituation session. During 10 days, and after 1 h habituation in the activity cages, animals received daily i.p. injections of either NaCl, amphetamine 2 mg/kg or cocaine 30 mg/kg. Immediately after the injections, rats were placed back in the activity cages and locomotor activity was measured for another 2 h.

Instrumental learning. Instrumental learning started ten days after the last day of sensitization (one day of abstinence per day of pretreatment) and was held in exactly the same way as in Experiment 7 (*limited* period of training) (see Instrumental learning, page 115). After the fourth VI-30 sec session, the first specific satiety devaluation took place. Then, animals were submitted to an *extended* period of training: six additional VI-30 sec sessions, with two sessions per day (sessions 5 to 10), before proceeding to the second specific satiety devaluation. Finally, animals were once again submitted to a second *extended* period of training, but this time we extended it to seven additional VI-30 sec sessions spaced in time, with one session per day (sessions 11 to 17), before proceeding to the third and last specific satiety devaluation. In order to perform the specific satiety devaluation (SSD) procedure, an alternative reinforcer was used as a control (non-devalued condition) (Nordquist et al. 2007). Therefore, two types of pellets were used in this experiment. Half of the rats of each group (NaCl, amphetamine and cocaine) were trained to lever press for GB-food pellets [45 mg Rodent Tablet, BioServ (Grain-Based)], whereas the other half were trained to lever press for 5TUT-food pellets [45 mg Rodent Tablet, Test Diet (5TUT)]. Preliminary experiments on other animals showed that the acute effect of devaluation by specific satiety was identical for these two types of food

[•] This dose of cocaine was chosen because of the results obtained in Experiment 6, where only the 30 mg/kg cocaine pretreatment seemed to lead to a habit-based behavior during the first three minutes of the extinction test.

pellets. These experiments also showed that when sated with one type of food pellets, animals were still motivated to consume the other type of food pellets, indicating similar levels of palatability of both types of food pellets (data not shown). Before the beginning of instrumental learning, and in order to avoid neophobia to the food reinforcer, animals were habituated to both types of food pellets (GB and 5TUT) in their home cages. Animals were divided into six homogenous groups on the basis of their locomotor activity measured during the pretreatment (depending on the difference in locomotor activity levels between injection 1 and injection 10): NaCl (GB n=8, 5TUT n=8), amphetamine (GB n=5, 5TUT n=5) and cocaine (GB n=5, 5TUT n=5).

Specific satiety devaluation and its effect on instrumental responding under extinction conditions. The first specific satiety devaluation procedure took place after the *limited* period of training (between the 4th and the 5th VI-30 sec session) and it was organized in two sessions performed in two different days. Animals were placed in individual feeding cages (the same cages used in Experiments 4, 5 and 6) (which were completely different from the chambers previously used during the instrumental training) made of clear plastic measuring 13 cm height x 30 cm length x 13 cm depth, with wire-mesh ceilings. During the first day of SSD (session 1), rats were placed in these individual cages and allowed to *ad libitum* consume one of the two different types of food pellets (GB or 5TUT) from a bowl placed in each individual cage for 1 h. For half of the rats, the food pellets presented in the bowl were the same food pellets obtained in the operant cages (devalued condition: GB for the GB groups and 5TUT for the 5TUT groups), whereas alternative food pellets were presented for the other half of the rats (non-devalued condition: GB for the 5TUT groups and 5TUT for the GB groups), inducing specific satiety for the type of food pellets consumed. Immediately after this hour had elapsed, rats were submitted to a 30 min extinction test during which lever presses no longer resulted in food pellets delivery. The amount of food pellets consumed was measured by weighing the bowl before and after the devaluation procedure. Finally, and in order to verify the effectiveness of the specific satiety devaluation, animals were submitted to two 15 min consumption tests in standard individual cages: one in the presence of a bowl containing 5 gr of GB-food pellets and the other in the presence of 5 gr of 5TUT-food pellets. The number of food pellets consumed was counted after each test. The following day, and in order to prevent extinction of the instrumental response, animals received a "reminder VI-30 sec session" (Nelson and Killcross 2006), which was identical to the last learning sessions. The second day of SSD (session 2) was held in the same way as the first session, but the food pellets available for specific satiety were inversed: rats that had *ad libitum* access to GB-food pellets in the first session now had *ad libitum* access to 5TUT-food pellets, and vice versa. Animals were then once again submitted to a 30 min extinction test and to the two 15 min consumption tests. The second specific satiety devaluation procedure took place after the *extended* period of training [after the last learning session (10th VI-30 sec session)] and continued to progress exactly as outlined for the first specific satiety devaluation, whereas the third and last specific satiety devaluation procedure took place after the second *extended* period of training [after the last learning session (17th VI-30 sec session)] and continued to progress exactly as outlined for the first and second specific satiety devaluation. Summarizing, each animal was submitted to six devaluation sessions: two after a *limited* period of training, two after an *extended* period of training and two after a second *extended* period of training, and each of them tested for the same food pellets instrumentally obtained (devalued condition) and for the alternative food pellets (non-devalued condition). This allows us to test and compare the instrumental responses of each animal in both conditions (devalued and non-devalued).

Statistical Analyses

Operant responses were analyzed with repeated measures analysis of variance (ANOVA), with between-subjects factors of treatment (three levels: NaCl, amphetamine, cocaine), type of food pellets (two levels: GB, 5TUT) and devaluation condition (two levels: devalued, non-devalued), and within-subjects factors of lever presses, session, time and food pellet consumption. Locomotor activity data were analyzed using an ANOVA, with between-subjects factors of treatment (three levels: NaCl, amphetamine, cocaine) and within-subjects factor of session. Post hoc analyses were carried out using the Newman-Keuls (NK) test. The level of $p<0.05$ was the criterion for statistical significance. Group data are presented as the mean $\pm$ SEM in the text and figures.

Results

Drug pretreatment

Animals received 10 daily NaCl, amphetamine-2 or cocaine-30 mg/kg injections. A two factor ANOVA (treatment x day) revealed a significant effect of treatment ($F(2,33)=75.06$, $p<0.000$) and day ($F(9,297)=2.27$, $p<0.017$), and a significant interaction between them ($F(18,297)=4.24$, $p<0.000$). Post hoc NK comparisons revealed that amphetamine and cocaine injections produced an increase in locomotor activity in comparison to NaCl injections through the 10 days of injections. However, in the cocaine group, this hyperactivity decreased through the days (result previously found in Experiment 7) (when compared to day one in the cocaine-30 group, NK test: $p<0.000$ in day ten), indicating the appearance of stereotypies which are in competition with locomotor activity, whereas in the amphetamine group this hyperactivity increased through the days (when compared to day one in the amphetamine-2 group, NK test: $p<0.010$ in day two, $p<0.000$ from day three to day nine and $p<0.002$ in day ten) (see Figure 1).

