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Mme FANNY MENUGE

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Directeur(s) de Thèse :

M. PATRICK PAGEAT

MME MIRIAM MARCET-RIUS

Rapporteurs :

M. ANGELO GAZZANO, UNIVERSITA DI PISA

M. LUC MOUNIER, VETAGRO SUP LYON

Membre(s) du jury :

M. DONALD BROOM, UNIVERSITE DE CAMBRIDGE, Président

M. ALESSANDRO COZZI, IRSEA, Membre

M. ANTOINE GUEDENEY, UNIVERSITE PARIS 7, Membre

M. JEAN-LUC CADORE, ECOLE NATIONALE VETERINAIRE DE LYON, Invité(e)

MME CÉCILE BIENBOIRE-FROSINI, IRSEA, Membre

MME MIRIAM MARCET-RIUS, IRSEA, Membre

M. PATRICK PAGEAT, IRSEA, Membre

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AUTEUR : Fanny MENUGE

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DIRECTEUR DE THESE : Prof Patrick PAGEAT

CO-DIRECTRICE : Dr Míriam MARCET-RIUS

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RESUME en français

Les chiens guides d'aveugles, qui constituent aujourd'hui un enjeu d'utilité public, améliorent le quotidien des personnes atteintes de déficience visuelle ainsi que la perception du handicap. Même si ces chiens sont élevés et éduqués de manière à les préparer à leur futur travail, le taux d'échec reste très élevé, près de 40%. De manière général, les écoles de chiens guides fonctionnent globalement de la même manière au niveau international : Les chiens sont formés par des associations, et leur financement repose essentiellement sur des dons. Diminuer ou détecter au plus tôt les échecs est donc important afin d'éviter au maximum les pertes financières, un chien coutant environ 25 000 euros.

Un chien guide est remis à son bénéficiaire à l'âge d'environ 2 ans. Avant cela, une fois sevré, il est placé en famille d'accueil où il va apprendre les bases éducatives,

mais la famille d'accueil va surtout avoir le rôle très important de le sociabiliser et de l'habituer à toutes sortes de stimulations. Ensuite, vers l'âge d'un an et demi, il va commencer sa formation auprès d'un éducateur canin pendant environ 6 mois. Au cours de cette période, différentes possibilités sont envisagées selon les écoles de chiens guides. Le chien peut soit rester à l'école 7 jours sur 7, soit revenir dans sa famille d'accueil pour les weekends, ou encore passer seulement la journée à l'école et rentrer tous les soirs. Dans l'école avec laquelle nous travaillons pour ce projet, la fondation Frédéric Gaillanne (L'Isle-sur-la-Sorgue, 84), les chiens sont à l'école du lundi au vendredi et retournent dans leur famille chaque weekend.

Au regard de ceci, plusieurs choses sont à souligner. Tout d'abord, le développement du jeune chiot, déterminant pour son équilibre émotionnel à l'âge adulte, est géré par les familles d'accueil. Ensuite, le chien est soumis à plusieurs transferts du lien d'attachement, et enfin, en période d'éducation, les ruptures hebdomadaires de routine et les séparations répétées avec la famille d'accueil pourraient s'avérer stressantes pour l'animal. Dans le cadre de ce projet de thèse, nous nous sommes donc penchés sur ce contexte particulier dans lequel les chiens évoluent, et nous avons formulé l'hypothèse que ce contexte pouvait influencer le bien-être des chiens et le développement de leur état émotionnel, et donc avoir un impact sur leur réussite en tant que chien guide.

Les résultats ont permis de mettre en évidence l'importance de la familiarisation à l'environnement mise en place par la famille d'accueil, et également l'importance de la relation d'attachement instauré entre les deux parties. Ces deux paramètres pourraient en effet avoir un impact sur le développement émotionnel et le succès des

chiens. Concernant l'organisation de la période d'éducation, les résultats ont démontré une bonne adaptation des chiens à la vie au chenil de l'école, ainsi qu'une facilité d'apprentissage et un bien-être plus important lorsque les chiens y restaient tout au long de cette période, en comparaison à ceux retournant dans leur famille d'accueil chaque weekend.

Ce projet apporte des pistes aux écoles afin de former des chiens plus équilibrés émotionnellement et de faire bénéficier au plus grand nombre de l'aide du chien guide. Ceci pourrait passer par la mise en place d'un contexte d'éducation plus adapté, un renforcement du suivi en famille d'accueil, ou encore par une professionnalisation de la socialisation des chiots.

Mots-clés : Attachement, Bien-être, Chien de travail, Equilibre émotionnel, Famille d'accueil, Ecole de chiens guides

ABSTRACT

Guide dogs, which have become a public utility issue, improve the daily lives of visually impaired people as well as their perceptions of their disability. Even if these dogs are raised and trained to prepare them for their future work, the failure rate of such training remains very high, i.e., close to 40%. In general, guide dog schools worldwide operate in the same way: the dogs are trained via association, and the financing of such schools is essentially based on donations. It is therefore important to reduce or detect failures as early as possible to avoid financial losses to the greatest extent possible. The training of each dog costs approximately 25,000 euros.

A guide dog is given to the relevant beneficiary when the dog is approximately 2 years old. Prior to that time, after the dog is weaned, he is placed in a foster family from whom he learn the basics of education. In particular, the foster family plays the very important role of socializing the dog and familiarizing him with all kinds of stimulation. Subsequently, when the dog is approximately one and a half years old, he begins training with a dog trainer, which lasts approximately 6 months. During this period, different possibilities are considered depending on the guide dog school in question. The dog can remain at the school 7 days per week, return to the foster family on weekends, or spend only the day at the school and return to the foster family every evening. In the school that is the focus of this project, the Frederic Gaillanne Foundation (L'Isle-sur-la-Sorgue, France), the dogs remain at the school Monday through Friday and return to their families each weekend.