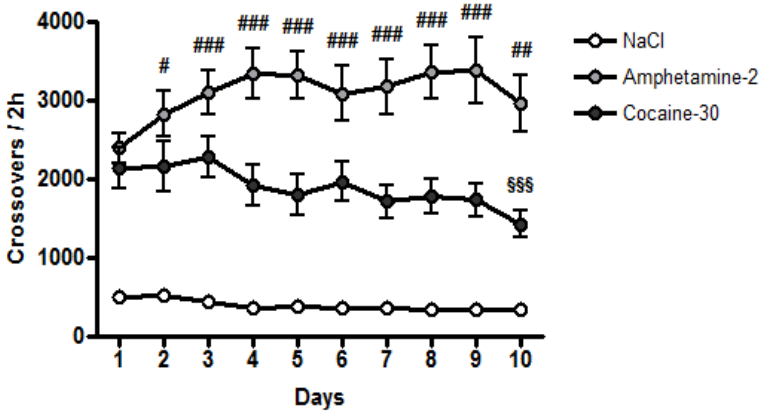


Figure 1 Amphetamine and cocaine pretreatment. Each point represents mean (± SEM) crossovers recorded in the locomotor activity cages during the 2 h session. Data is represented according to the three different pretreatments (NaCl, amphetamine-2 or cocaine-30 mg/kg). # significantly different from day 1 in the amphetamine-2 mg/kg group, § significantly different from day 1 in the cocaine-30 mg/kg group (#/§ $p<0.05$, ##/§§ $p<0.01$, ###/§§§ $p<0.001$).

Acquisition of the instrumental response

Limited period of training (four sessions)

The total number of lever presses from the four learning sessions (VI-30 sec) is expressed by minute. A three factor ANOVA (treatment x type of food pellets x session) revealed only a significant effect of session ($F(3,90)=48.99$, $p<0.000$), indicating that the instrumental response was easily acquired in all

animals independently of previous amphetamine and cocaine treatment and independently of the type of food pellets they experienced as a reward (GB-food pellets or 5TUT-food pellets). In fact, post hoc NK comparisons on this session effect revealed that the number of lever presses started to increase from session two onwards in a similar manner across all groups (when compared sessions two, three and four to session one, NK test: p<0.000), independently of previous pretreatment (NaCl, amphetamine-2 and cocaine-30 mg/kg) and independently of the type of food pellets they experienced as a reward (GB or 5TUT) (see Figure 2).

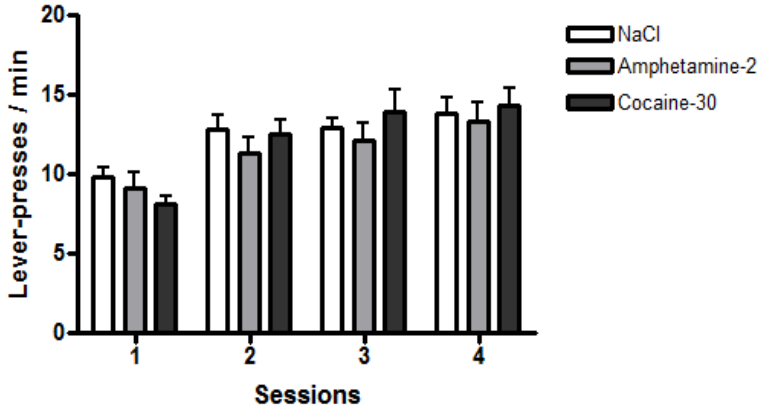


Figure 2 Acquisition of an operant food-reinforced behavior during a *limited* period of training. Histograms represent mean (± SEM) lever presses responses per group (NaCl, amphetamine-2 and cocaine-30 mg/kg). The total number of lever presses is expressed by minute and in function of learning sessions (four VI-30 sec sessions).

Effect of drug pretreatment on instrumental responses in extinction conditions

<u>After a limited period of training (four sessions)</u>

The first specific satiety devaluation procedure was performed one day after the fourth VI-30 sec session (after the *limited* period of training) and consisted of two sessions conducted in the same way: animals consumed one of the two types of food pellets (GB-food pellets in one session and 5TUT-food pellets in the other) until sated. Immediately after, rats were submitted to a 30 min extinction test in which the lever was available but no reward was obtained. The results obtained are expressed by 3 minutes blocks and for each block the total number of lever presses is expressed by minute and percentage of the baseline, that is to say, the mean of lever presses per minute from the last two instrumental learning sessions (VI-30 sec) (as in Experiment 7).

The reinforcer devaluation by specific satiety resulted in a decrease on the number of lever presses in the devalued condition (where the instrumental reinforcer was devalued) in comparison to the non-devalued condition (where the alternative reinforcer was devalued), independently of drug pretreatment. A three factor ANOVA (treatment x devaluation condition x time block) revealed a significant effect of devaluation condition ($F(1,66)=29.19$, $p<0.000$), time block ($F(9,594)=148.07$, $p<0.000$) and a significant interaction between them ($F(9,594)=7.51$, $p<0.000$). Post hoc NK comparisons on this interaction revealed that outcome devaluation by specific satiety induced a gradual decrease in the number of lever presses across the 30 min. extinction test in all animals in both conditions (devalued and non-devalued). Specifically, animals in the devalued condition showed lower levels of lever pressing in comparison to animals in the non-devalued condition during the first 9 minutes of the extinction test [when comparing devalued to non-devalued groups, NK test: p<0.003 in time block 1 (from minute 1 to 3), p<0.006 in time block 2 (from minute 4 to 6), p<0.049 in time

block 3 (from minute 7 to 9)], independently of pretreatment. Thus, all groups (NaCl, amphetamine-2 and cocaine-30 mg/kg) remained in a goal-directed behavior (sensitive to the current value of the outcome) after a specific satiety devaluation procedure and after a *limited* period of training (see Figure 3).

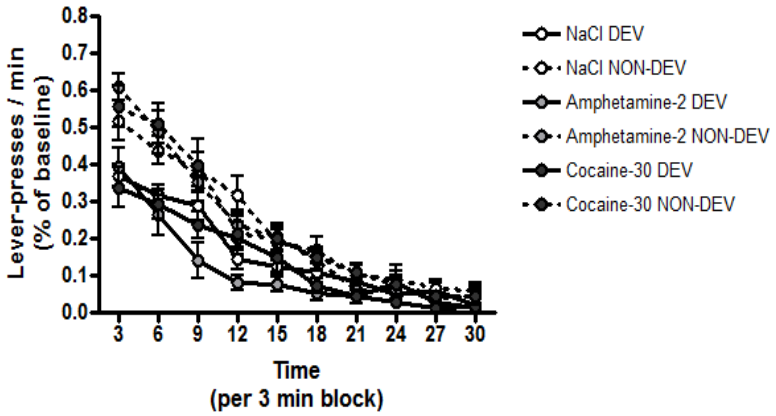


Figure 3 Effect of amphetamine and cocaine pretreatment on lever pressing after a first SSD in extinction conditions (after a *limited* period of training). Each point represents mean (± SEM) lever presses during the 30 min extinction test. The number of lever presses is expressed per minute as a proportion of baseline over time (mean of lever presses per minute from the last two instrumental learning sessions) (± SEM).

Instrumental responding

Extended period of training (six sessions: two sessions per day)

After the first specific satiety devaluation procedure, instrumental learning was extended to six additional VI-30 sec sessions (two sessions per day). As during the limited period of training, the total number of lever presses is expressed by minute. A three factor ANOVA (treatment x type of food pellets x session) revealed a significant effect of session ($F(5,150)=84.01$, $p<0.000$), and a significant treatment x type of food pellets x session interaction ($F(10,150)=2.15$, $p<0.023$). Post hoc NK comparisons revealed that the number of lever presses gradually increased throughout the sessions in all animals. Specifically, the NaCl group started to increase the number of lever presses at session seven, followed by the cocaine group at session eight and the amphetamine group at session nine (when compared NaCl-GB to session five, NK test: $p<0.039$ in session 7, $p<0.000$ in sessions 8, 9 and 10; when compared NaCl-5TUT to session five, NK test: $p<0.043$ in session 7, $p<0.003$ in session 8, $p<0.000$ in sessions 9 and 10; when compared amphetamine-GB to session five, NK test: $p<0.021$ in session 9, $p<0.000$ in session 10; when compared amphetamine-5TUT to session five: $p<0.032$ in session 9, $p<0.002$ in session 10; when compared cocaine-GB to session five: $p<0.041$ in session 8, $p<0.000$ in sessions 9 and 10; when compared cocaine-5TUT to session five: $p<0.002$ in session 8, $p<0.000$ in sessions 9 and 10), independently of the type of food pellets they experienced as a reward (GB-food-pellets or 5TUT-food-pellets) (see Figure 4).

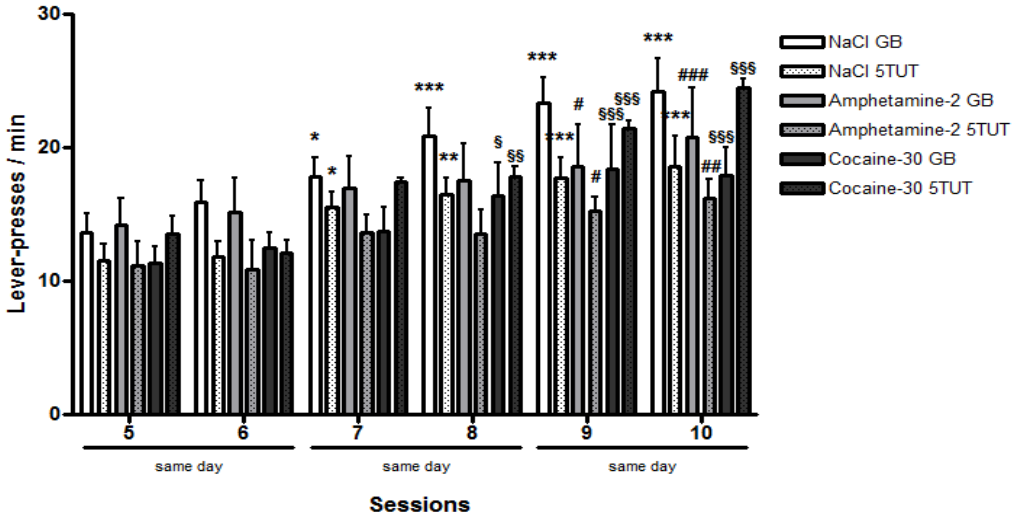


Figure 4 Operant food-reinforced behavior during an *extended* period of training. Histograms represent mean (± SEM) lever presses responses per treatment group (NaCl, amphetamine-2 and cocaine-30 mg/kg) and per food pellets type (GB and 5TUT). The total number of lever presses is expressed by minute and in function of learning sessions (six additional VI-30 sec sessions). * significantly different from session 5 in the NaCl group, # significantly different from session 5 in the amphetamine-2 mg/kg group, § significantly different from session 5 in the cocaine-30 mg/kg group (*/#/§ $p<0.05$, **/##/§§ $p<0.01$, ***/###/§§§ $p<0.001$).

Effect of drug pretreatment on instrumental responses in extinction conditions

<u>After an extended period of training (six sessions: two sessions per day)</u>

The second specific satiety devaluation procedure was performed one day after the tenth VI-30 sec session (after an *extended* period of training) and was conducted in the same way as the first specific satiety devaluation procedure. The results are expressed as previously described.

The reinforcer devaluation by specific satiety resulted in a decrease on the number of lever presses in the devalued condition (where the instrumental reinforcer was devalued) in comparison to the non-devalued condition (where the alternative reinforcer was devalued), independently of drug pretreatment. A three factor ANOVA (treatment x devaluation condition x time block) revealed a significant effect of devaluation condition ($F(1,66)=38.16$, $p<0.000$), time block ($F(9,594)=89.78$, $p<0.000$) and a significant interaction between them ($F(9,594)=12.00$, $p<0.000$). Post hoc NK comparisons on this interaction revealed that outcome devaluation by specific satiety induced a gradual decrease in the number of lever presses across the 30 min extinction test in all animals in both conditions (devalued and non-devalued). Specifically, animals in the devalued condition showed lower levels of lever pressing in comparison to animals in the non-devalued condition during the first 9 minutes of the extinction test [when compared devalued to non-devalued groups, NK test: $p<0.000$ in time block 1 (from minute 1 to 3) and time block 2 (from minute 4 to 6) and, $p<0.004$ in time block 3 (from minute 7 to 9)], independently of pretreatment. Thus, all groups (NaCl, amphetamine-2 and cocaine-30 mg/kg) remained in a goal-directed behavior (sensitive to the current value of the outcome) after a specific satiety devaluation procedure and even after an *extended* period of training (see Figure 5).