Accordingly, several factors should be emphasized in this context. First, the development of the young puppy, which is crucial to the dog’s emotional balance in adulthood, is managed by foster families. Second, the dog is subjected to several transfers of the attachment bond. Finally, during the training period, weekly breaks in routine and repeated separations from the foster family can be stressful for the animal. In this thesis, we therefore examined the particular context in which the dogs develop and formulated the hypothesis that this context can influence the dogs' welfare and the development of their emotional states and can thus have an impact on their success as guide dogs.

The results of this investigation highlighted the importance of the familiarization to the environment facilitated by the foster family and the importance of the attachment relationship established between the two parties. These two parameters can indeed have an impact on the emotional development and success of the dogs. Regarding the organization of the dog’s education, the results showed that the dogs adapted well to life in the school kennel and highlighted the greater ease of learning and improved welfare that resulted when the dogs remained in the kennel throughout this period rather than returning to the foster family each weekend.

This project provides guidance to schools that can help them train more emotionally balanced dogs and that can allow more people to benefit from the assistance of a guide dog. This goal could be achieved by establishing a better-adapted educational context, by reinforcing follow-up measures with foster families, or by professionalizing the socialization of the puppies.

Keywords: Attachment bond, Emotional balance, Foster family, Guide dog school, Welfare, Working dog

LIST OF PUBLICATIONS

1. Articles

- Menuge, F., Marcet-Rius, M., Jochem, M., François, O., Assali, C., Chabaud, C., Teruel E., Guillemot J. and Pageat, P. (2021). Early Evaluation of Fearfulness in Future Guide Dogs for Blind People. Animals, 11(2), 412. https://doi.org/10.3390/ani11020412

- Menuge, F., Descout, E., Arroub, S., Lafont Lecuelle, C., Gautier, E., Mengoli, M. and Pageat, P. (2021). Attachment: a new approach to predict the success of future guide dogs? A pilot study. Scholarly journal of Psychology and Behavioral Sciences. DOI: 10.32474/SJPBS.2021.06.000228

- Menuge, F., Marcet-Rius, M., Chabaud, C., Teruel E., Berthelot C., Kalonji G., Bienboire-Frosini C., Mendonça T., Lascar E. and Pageat, P. (2021). Repeated separations between a future guide dog and its foster family modify stress-related indicators and affect dog's focus. Applied Animal Behaviour Science, 105486. https://doi.org/10.1016/j.applanim.2021.105486

- Menuge, F., Pageat, P., Chabaud, C., Teruel E., Ambielle, B., Kalonji G., Bienboire-Frosini C., Mendonça T., Poupée, A. and Marcet-Rius, M. (2022). Home or boarding school for the weekend: Which is best for future guide dogs involved in an ongoing training programme? Dog Behavior.

- Mendonça T., Bienboire-Frosini C., Menuge F., Leclercq J., Lafont-Lecuelle C., Arroub S. and Pageat P. (2019). The Impact of Equine-Assisted Therapy on

Equine Behavioral and Physiological Responses. Animals, 9(7) 409. https://doi.org/10.3390/ani9070409

- Kasbaoui, N., Marcet-Rius, M., Bienboire-Frosini, C., Menuge, F., Monneret, P., Descout, E., Cozzi, A. and Pageat, P. (2022). A feline semiochemical composition influences the cat's toileting location choice. Animals 12, 938. https://doi.org/10.3390/ani12070938

2. International congresses, proceedings, oral presentations, and posters

2.1. Oral presentations and proceedings

- Menuge F., Marcet-Rius, M., Chabaud, C., Teruel, E., Berthelot, C, Kalonji, G., Bienboire-Frosini, C., Mendonça, T., Lascar, E. and Pageat, P. Go back to the foster family every weekend: what is the impact on stress of future guide dogs in an ongoing training program? ISAE, Online congress, 2021.

- Menuge F., Marcet-Rius, M., Teruel E., Gautier E. and Pageat P. Could the success of guide dogs be influenced by the bond they developed with their puppy raisers? EVCBMAW, Online congress, 2021.

- Mengoli M., Mendonça T., Menuge F., Codecasa E., Marcet-Rius M., Royer D., Cozzi A., and Pageat P. Welfare of assistance dogs during lifelong working activity: an ethical review. IVBM, Washington, 2019.

- Mendonça T., Bienboire-Frosini C., Menuge F., Lafont-Lecuelle C., Arroub S. and Pageat P. How do horses perceive equine-assisted therapies: as negative or a positive event? EVCBMAW, Eindhoven, 2019.

2.2. Posters and proceedings

- Menuge, F., Marcet-Rius, M., Chabaud, C., Teruel, E., Kalonji, G., Bienboire-Frosini, C., Mendonça, T., Cozzi, A. and Pageat, P. Research program about the development of future guide dogs for blind people: How can we optimise the breeding programme, from weaning to the end of training? ISAE, North Macedonia 2022.

- Menuge F., Pageat P., Marcet-Rius M., Teruel E., Arroub S., Cozzi A. Effect of an avoidance semiochemical on cat's interest in garbage can: a pilot study. EVCBMAW, Mallorca, Spain, 2022.

- Mendonça T., Bienboire-Frosini C., Arroub S., Menuge F., Lafont-Lecuelle C., and Pageat P. Influence of the patient's expectation on equine emotional responses during equine-assistes therapy sessions. EVCBMAW, Eindhoven, 2019.