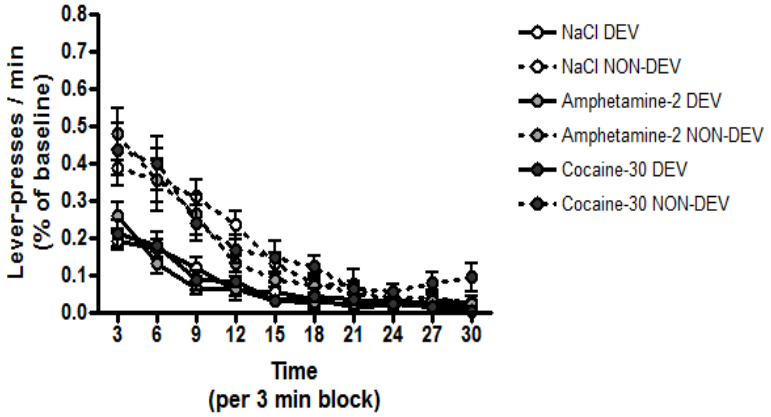


Figure 5 Effect of amphetamine and cocaine pretreatment on lever pressing after a second SSD in extinction conditions (after an *extended* period of training). Each point represents mean (± SEM) lever presses during the 30 min extinction test. The number of lever presses is expressed per minute as a proportion of baseline over time (mean of lever presses per minute from the last two instrumental learning sessions) (± SEM).

Instrumental responding

Second extended period of training (seven sessions: one session per day)

As we have previously mentioned, in normal conditions, the process of behavioral automatism is observed after *extended* learning. However, our results obtained from the extended period of training showed that all animals remained in a goal-directed behavior. In order to test if the length of our extended period of training was not the adequate and/or if the fact that the sessions were grouped in two per day prevented the development of behavioral automatisms, we decided to extend instrumental learning once again, but this time we extended it to seven additional VI-30 sec sessions spaced in time, with one session per day (the previous extended period of training consisted on six VI-30 sec sessions, with two sessions per day). According to Adams (1982), distribution of training sessions over time plays a role in the formation of behavioral automatisms: the implementation of an automatic behavior benefits from training sessions spaced and not grouped over time.

After the second specific satiety devaluation procedure, instrumental learning was extended to seven additional VI-30 sec sessions (one session per day). As during the limited and the first extended period of training, the total number of lever presses is expressed by minute. A three factor ANOVA (treatment x type of food pellets x session) revealed a significant effect of session ($F(6,180)=24.88$, $p<0.000$), and a significant treatment x type of food pellets x session interaction ($F(12,180)=1.97$, $p<0.028$). Post hoc NK comparisons revealed that the number of lever presses gradually increased throughout the session in all animals; however, this increased in the number of lever presses was dependent on the type of food pellets experienced as a reinforcer during instrumental training. Specifically, the amphetamine group started to increase the number of lever presses at session fourteen, but only applied to the animals that experienced GB-food pellets during instrumental training (when compared amphetamine-GB to session eleven, NK test: $p<0.045$ in session 14, $p<0.023$ in session 15, $p<0.000$ in sessions 16 and 17). The NaCl group started to increase the number of lever presses at session fifteen and only applied to the animals that experienced GB-food pellets during instrumental training (when compared NaCl-GB to session eleven, NK test: $p<0.000$ in sessions 15, 16 and 17). Finally, the cocaine group started to increase the number of lever presses at session fifteen and only applied to the animals that experienced 5TUT-food pellets during instrumental

training (when compared cocaine-5TUT to session eleven, NK test: p<0.000 in session 15, p<0.001 in session 16, p<0.000 in session 17) (see Figure 6).

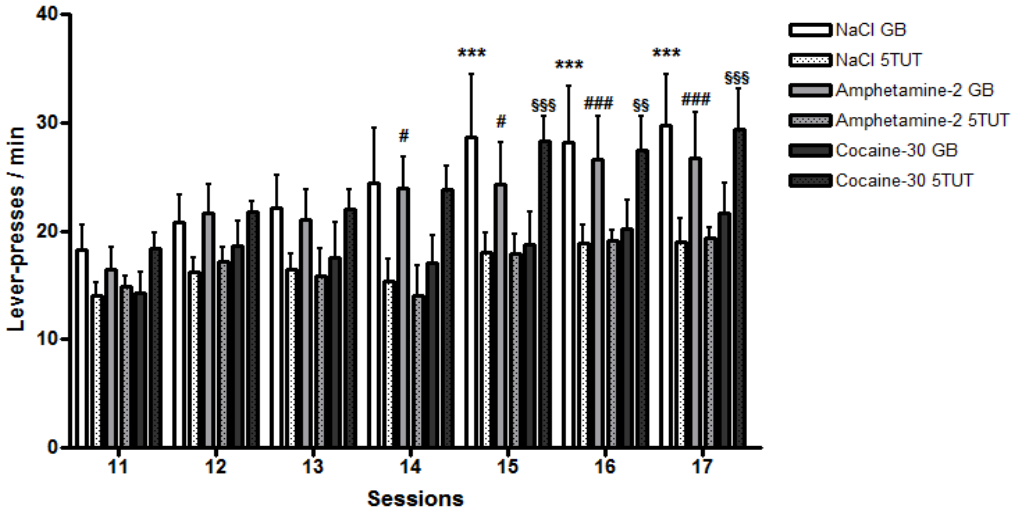


Figure 6 Operant food-reinforced behavior during a second *extended* period of training. Histograms represent mean (± SEM) lever presses responses per treatment group (NaCl, amphetamine-2 and cocaine-30 mg/kg) and per food pellets type (GB and 5TUT). The total number of lever presses is expressed by minute and in function of learning sessions (seven additional VI-30 sec sessions). * significantly different from session 11 in the NaCl group, # significantly different from session 11 in the amphetamine-2 mg/kg group, § significantly different from session 11 in the cocaine-30 mg/kg group (*/#/§ p<0.05, **/##/§§ p<0.01, ***/###/§§§ p<0.001).

Effect of drug pretreatment on instrumental responses in extinction conditions

<u>After a second extended period of training (seven sessions: one session per day)</u>

The third specific satiety devaluation procedure was performed one day after the seventeenth VI-30 sec session (after the second *extended* period of training) and progressed in the same way as outlined for the first and the second specific satiety devaluation procedures. The results are expressed as previously described.

The reinforcer devaluation by specific satiety resulted in a decrease on the number of lever presses in the devalued condition (where the instrumental reinforcer was devalued) in comparison to the non-devalued condition (where the alternative reinforcer was devalued), independently of drug pretreatment. A three factor ANOVA (treatment x devaluation condition x time block) revealed a significant effect of devaluation condition ($F(1,66)=16.36$, $p<0.000$), time block ($F(9,594)=40.49$, $p<0.000$) and a significant interaction between them ($F(9,594)=7.97$, $p<0.000$). Post hoc NK comparisons on this interaction revealed that outcome devaluation by specific satiety induced a gradual decrease in the number of lever presses across the 30 min extinction test in all animals in both conditions (devalued and non-devalued). Specifically, animals in the devalued condition showed lower levels of lever pressing in comparison to animals in the non-devalued condition during the first 6 minutes of the extinction test [when compared devalued to non-devalued groups, NK test: p<0.026 in time block 1 (from minute 1 to 3), p<0.008 in time block 2 (from minute 4 to 6)], independently of pretreatment. Thus, all groups (NaCl, amphetamine-2 and cocaine-30 mg/kg) remained in a goal-directed behavior (sensitive to the current value of the outcome) after a specific satiety devaluation procedure and even after a second *extended* period of training spaced in time (see Figure 7).

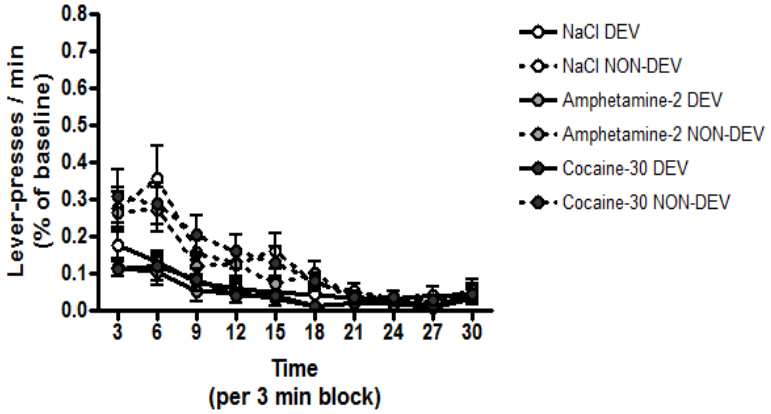


Figure 7 Effect of amphetamine and cocaine pretreatment on lever pressing after a third SSD in extinction conditions (after a second *extended* period of training). Each point represents mean (± SEM) lever presses during the 30 min extinction test. The number of lever presses is expressed per minute as a proportion of baseline over time (mean of lever presses per minute from the last two instrumental learning sessions) (± SEM).

Discussion

This study showed that neither amphetamine nor cocaine pretreatment accelerates the formation of habits, even after extended periods of instrumental training. Concretely, the three extinction tests revealed a goal-directed behavior in amphetamine-2 mg/kg and cocaine-30 mg/kg pretreated animals and, importantly, instrumental behavior also remained goal-directed in NaCl pretreated rats, which is in disagreement with the well known fact that instrumental performance becomes habitual as training proceeds (Adams 1982; Dickinson et al. 1995).

No different effects of amphetamine and cocaine pretreatment in acquisition of operant responding
During acquisition of an instrumental response for two different types of food pellets, the drug pretreated groups (amphetamine-2 and cocaine-30 mg/kg) did not show different levels of instrumental responding when compared to the NaCl pretreated group. Furthermore, no differences were observed between animals pretreated with amphetamine 2 mg/kg and animals pretreated with cocaine 30 mg/kg. These results are in accordance with previous results from the literature showing that prior sensitization to psychostimulants (amphetamine and cocaine) does not affect the acquisition of an instrumental response (Nelson and Killcross 2006; Nordquist et al. 2007; Schoenbaum and Setlow 2005). However, instrumental responses after a first and a second extended period of training were influenced by the type of food pellets experienced as reinforcers. We hypothesize that this variability in responding was due to the fact that animals became sated to the food pellets (either GB or 5TUT), since this variability was higher in the second extended period of training (after two specific satiety devaluation procedures) than in the first extended period of training (after one specific satiety devaluation procedure). Nonetheless, it can also be argued that, by being on a food-deprived state, the increase in the number of lever presses was higher for the nutritive outcome (GB food pellets) instead of the only rewarding outcome (5TUT food pellets). Another possibility is that rats lever press more for one type of food pellets (either GB or 5TUT) simply because they do not like the taste of one type of food pellets as much as they like the taste of the other type of food pellets (it is less hedonically rewarding) (Beeler et al. 2012).

Specific satiety devaluation procedure

This devaluation procedure leads to a change in the hedonic value of the food consumed: a temporary decrease in the pleasurable sensation derived from consuming a certain type of food, while maintaining animals' motivation to consume another type of food. The results from the three specific satiety devaluation procedures demonstrated that neither pretreatment (NaCl, amphetamine-2 and cocaine-30 mg/kg) nor instrumental training affected the changes in the value of the outcome induced by a devaluation procedure, since the consumption tests showed that after consuming one type of food pellets until sated, the satiety was specific to that type of food pellets in all animals (data not shown).

Neither amphetamine 2 mg/kg nor cocaine 30 mg/kg induce a habit-based lever pressing responding during extinction tests

The results from the three extinction tests revealed that the devalued saline, amphetamine-2 and cocaine-30 mg/kg pretreated rats showed lower levels of lever pressing than the non-devalued saline, amphetamine-2 and cocaine-30 mg/kg pretreated rats, indicating that lever pressing responding during the extinction tests was goal-directed in all groups of animals.