GENERAL INTRODUCTION

1. The human-dog relationship

Domestication of dogs

The dog (*canis familiaris*) is known as humanity's best friend and was the first animal to be domesticated by human beings. According to current knowledge and archaeological archives, the dog has been domesticated for at least 15,000 years (Jensen, 2007). Before the current peculiar relationship between human beings and dogs developed, a long process occurred, causing numerous scientists to ask the question of "Why and How domestication happened?". Genetic studies have identified the wolf as the ancestor of the dog. A large number of studies and evidence from the fields of biology, morphology, genetics, and behavioural studies support this hypothesis, to avoid the term conclusion (Larson et al., 2012; Lindblad-Toh et al., 2005). In this case, how could the human and the wolf, a wild species, develop a closer relationship? A probable hypothesis to explain this situation is that both parties benefitted from working together to locate food resources. Indeed, the wolf is an opportunist, and by remaining close to humans, he would be able to prey on game wounded by human hunters or consume humans' leftovers (Miklosi, 2007). The same reasoning applies to the human, who could have followed wolves' tracks towards bigger game more easily, thus resulting in their hunting-based partnership.

Cooperation and mutual benefit would then be the origin of this process of domestication (Stafford, 2006).

Subsequently, a process of selection occurred, which progressively endowed the wolf with a temperament and a morphology that is well-known today to be typical of dogs. Specific breeds emerged from this process to adapt to human needs, such as for protection or transport (Miklosi, 2007). Currently, the dog is more of a companion than a working animal, although humans have preserved and expanded the scope of dogs' work. Police dogs or assistance dogs are clear examples of human-animal partnership that highlight the aptitudes of dogs substantially, although these identities would not be possible without a strong and reciprocal bond developed patiently over the past 15,000 years (Stafford, 2006).

Attachment theory

It can be difficult to discuss the development of the human-dog relationship without evoking the notion of attachment. All of the cooperative work accomplished jointly by humans and dogs, which will be discussed later in this thesis, could not be effective without a unique and strong bond developed over the past 15,000 years. In the following section, "Benefits of having a pet dog for a child", the term attachment will be used frequently, and so it seems to be essential to introduce this concept here.

The development of the theory

The term attachment originated recently and was formulated by John Bowlby in the 1950s (the origins of attachment theory). Bowlby proposed that early relationships are decisive for emotional, social and cognitive development and can have repercussions in adulthood. According to Bowlby (1978), attachment aims at maintaining a degree of proximity between a child and his or her first attachment figure, usually his or her mother, to ensure the survival of the species. The attachment system is activated during stressful situations. The baby then adopts strategies that promote this proximity, such as crying, smiling, clinging, following and sucking. The responsiveness of the caregiver influences the baby's establishment of internal working models, and the development of the child coincides with the integration of these internal working models in his or her relationships with others.

Mary Salter Ainsworth completed attachment theory by introducing the notion of the secure base effect (Ainsworth, 1967). Ainsworth believed that a child must develop a secure dependence on a parent to become capable of adapting to nonfamiliar situations: an attachment figure serves as a secure base effect from which to explore the world. In fact, the world becomes easier to explore when one is aware that someone will be present if needed to serve as a refuge. The responsiveness of a caregiver is very important to individuals' self-concepts and self-perceptions. Such responsiveness shows that we can trust someone but also that we are worthy of support and affection. These notions are essential to the development of healthy relationships in adulthood.

In 1978, Mary Salter Ainsworth designed a behavioural test, the Strange Situation Test (SST), to activate and observe the system of attachment between a mother and her one-year-old child. The test has been described completely in many papers. The aim of the test is to observe the reaction of the child to a strange and stressful event—i.e., instances of separation and reunion with their first attachment figure—and to classify the attachment styles thus observed into three categories: secure, insecure-avoidant, and insecure-ambivalent (Ainsworth et al., 1979). A fourth category (insecure-disorganized) was added later by Main and Solomon (1986).

- **Secure:** This term refers to children who activate their attachment system during stressful situations and deactivate the system as soon as a response from the attachment figure is elicited. The child thus becomes capable of exploration once again. The period of the activation of the attachment system and that of exploration thus alternate. This attachment pattern occurs when a child is easily able to obtain attention and care when he or she needs them. Thus, distress at the time of separation is observed, but this distress quickly disappears when the parent returns.

- **Insecure-avoidant:** In this case, the expression of the attachment system is inhibited. Such children do not exhibit signs of distress when the mother leaves and do not seek comfort when she returns. Physical contact is not sought.

- **Anxious-ambivalent:** Conversely, in this case, the attachment system is overactive. As in the case of secure children, distress is observed at the time of separation, but in this case the distress is more intense. The child is difficult to console when the parent returns and can regain his or her composure only with difficulty.

- **Insecure-disorganized:** This category refers to children whose attachment strategy is inconsistent. The behaviours of such children are contradictory, alternating between avoidance and contact-seeking behaviours.

In parallel with the work of Bowlby and Ainsworth, other scientists have emphasized the importance of comfort resulting from the presence of an attachment figure in broader terms than merely as a source of food.

Konrad Lorenz, in his work on imprinting, discovered in the 1930s that birds exhibit signs of distress in the absence of their mothers and engage in more exploration when their mothers are present, while these birds were autonomous with respect to their search for food. In another context, Harlow's experiment on rhesus macaques highlighted the primordial character of comfort and security provided by the mother in the context of the psychological development and health of the young.

The theory of attachment, therefore, cannot be attributed solely to the human species but can also be applied to other animals via the links between ethology and psychology.

Based on ethological knowledge of various species, researchers have also been interested in interspecies links, particularly with respect to human-animal relationships. To investigate such phenomena, the Ainsworth strange situation test was conducted on various species, such as the chimpanzee, the cat, and naturally the dog.