The finding that the cocaine-30 mg/kg pretreated rats showed a goal-directed behavior during the first extinction test is in disagreement with our previous finding in Experiment 7, where we found that cocaine-30 mg/kg pretreated rats showed a habit-based lever pressing responding during an extinction test. We think that these different findings are the result of using different devaluation paradigms. Specifically, in Experiment 7, we devalued the reinforcer by pairing its consumption with LiCl-induced gastric illness (conditioned taste aversion), whereas in this experiment we devalued the reinforcer by specific satiety [motivational shift: hungry (food tastes pleasant) $\rightarrow$ sated (food tastes hedonically neutral)] (Rolls 2007), which suggests that conditioned taste aversion by LiCl is more efficient in changing the value of the outcome than a motivational shift by specific satiety. Then, the finding that amphetamine-2 mg/kg pretreated rats did not show a habit-based instrumental responding is in disagreement with previous reports from the literature, where it was shown that amphetamine pretreatment accelerates the formation of habits (Nelson and Killcross 2006; Nordquist et al. 2007). However, there are differences between protocols. First, our amphetamine pretreatment lasted 10 days, whereas Nelson and Killcross' lasted 7 days and Nordquist and colleagues' lasted 5 days (2.5 mg/kg). Second, we started operant training after a 10 days abstinence period, whereas Nelson and Killcross started theirs after a 7 days abstinence period and Nordquist and colleagues after two weeks abstinence period. Third, we used food pellets as reinforcers (GB and 5TUT), whereas Nelson and Killcross used liquid solutions as reinforcers (maltodextrin solution with a cherry flavor and sucrose solution with a grape flavor) and Nordquist and colleagues used different food pellets as reinforcers (Noyes formula P and Noyes Formula F). Thus, even though these differences in protocols might explain the results observed in the amphetamine pretreated rats in the first extinction test (after a *limited* period of training), they cannot explain the results observed in all rats (NaCl, amphetamine-2 and cocaine-30 mg/kg) after the *extended* periods of training (i.e. all groups remained in a goal-directed behavior after a specific satiety devaluation procedure and even after a second *extended* period of training). One hypothesis is that instrumental responding sensitivity after a specific satiety devaluation procedure is dependent on the context, as indicated by Killcross and Coutureau (2003). These authors suggest that the use of a context different from the operant cages might maintain operant behavior under the control of the representation of the outcome, resulting in sensitivity to its devaluation. By reassessing each time the link between context

and action, the animal revalues the outcome and maintains a goal-directed behavior. Although we cannot exclude this possibility, Nelson and Killcross (2006) and Nordquist and colleagues (2007) performed the specific satiety devaluation procedure in a different context than the operant cages (individual cages) and found that operant behavior was under the control of automatic, stimulus-response associations. Finally, since lever pressing responding in the devalued and the non-devalued animals started to decrease in the last two extinction tests (after the extended periods of training), we cannot exclude the possibility that, with further training, the animals might have shown a habit-based responding.

In summary, we concluded that drug pretreatment does not accelerate the formation of habits (in an instrumental task). However, the finding that saline pretreated rats maintained a goal-directed behavior even after two extended periods of training (where the behavior is habit-based), raise the question of what is the length of an extended period of training in comparison to a limited period of training (where the behavior is goal-directed). Thus, it would be necessary to perform a replication of the protocols followed by Nelson and Killcross and Nordquist and colleagues in order to verify the hypothesis that drug pretreatment accelerates the formation of habits.

General Discussion

1. Reminder of the aim

The general aim of my PhD was to study if different drugs of abuse with different interoceptive properties can trigger habit-based behavior depending on their discriminative stimulus properties. We worked with three different drugs of abuse: cocaine, heroin and nicotine.

Thus, in order to study if the discriminative stimulus properties of cocaine, heroin and nicotine induce habit-based reinstatement, three specific questions were developed:

1) The exact behavioral nature of drug-induced reinstatement is still in debate. One hypothesis suggests that, as incentives, drugs of abuse can increase the incentive value of environmental stimuli and influence appetitive behaviors; whereas another hypothesis suggests that, as an interoceptive stimulus, drugs of abuse can acquire discriminative stimulus properties and control behavior. Thus, in the first question, we wanted to identify two things: 1) whether the discriminative stimulus properties of cocaine, heroin and nicotine play a crucial role in relapse and 2) whether the discriminative stimulus properties of heroin and nicotine are different from the discriminative stimulus properties of cocaine.

2) Relapse to drug-seeking and drug-taking can be either a voluntary behavior, motivated by the desire to re-experience the positive hedonic effects of drugs of abuse [a goal-directed behavior: action-outcome (A-0)] or can be an automatic response triggered by certain stimuli previously associated with drug consumption [a habit-based behavior: stimulus-response (S-R)]. Thus, in the second question, we wanted to identify if reinstatement induced by the discriminative stimulus properties and/or the neuroadaptations produced by cocaine, heroin and nicotine is voluntary (a goal-directed behavior) or automatic (a habit-based behavior).

3) It has been suggested that the neuroadaptations that take place following repeated exposure to drugs of abuse accelerates the formation of habits. Thus, in the third question, we wanted to identify the way drugs of abuse lead to the formation of habits by examining the effects of cocaine, heroin and nicotine sensitization on the temporal transition from action-outcome processes to stimulus-response habits for a food reward after outcome devaluation.

2. Summary of results

1) **Question 1.** In the first question we demonstrated that, when comparing different drugs of abuse, the discriminative stimulus properties of cocaine and nicotine, but not heroin, play a significant role in driving reinstatement of an extinguished operant behavior not directed towards the drug itself but towards a natural reinforcer (5% sucrose solution), indicating that animals are using the discriminative stimulus properties of the drugs to guide their behavior.

2) **Question 2.** In the second question we demonstrated that re-exposure to the discriminative stimulus properties of cocaine and nicotine reinstates an automatic, habit-based food-

seeking behavior, independent of the current value of the outcome, whereas re-exposure to a discriminative tactile and olfactory stimulus reinstates a goal-directed food-seeking behavior, dependent of the current value of the outcome.

3) **Question 3.** Finally, in the third question we could not demonstrate that cocaine pretreatment accelerates the progression from goal-directed to habit-based behaviors.

3. How do the discriminative stimulus properties of drugs induce reinstatement?

It has been demonstrated that drugs can be recognizable stimuli and as result, identified by the individual. For example, the interoceptive effects induced by the drug may act as a stimulus, signaling the rat that lever pressing will lead to a food reward (Young 2009). It was at the end of the 1960s that Overton (in Drug Discrimination studies) was able to report on the relative discriminability of about ten different types of drug states. Since the work performed by Overton, it was generalized that each different type or class of drugs produce a different discriminative effect.

Drug exposure, through its central and/or peripheral effects, leads to physiological changes like heart rate increase, vasoconstriction, salivation, disruption of thermoregulation and glucose levels, and changes in visual, auditory and olfactory perception. These physiological changes are detected by one or more sensorial organs and as a result, can be identified by the organism (Cameron 2001; Craig 2002; 2003). Once detected, these interoceptive stimuli can acquire conditioned properties like any other type of stimuli (i. e. auditory tone, visual cue, etc.) (Overton 1988). Accordingly, it seems that interoceptive pharmacological cues as well as exteroceptive environmental cues can be paired with a drug effect. That is, interoceptive and exteroceptive cues can enter into association (following the principles of contingency, contiguity and competition between stimuli) and can even enter in competition for the control of a conditioned response (Kim et al. 1999; Siegel et al. 2000).

Our results suggest that there is an action mechanism through which cocaine and nicotine induce reinstatement. We propose that the interoceptive properties of cocaine and nicotine act as discriminative stimulus explicitly paired with behavioral consequences. Specifically, re-experiencing cocaine or nicotine, guides animal's behavior and induces reinstatement.

However, the finding that re-exposure to heroin did not induce reinstatement of an extinguished sucrose-seeking behavior raise the question why? Could it be that the ability of the discriminative stimulus properties of drugs of abuse to induce reinstatement is related to the amount of drug exposure? It might be that the heroin rats, unlike the cocaine and nicotine rats, needed a longer period of heroin exposure in order to see a sucrose-seeking reinstatement induced by re-exposure to heroin. This hypothesis is in accordance with a previous report from the literature where it was shown that the ability of heroin to induce reinstatement of drug-seeking is related to prolonged, but not limited, access to heroin self-administration (Lenoir and Ahmed 2007).

Whether central or peripheral, short-term or prolonged exposure, the exact nature of the discriminative stimulus properties of cocaine and nicotine (and drugs of abuse in general) that induce reinstatement remains to be determined.

4. What is the nature of the reinstatement induced by the discriminative stimulus properties of cocaine and nicotine? Goal-directed or habit-based behavior?

Our results showed that the discriminative stimulus properties of cocaine and nicotine reinstate an automatic, habit-based food-seeking behavior (independent of volition). We propose that by re-experiencing the drug effects in the body, the behavior performed during previous drug exposure (lever pressing for a food reward) is reinstated. This lever pressing reinstatement reflects the stimulus-response associations previously acquired under the cocaine and nicotine effects during instrumental conditioning.

More specifically, Tiffany (1990) proposed that automatic processes could control habitual drug-use behaviors in absence of explicit motivation to take the drug. Our results are in agreement with this hypothesis. Furthermore, previous work performed in our team, where cocaine intake and operant training were dissociated by using experimental procedures generating different histories of operant training but almost identical histories of cocaine intake, showed that the magnitude of cocaine reinstatement did not depend neither on the previous amount of cocaine intake nor on the intensity of the expected cocaine reward, but on the extent of motor training before the extinction of the behavior (Keiflin et al. 2008b), which suggests that cocaine reinstatement is the expression of the automatic resumption of fixed action patterns previously performed during cocaine exposure periods.

Furthermore, in another study, Norman and Tsibulsky (2006) showed that in rats given cocaine either contingently (self-administered by the rat) or non-contingently (infused by the experimenter), rats continued responding even in the absence of contingency, and this lack of contingency between action-outcome is a key characteristic of automatic behaviors (Balleine and Dickinson 1998). At the neurobiological level, this hypothesis is consistent with the work of McFarland and Kalivas (2001), in which they showed that the circuitry mediating cocaine-induced reinstatement is part of a motor circuitry known to be involved in automatic behaviors (the cortico-striato-pallidal circuitry). Both of these studies are in agreement with our hypothesis that cocaine-induced reinstatement is a habit-based behavior and confirms to a certain extend the habit theory of drug addiction by Everitt and Robins (2005). The fact that reinstatement by nicotine is also a habit-based behavior suggests that the circuitry mediating nicotine-induced reinstatement is also part of the cortico-striato-pallidal circuitry.

5. How do the discriminative stimulus properties of cocaine and nicotine reinstate an automatic, habit-based food-seeking behavior?

Our results indicate that learning of an instrumental response under the effects of cocaine and nicotine promotes the acquisition and expression of behavioral automatisms. Furthermore, our results are in agreement with previous studies showing that exposure to psychostimulant drugs results in enhanced habit formation (Nelson and Killcross 2006; Nordquist et al. 2007; Schoenbaum and Setlow 2005). However, these authors suggest that cocaine and amphetamine accelerate the formation of habits by the neuroadaptations induced by these drugs.

Importantly, in the studies previously mentioned, the animal's behavior was never performed under the drug effects, suggesting that another process (in addition to the neuroadaptations induced by drug exposure) might mediate the acceleration from goal-directed to habit-based behaviors. Specifically, we had two groups of rats in our experimental setting that received the same amount of drug exposure, with the difference being that the *inside* groups experienced the drug effects while performing the operant task, whereas the *outside* groups experienced the drug effects in another environment. Interestingly, only the *inside* groups reinstated operant responding when re-exposed to the drug. Thus, if the neuroadaptations induced by drug exposure are the only factors necessary to promote habit-based behaviors, why did the *outside* groups not show reinstatement? Our results suggest that the effect of cocaine and nicotine priming injections on habit-based food-seeking behavior is dependent of the pairing of the drug effects with food reinforced responding during training of an instrumental response.

However, this pairing of the drug effects with food reinforced responding raises an interesting question: which drug effects? central or peripheral? Interestingly, a recent study suggests that the interaction of nicotine with peripheral neural substrates is the primary factor responsible for its powerful activating effects in the brain. However, they also suggest that the peripheral actions of nicotine alone without subsequent central actions are unable to produce large central effects (Lenoir and Kiyatkin 2011). Accordingly, Wise and Kiyatkin (2011) suggest that cocaine activates the reward system in response to its peripheral actions that precede the direct effects in the brain. Thus, these authors agree that both peripheral and central actions of drugs of abuse are critical for its rewarding effects.