Attachment bonds in dog-human relationship

The first study that demonstrated the attachment behaviour of dogs towards humans by employing an ethological method was conducted by Topal et al. (1998). These authors used a revised version of the strange situation test to adapt the analysis and interpretation associated with this test to the human-dog relationship. They showed that this test could activate the attachment behaviour of a dog towards his or her owner. More recently, several researchers have published papers concerning attachment in the context of dog-owner dyads using the strange situation test and have highlighted the existence of an expression of attachment that is similar to that the bond between a mother and her child (Palmer and Custance, 2008; Prato-Previde et al., 2003). In fact, the dogs expressed distress during separation and excitement upon reunion. In a secure attachment, the owner acts as a secure base effect, as in human, and facilitates exploration in his or her presence. However, the methods employed by

these authors involved observation of the behaviour of the dogs in a quantitative manner. In 2014, an adaptation of the original classification of the Ainsworth strange situation test to suit the context of dogs was proposed at a conference (Solomon et al., 2014) and published later as a paper (Solomon et al., 2019). These authors standardized a method of classifying the attachment of dogs towards their caregiver according to the four attachment patterns (i.e., secure, insecure-avoidant, insecure-ambivalent, insecure-disorganized). This classification procedure is currently used to test human-dog relationships (Schöberl et al., 2016; Thielke et al., 2017; Thielke and Udell, 2019; Wanser and Udell, 2018).

The special relationship between dogs and humans is not without consequences for the humans who live with dogs on a daily basis. In the subsequent part of this thesis, we discuss the benefits of the presence of a dog in our homes, particularly for children and teenagers, who represent the public to whom the subjects of this thesis are to be entrusted.

Benefits of having a pet dog for a child

After initially serving merely as a source of food and work for humans, animals have become an integral part of the family circle, as pets (Mueller et al., 2015). This phenomenon is more prevalent among families with children (Cendrier, 2016), as these children often need a companion (Kidd and Kidd, 1985). This new daily presence, to which individuals develop a strong attachment, is of interest to researchers, who

increasingly focus on the impacts of this interspecies relationship on people (McCune et al., 2014). Studies focusing on adults are as common as those that focus on children, if not more so. The impact of these interactions on young people is interesting, however, since the preadolescent and adolescent periods are key times in an individual's life due to the occurrence of many physical, psychological and moral changes during these periods (Coslin, 2017). This thesis focused on dogs who were intended to help children with visual impairments. This chapter therefore highlights the typical effects of an animal on young people during their development. Four main categories of contributions related to the presence of a pet have emerged in the literature, which pertain to emotional, social, and individual development as well as physical health.

Individual development

A decrease in self-esteem is a common phenomenon during adolescence, which is a period of reshuffling during which individuals engage in a complex process of self-assessment (Wallace et al., 1984). Van Houtte and Jarvis (1995) demonstrated that having a dog or cat can help young people in this regard. Children with one such an animal at home tend to have higher self-esteem. Once again, the influence of such an animal becomes more pronounced during adolescence. These results were confirmed by the research of Covers et al. (1985), which reported that young adolescent pet owners had higher self-esteem scores than nonowners.

The ability to engage in intentional self-regulatory behaviours is important to the establishment of positive development (Gestsdottir et al., 2009; Gestsdottir and Lerner, 2008). Self-regulation involves skills such as those required to optimize the resources needed to achieve an objective or compensate effectively when the goal is impossible to achieve by reorganizing one's objectives (Mueller, 2014a). Based on a previously conducted study (Mueller, 2013), these authors suggested that a pet can facilitate the acquisition of these skills. Fifteen-year-olds who are involved in a relationship with an animal are able to regulate themselves more effectively than are others. The animal provides the child with objectives, which must constantly be reassessed due to the unpredictable nature of the animal (Mueller, 2014a). The young person therefore faces regular challenges, which he or she must manage and for which he or she must compensate in order to maintain a good relationship with his or her pet.

Social development

During development, especially in adolescence, the relationship that an individual may develop with his or her dog has an impact on his or her cognitive and socioemotional development. This claim was made by Vidovic et al. (1999), who added that a prerequisite for developing and maintaining good relationships with others is the establishment of mutual attachment. It must be noted that attachment, the theory of which was developed by Bowlby (1978), is defined as the product of behaviours aimed at ensuring and maintaining the proximity of a specific person. It will now become

apparent that the presence of a familiar dog has consequences for the development of empathy, caring behaviours, and relationships with others.

The definition of empathy most commonly found in the literature is broadly characterized in terms of the ability to put oneself in the positions of others. The results of a study by Vidovic et al. (1999) suggested that children aged 10 to 15 who own dogs are more empathetic than are nonowners. Daly and Morton (2006) supported these findings by highlighting the impact of animals on this personality trait. Indeed, comparing children aged 8 to 14 years old who are either pet owners or not produces interesting results. Such children with a dog or cat at home are not more empathetic than children without pets. However, children who have both a dog and a cat at home do exhibit increased empathy. Daly and Morton (2006) also investigated whether children's preference for an animal species could also play a role in this context. Indeed, children who exhibit no preference between dogs and cats, that is, children who love both, are more empathetic than children who prefer one animal or the other. Finally, young people who exhibit a particular interest in horses have the highest empathy scores. In any case, this study noted that the stronger the child’s attachment to the animal is, the more empathetically the child is. However, care must be taken with respect to the way in which these results were collected. That is, the author relied solely on questionnaires. Even if these questionnaires have been verified and validated in the literature, it would be interesting to supplement these results with more objective measures of behaviour. Empathy is an important quality for understanding others, and Melson (2003) claimed that empathy is the foundation of the implementation of care behaviours.

Melson conducted several studies to investigate care behaviours. In one of the first such studies, he developed the idea that children cultivate their knowledge regarding these behaviours by learning to care for their pets (Melson and Fogel, 1989). Indeed, beginning at the age of 3, children are able to understand the appropriate care methods that a mother ought to employ when interacting with her young child. These results were also found for children who are not pet owners. According to these authors, a pet provides the child with an opportunity to demonstrate care and affection. The relationship established between the young person and his or her animal is therefore important insofar as this relationship can function as a path towards the development of an understanding of reciprocity and the establishment of care behaviours. These two skills, which are crucial for developing social skills, can subsequently be transferred to future relationships with humans (Mueller et al., 2015).