However, these authors are examining the rewarding effects of drugs, whereas we are examining the role of the discriminative stimulus properties of the drugs. In our experimental setting we dissociated the reinforcing from the discriminative stimulus properties of the drug by training the animals to self-administer a non-drug reward. In order to verify our hypothesis that the discriminative stimulus properties of the drugs are responsible for inducing habit-based food-seeking reinstatement, it would be necessary to dissociate the peripheral from the central actions of drugs of abuse. For example, it would be interesting to compare the contribution of the central effects of cocaine hydrochloride with the peripheral effects of cocaine methiodide (a cocaine analogue that does not cross the blood brain barrier) (Wise et al. 2008) to verify which cocaine effect is the responsible to induce habit-based food-seeking reinstatement.

6. Relationship between craving and relapse

Our results indicate that reinstatement of a food-seeking behavior induced by cocaine and nicotine is an automatic response, independent of the representation of the outcome. In our experiments, reinstatement is not directed towards drugs but towards food. Thus, this reinstatement cannot be interpreted in terms of craving. Indeed, the definition of drug craving involves anticipation and representation of the drug effects. However, relapse is not always associated with craving, since relapse might occur when craving is weak or even non-existent and, in the other hand, high levels of craving does not necessarily lead to relapse (Anton 1999).

According to the Incentive Sensitization Theory of Addiction, craving is the subjective experience associated with incentive salience attribution, which can persist long after withdrawal ends (Robinson and Berridge 1993), whereas according to Tiffany (1990), craving is not the direct cause of drug-seeking behavior, but a reaction to highly automatized behaviors which are controlled by stimulus-response associations independent of an explicit desire to consume drugs and, therefore, do not require complex cognitive processes.

Thus, one possibility to explain these conflicting results between craving and relapse is that craving is a multidimensional state that includes cognitive, affective, motivational and physiological components (Anton 1999; Mezinskis et al. 2001; Sayette et al. 2000); so, selecting an appropriate craving measure (e.g. self-reports and non-verbal approaches) is important, since craving can reflect less or more components associated with relapse depending on the craving measure used.

7. Limitations of our protocols

We have to outline some methodological limitations in the experimental protocols used in our experiments.

First, the participation of the discriminative stimulus properties of cocaine, heroin and nicotine in reinstatement of a food-seeking behavior was demonstrated by one route of drug administration (i.p. for cocaine and s.c. for heroin and nicotine) during training. However, in the traditional model of reinstatement, drugs are intravenously self-administered during training. Although, the discriminative effects of drugs are generally independent of the route of administration (De la Garza and Johanson 1986; Lamb et al. 1991), this difference in routes of administration may limit the generalization of our findings.

Second, our rats were trained to self-administer different types of food that varied across experiments. Specifically, 5% sucrose solution was used as a reinforcer for Experiments 1, 2 and 3, whereas food pellets [45 mg Rodent Tablet, Test Diet (5TUL)] were used as reinforcers for Experiments 4, 5, 6 and 7, and other type of food pellets [45 mg Rodent Tablet, BioServ (Grain-Based) and 45 mg Rodent Tablet, Test Diet (5TUT)] for Experiment 8. Our results suggest that the food pellets and the sucrose solution functioned qualitatively differently in the strength of the instrumental response. We believe that some of the factors that made the food pellets qualitatively different from the sucrose solution are the palatability, the texture (taste and smell) and the caloric value (Nair et al. 2009).

Third, we used standard methods of reward devaluation (LiCl-induced gastric illness and specific satiety), but these methods are not effective with a reward that has no consummatory or gustatory component (i.e. intravenously administered drugs) (Holmes and Clemens 2011; Zapata et al. 2010). As a result, studies assessing the development of habitual drug seeking are limited. One of the methods used to circumvent these issues is the use of a drug seeking/taking chained schedule to obtain a cocaine reward (Olmstead et al. 2001; Zapata et al. 2010); however, it must be noted that cocaine itself is never devalued, nor associated with any adverse consequences.

Fourth, our rats were trained, extinguished and tested in the same context (i.e. AAA renewal). However, it is known that contexts play an important role in regulating the performance of learned

associations (Hamlin et al. 2006). Nonetheless, contextual control over extinguished responding in instrumental conditioning (learning about the relation action-outcome) has been less studied than in pavlovian conditioning (learning about relation CS-US). There is a possibility that if our rats were trained for a sucrose reward in one context, then submitted to instrumental extinction in a different context from training, and finally tested in the original training context or in the extinction context (i.e. ABA versus ABB), the behavior observed during the reinstatement of the extinguished food-seeking behavior would indicate if food-seeking reinstatement is context-specific.

8. Future perspectives

Interoceptive awareness, the ability to perceive bodily changes, differs substantially across individuals (Pollatos et al. 2007). Interestingly, people classified as *viscerally aware* are described as more emotionally expressive (Ferguson and Katkin 1996) and may experience emotions with higher-intensity. Thus, since our results showed that the discriminative stimulus properties of drugs of abuse induce reinstatement, it would be interesting to study if these people are more prone to develop an addiction and/or to relapse.

Furthermore, it has been suggested that the effect of insula lesions observed on smoking addiction (i.e. smoking addiction is attenuated or abolished following focal damage to insula cortex) is attributable to disruption of the capacity to represent or respond to internal interoceptive signals (Gray and Critchley 2007). This study by Naqvi and colleagues (Naqvi et al. 2007) emphasizes the critical role of conscious feelings states in driving habitual behavior. Thus, further research is needed to see if the contribution of the insula cortex to neural processes that maintain addictive behaviors applies to other drugs of abuse.

9. Conclusion

The aim of this thesis was to study the behavioral processes involved in reinstatement induced by drugs of abuse. With our results obtained we can say that reinstatement induced by cocaine and nicotine involves both the discriminative stimulus properties of the drug as well as stimulus-response associations acquired under the effects of the drug. However, in order to actually assign a key role to behavioral automatisms in relapse and in drug addiction, it is necessary to know it the formation of habits can be generalized to other drugs of abuse. Our work was a first step in the comparison of automatic processes produced by cocaine and nicotine. If the activation of automatic, habit-based behaviors can be generalized to other drugs of abuse, we could consider that relapse to drug-seeking and drug-taking is partly under the control of automatic processes, which could explain the high probability to relapse during abstinence, despite the knowledge of the adverse consequences.

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