In addition to the positive consequences of a pet on empathy, Daly and Morton (2006) noted that the effects of such a pet are exhausted by those consequences. These authors showed that children with a strong attachment to their pet, who are therefore more empathetic, are also more likely to have a better attitude towards other pets. This finding was only significant for children with a dog, i.e., not for children with a cat. Some researchers have investigated whether this relationship can help the child develop more satisfying relationships with human beings. The results in question allow us to answer this question positively. In the study by Vidovic et al. (1999), the prosocial behaviours of children who do not own an animal decrease between the ages of 10 and 15, which does not occur in the case of children who do own an animal, for whom these behaviours remain stable. The presence of the animal therefore has a beneficial

effect in this context. This effect is more visible when the child's attachment to the animal is strong. The same study also highlighted the fact that children who are attached to their pets report their family climates to be more favourable than do those with less attachment or whose homes do not contain pets. Additionally, Mueller (2014b) found that the attachment bond that develops between the child and his or her animal is associated with greater social ties with the child's peers as well as with his or her family. More recently, Kerns et al. (2017) reported that children aged 9 to 11 who report more positive relationships with their dogs also report a more secure attachment to their parents and engage in less conflict with their best friends. According to Poresky and Hendrix (1990), pets are therefore a factor associated with the development of social skills and values by teaching children to be more empathetic and encouraging prosocial behaviour.

As early as 1993, Paul and Serpell noted the benefits of the presence of animals. According to these authors, involving children in a relationship with an animal at an early age can impact their attitudes towards others positively, leading to lasting and observable consequences extending into adulthood.

Emotional development

Emotions appear to be influenced positively by the presence of a pet. This conclusion is drawn by Kerns et al. (2017). In the presence of their pets (in this context, their dogs), children aged between 9 and 11 reported experiencing more positive

emotions when performing a social stress task than did children whose dogs were absent, especially during the phase before the test was taken. However, the negative effects induced by the test were not mitigated by the presence of the dog. It is possible that such children may have ignored the animal to focus on the task. Other recent research has shown that when the dog is receptive to the child's communicative gestures, the child tends to experience more positive emotions towards the dog (Hall et al., 2016). The fact that the presence of a canine companion can promote positive emotions suggests that a dog can help children through difficult times, such as when major changes affect their lives, which can be stressful. The type of interaction in question is thus decisive and influences children's emotional responses. According to the interesting work of Kerns et al. (2017), the more physical contact in which the child engages with the dog, the more positive emotions the child reports. The presence of the dog therefore provide supports and a social bond, thereby promoting positive emotions.

This induction of positive emotions may be the result of an increase in certain neurochemical substances that are associated with attachment or affiliation. Odendaal and Meintjes (2003) tested this claim on the assumption that a pet can help reduce anxiety by activating the sympathetic system and inducing a sense of security, comforting contact, and reduced loneliness and depression by enriching the human's environment and encouraging a varied lifestyle. Oxytocin, which as commonly referred to as the attachment hormone, induces positive emotions by promoting social interactions, reducing stress and improving health, for example (Beetz et al., 2012). As a result, an increase in this hormone may explain many of the positive effects induced

by pets. In adults, an increase in oxytocin levels has been observed when the individual caresses a dog (Odendaal, 2000; Odendaal and Meintjes, 2003). This increase was even more noteworthy when the person was in physical contact with his or her own dog than when caressing an unfamiliar animal (Curry et al., 2015; Odendaal and Meintjes, 2003). The quality of the relationship maintained in this way is therefore a key element associated with the secretion of this hormone (Beetz et al., 2012). Additionally, an increase in oxytocin in both the owner and the dog when the dyad shares a mutual gaze for long periods of time have been reported (Nagasawa et al., 2015). A study by Odendaal and Meintjes (2003) also showed an increase in endorphins, dopamine and phenylethylamine in these adults. According to these authors, a higher level of the secretion of these hormones suggests an association with pleasant interactions that can be induced by the animal, in this context, specifically a dog. To date, no study of this type concerning children has been published. Accordingly, there is a gap in the literature pertaining to this subject, which must be filled to verify the benefits of a pet on a child's neurohormonal system.

Physical health

Physical health is also improved by the presence of a pet. One study suggested that following a heart attack, patients with pets are less likely to experience a repeat seizure the following year than are patients without pets (Friedmann et al., 1980). In the context of children, dog owners suffer less ill health 6 months after adoption (Paul

and Serpell, 1996). Once again, this study demonstrated the importance of the child's attachment to his or her companion by highlighting the fact that the closer the child is to his or her dog, the more likely the child is to experience a decrease in disease symptoms from the first month. More specific research is consulted in this review to show that pets can have an impact on the development of allergies and asthma as well as on obesity and immune functions.

Three different studies have shown similar results regarding the influence of pets on the development of asthma and allergies. De Meer et al. (2004) first conducted a longitudinal study indicating that if a person owns a cat prior to the age of 18, his or her risk of suffering from asthma or allergies at 28 is reduced. According to the authors, exposure to microbes early in life protects against allergenic diseases by altering the immune response. Liccardi et al. (2005) found essentially the same results. Having a cat during the first months of one's life, according to the results of their study, has a protective effect against the development of allergies and asthma. Finally, Fujimura et al. (2014) showed that exposure to house dust associated with dogs during early childhood has a positive impact with respect to protecting against allergies in the respiratory tract.

Owning a dog is associated with increased physical activity (Timperio et al., 2008). Dog walking provides people with the motivation to engage in regular physical activity, with benefits for the health of the owner as well as for the health of the animal (Bartges et al., 2017). Timperio et al. (2008) therefore investigated the impact of an animal on the weight of children aged 5 to 6 years as well as on that of children aged 10 to 12 years. The results showed that for younger children, the dog-owning group

exhibited fewer cases of overweight children than the other group. According to these authors, this distinction did not result from the fact that the children walked the dog but rather from the games that they played with the animal. Moreover, these results were not found for older children, which was explained by Timperio et al. (2008) by reference to a decrease in interactions with the animal as the children aged. The presence of a pet dog therefore provides protection against obesity for young children. However, a longitudinal study would be interesting in this context since it remains unknown how long children aged 10 to 12 years have owned a dog.

Owning a pet provides physiological benefits, causes changes in the neurovegetative and hormonal systems, and promotes physical health. These changes are also associated with psycho-behavioural changes, such as the promotion of good individual development and improved social skills. However, dogs are not merely precious as companions. In fact, they put their abilities to use by helping humans with their work, and together, in the form of a partnership, beautiful results can emerge.

The dog, a valuable ally

In this section, some examples of different works performed by dogs in close collaboration with humans are presented.

Security, search, and rescue

Since approximately the 1800s, dogs have been used to search for and rescue people (Jones et al., 2004). The contribution of dogs to human life thus increased and facilitated the task of saving people's lives due to the dogs' sense of smell, which allow them, for instance, to locate victims who have been swept away by an avalanche or buried in rubble. During the first world war, dogs were used by the army to find soldiers buried in the trenches; however, the first training structures for such dogs emerged later, i.e., in 1954 for the United States, Switzerland and Germany and in 1982 for France, whose first training centre was established in Briançon. At present, trained dogs work worldwide to address disasters such as earthquakes or cyclones.

Additionally, the dog has proven to be a valuable resource for the police with respect to maintaining security. In 1943, dogs were first incorporated into the gendarmerie "police dogs". The main mission of these dogs is to accompany the police officers during their interventions on public highways to dissuade, attack, defend or secure. Thus, such dogs are present during searches, arrests, and public demonstrations. Some dogs are also specialized to assist in the search for drugs, weapons, explosives, or counterfeit money. These dogs are selected for their capabilities. Such animals are indeed capable of recognizing more than 10 different odours. The olfactory capacities of these dogs are even more impressive due to the fact that the more odours the dog recognizes, the easier it becomes for the dog to learn new ones (Williams & Johnston, 2002). The dog is therefore a formidable ally, applying its skills in cases where those of humans are limited or nonexistent.

Biodetection

The olfactory capacities of dogs are not exhausted by this task, and their field of expertise also pertains to the field of health. Indeed, dogs are used to identify the presence of disease by detecting the volatile organic compounds that such diseases emanate (Edwards et al., 2017).

These animals are called biodetection dogs (Salgirli Demirbaş et al., 2021). In a case report published in 1989 by Williams and Pembroke, a woman's dog constantly sniffed at a lesion on her skin. A few months later, the behaviour of the dog encouraged the woman to seek medical advice, and it was revealed that the lesion was a malignant melanoma. The dog was able to detect this cancer at a curable stage, thus facilitating the treatment and recovery of the patient. This event raised new possibilities for cancer research with respect to early diagnosis. This approach is an efficient and interesting complementary method because dogs can be trained rapidly and in a cost-effective manner (Pirrone and Albertini, 2017).

At present, dogs are trained to detect several diseases, such as various types of cancer (skin, lung, breast, prostate, ovarian, etc.) or, more recently, COVID-19. Additionally, such dogs can assist people suffering from diabetes or epilepsy during a crisis. In fact, these dogs can react to a hypoglycaemic or future epileptic attack and alert their owner prior to the onset of symptoms.

Unfortunately, the use of biodetection dogs remains marginal, which represents a loss to the medical world and to our ability to diagnose cancer.

Assistance

Another category of working dogs is assistance dogs. This category is a general term that refer to dogs tasked with the mission of assisting humans with disabilities (Bremhorst et al., 2018; Sachs-ericsson et al., 2002). Like the dogs who accompany people suffering from diabetes or epilepsy, assistance dogs live with the person who has the disability. Different types of assistance dogs exist, as follows:

- Service dogs for people who suffer from mobility impairments. These dogs are trained to respond to approximately 50 orders to make everyday life easier for such a person. For example, they can open and close doors, drawers, pick up or fetch objects, and bark on command to warn others in case of trouble.
- Hearing dogs. These dogs assist humans who suffer from hearing impairments. During their training programme, they learn approximately thirty orders in sign language. They are trained to make physical contact with the “owner” when they hear a specific sound and to direct the person to the source of the noise. They are able to indicate the ringing of the alarm clock, the telephone, the doorbell, the whistle of a pressure cooker, a child crying, a car approaching from behind, a person calling out to the person in the street, the arrival of a bicycle or tram, etc.
- Autism assistance dogs. Children, teenagers and adults with autism spectrum disorders can apply to obtain an assistance dog especially trained for such cases. These dogs relieve the person during stressful situations, for

example, in public places, and can guide him or her towards the exit. They are able to prevent imminent crises, thus allowing the person to anticipate and manage such a situation in a better way. They can prevent behaviours that could be dangerous (slapping, head-butting, etc.). These dogs act as sources of strong emotional support, i.e., as anchors, by providing security and company. Once again, the assistance dog helps destigmatize the disorder and promotes social interaction, thereby combatting isolation and exclusion.

Guide

Guide dogs are a subcategory of assistance dogs, but since they are the subject of this thesis, I have devoted a separate section to discussing them.

The missions of guide dogs are to improve the autonomy, safety and independence of people with visual impairment. Journeys become more fluid thanks to such dogs, who are superior to the use of a white cane. In fact, these dogs are trained to avoid obstacles and to assist with the tasks of ascending or descending stairs. They obey orders regarding specific directions and can locate crosswalks or certain pieces of infrastructure, such as a mailbox or a bus stop. Beyond these practical aspects, guide dogs also improve the relationships of people with disabilities and facilitate their social relations (Gaunet and Milliet, 2010). This task is an important aspect of such guide dogs because they can allow people with a visual disability to travel outside with more confidence and meet more people. Typically, guide dogs are

entrusted to visually impaired or blind adults, but some schools have also decided to help teenagers in their struggle to live with their disabilities.

In the following section, we describe the history, development, and management of guide dogs.

2. Guide dogs for blind people

Blind children: Left behind?

A spot of history: The development of the concept of a guide dog

The first record of a dog accompanying a blind person dates to the first century A.D. A fresco was discovered in the ruins of the Roman city of Herculaneum (Fig. 1).

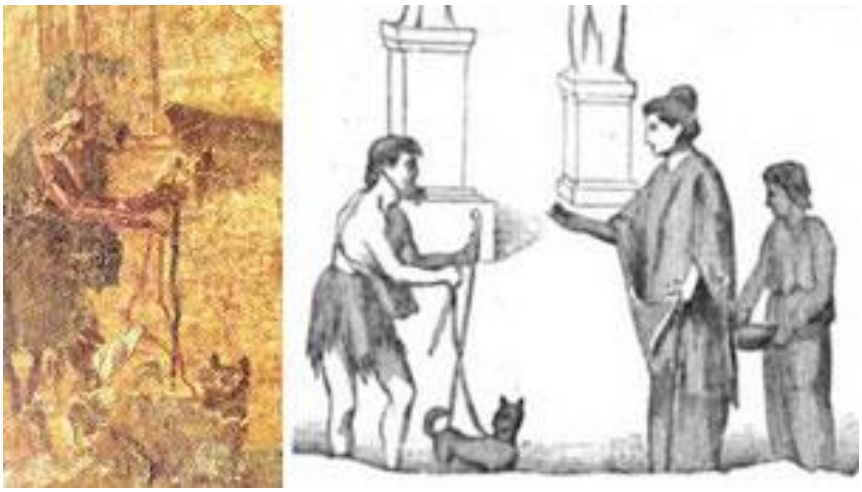

Fig. 1. First known fresco of a guide dog: a beggar with a cane holds a dog on a leash while a woman and her servant give him a coin. Source: https://www.canidea.fr/un-peu-d-histoire/

In 1750, the first experiments with the education of guide dogs for the visually impaired were conducted at the Quinze-Vingt Hospital in Paris. However, the guide dog movement was inaugurated much later, during the First World War, to help soldiers who had lost their vision. The Germans were the initiators of this movement, which was joined by the United States (the Seeing Eye), Italy, Switzerland, and Great Britain. In 1951, Paul Corteville introduced the movement to France and trained a dog, Dickie, for a visually impaired friend, thus creating the Club des Chiens Guides d'Aveugles. Since that time, related schools have continued to develop, both in France and abroad. However, guide dogs were intended for the use of adults only, and due to their supposed immaturity and the common belief that guide dogs would be useless for them, children were not permitted to have a guide dog until 1990 (Gravrok et al., 2018).

The Frederic Gaillanne Foundation

The extension of guide dogs to children started in Quebec and was particularly the responsibility of one man, Eric St-Pierre. He founded Canada's first guide dog school in 1981, and 10 years later, he created the world's first guide dog programme to focus on minors below the age of 15. Whereas existing schools assumed that children were not sufficiently mature to handle a guide dog, St-Pierre conducted a

study at the Mira Foundation that demonstrated the benefits of the dog for young people and revealed how quickly they were able to move around with the aid of the dog. With the support of the parents of the young beneficiaries, the programme was developed, and it became possible for young people to obtain a guide dog.

For many years, Mira remained the only guide dog school to serve blind and visually impaired children. France was no exception to this rule, and no school in the country trained guide dogs for children. It was only in 2008 that Frédéric Gaillanne laid the first stone of the foundation of the same name after noticing the injustice caused by this lack for children and teenagers seeking autonomy and independence. Since that time, the Frederic Gaillanne Foundation (FGF) has trained guide dogs for young people aged 12 to 18 years old. The foundation also participates in a unique research programme that evaluates the impact of the presence of an assistance dog on the development of blind babies.

« Un chien guide auprès de soi c'est de la lumière sous les pas. En offrant un chien guide à un enfant aux yeux éteints, nous lui apportons peut-être cette lumière qu'il n'a plus. Un chien, un enfant, une image de bonheur où le cœur de ces deux êtres bat à l'unisson au rythme d'une chanson. La chanson de la vie. »

Frédéric Gaillanne

Benefits of having a guide dog for young people

Visually impaired adults describe the benefits of guide dog ownership clearly (Craigon et al., 2017; Whitmarsh, 2005). First, these dogs can improve the mobility and independence of most of these individuals notably. However, the advantages of such a dog do not stop there. In fact, guide dogs provide social and psychological benefits similar to those provided by companion animals. Additionally, guide dogs decrease social isolation by facilitating social interactions.

Nevertheless, these benefits have not been recognized clearly in the context of visually impaired children, which is why it remains rare for guide dog organizations to train guide dogs for young people (Gravrok et al., 2018). However, we can now affirm by reference to the testimonies of teenagers and their parents that guide dogs increase the quality of life of the young at different points in time, just as they do for visually impaired adults. The mobility of these individuals becomes more efficient, and they can walk more quickly and with more fluid movements. Their feeling of safety also increases due to these dogs, and people are actually safer when accompanied by such dogs. In fact, dogs can notice and avoid obstacles in cases in which a white cane provides little help. This assistance encourages adolescents to become more independent by journeying outside and doing things without the presence of a parent. Additionally, these children feel more confident when moving with a dog, thus increasing their self-confidence in general, which is very important during this period of life. Finally, guide dogs provide social benefits, such as companionship and social

interaction. Peers talk more easily with the adolescent when he or she has a guide dog than a white cane, thus shifting the disability into the background.

Delivery to visually impaired children

FGF offers free guide dogs to minors between the ages of 12 and 18. Visually impaired or blind teenagers who desire such an animal can apply to the school to receive one. To be eligible to receive a guide dog, certain conditions must be satisfied:

- The parents must be involved in assisting the young person with the task of caring for the dog.
- The young person must have knowledge of everyday routes and the ability to travel them on their own. If necessary, a locomotion instructor can accompany the young people to the site to help them visualize their routes. It is necessary to bear in mind that the guide dog does not have the function a GPS and must thus be guided. This process is therefore a team effort that requires the active participation of both partners.

Concerning the process of obtaining a guide dog at FGF, 3 steps must be followed:

1. Two-day discovery courses: These courses allow the child to have a first encounter with the dog and to experience moving with the dog's

assistance. The staff also raise the child's awareness of animal welfare and discuss what that notion means in everyday life.

2. One-week preclass: It is necessary for the child to exercise autonomy in his or her movements prior to obtaining a guide dog, i.e., he or she must be able to orient himself or herself and know his or her frequent routes. Therefore, locomotion instructions are presented to help the child acquire these skills if he or she does not already possess them. This course is mainly dedicated to this task. During this week, a trained dog is assigned to each child to familiarise him or her with the constant presence of the dog and the daily management the animal requires.

N.B. During the first two courses, the dogs employed have been trained as guide dogs but are not given to the children. These dogs live with their foster families and visit the foundation occasionally to facilitate these courses by exhibiting their knowledge.

3. Three-week handover class: During this three-week period, the children are in contact with all dogs that have recently completed their training and are ready to accompany a young person. The aim of this course is for the children to learn how to move safely and independently and to manage the dog in any situation. The ability of these adolescents to meet the physiological and emotional needs of these dogs is also an essential requirement that the trainers emphasize.

At the conclusion of these three courses, if the team judges the child to be suitable to receive a guide dog, he or she will leave with the dog that has been assigned to him or her by the dog trainers. During the class in which this transfer takes place, affinities emerge between children and dogs. There are always more dogs available than there are children who have the opportunity to form the dyad in accordance with these affinities due to technical reasons (the size of the dog, walking speeds, etc.). However, even if a child may occasionally not receives the dog that he or she would have liked to receive, disappointment soon gives way to enthusiasm once the bond between the two is established. The professionalism and experience of the trainers often leads them to make the correct choice regarding appropriate duos.

The dog is given to the children free of charge, and the training courses are paid for by the foundation (including accommodation at the school, meals, and teaching costs) to guarantee access to this aid for all people regardless of income. However, it is necessary to ensure that the beneficiary's family can afford the purchase of kibble and routine veterinary care for the animal, such as deworming and vaccination reminders.

A follow-up visit is conducted at the beneficiaries' homes to ensure that the animal is properly managed and that the children can make their journeys independently. A locomotion instructor is also present to help the children make these journeys safely. Control visits are subsequently scheduled to verify that everything is going well and to readjust the dog if any concerns arise with respect to the dog's responses to commands.

Strong bonds are formed during the years in which guide dogs provide loyal service, and when the time of retirement comes (which occurs when the dog is between the ages of 8 and 10 years), most dogs are adopted by a member of the beneficiary's family. It would be disruptive for the dog to remain with its "master" while the latter was being guided by another dog.

During the developmental period that constitutes teenagerhood, guide dogs can provide additional tools to combat one's disability. However, for the dyad to function properly, it is important for the dog's temperament and physique to match what is expected of them in both morphological and emotional terms. For this reason, certain breeds are preferred for this work.

The most commonly used breeds

Human selection of dogs has led to certain breeds specialize in specific areas. Current knowledge concerning behavioural genetics allows us to assert that behavioural traits have a genetic basis (Wayne and Ostrander, 2007). To understand the domestication of dogs more clearly, a Russian researcher, Belyaev, conducted an experiment beginning in the 1960s to illustrate this claim (Trut et al., 2004). By selecting the most docile foxes, their behaviour and appearance were modified after only a few generations. This physical transformation allows us to advance our understanding of this situation by establishing links among genetics, phenotypes, and behaviours.

The selection procedure is the same for working dogs. The goal of this process is to select dogs that have the attributes necessary to perform a specific task (Landsberg et al., 2013). Thus, we associate the border collie with herding, the beagle with hunting, the husky with transportation, and the German shepherd with search and rescue. The assistance dog, in particular the guide dog, is no exception to this rule, and different breeds have been selected to meet the needs of this work. The most common such breeds are the Labrador, the golden retriever, the German shepherd and the golden retriever/Labrador cross. The last of these breeds tends to be the most suitable for graduation as a guide dog (Ennik et al., 2006; Jones, 2016a).

The Mira Canada foundation decided to develop its own cross breed and to name this breed after its founder: St-Pierre. The St-Pierre is at least the second generation of a cross between a Bernese mountain dog and a Labrador (the first generation is called a Labernois). This cross allows the dog to thrive in the harsh conditions of a Canadian winter, but its advantages are not exhausted by that ability. In fact, the Labrador was chosen for its liveliness, responsiveness to the environment, confidence and friendliness, while the Bernese mountain dog was selected for its tendency to seek human contact, alertness, protective instincts, intelligence, and ability to resolve problems. This mix results in a dog that exhibits good capacities with respect to working with people with disabilities. In addition, the temperament and physicals aspect of this dog make this breed suitable for working with children. The individuals chosen to participate in the breeding programme are selected due to their good health, physiology (hips dysplasia) and temperament and with an eye to maintaining genetic

diversity (Ennik et al., 2006). As a result of their close collaboration with Mira Canada, FGF trains this breed of dog to guide their young beneficiaries.



Fig. 2. Picture of two St-Pierre dogs during puppyhood and adulthood.

Dogs' developmental context

In this section, I mainly present the developmental process of dogs at FGF. This form of management is similar to practices used worldwide to produce prospective guide dogs.

From birth to weaning

After selecting the parents, the puppies are born and develop in an appropriate environment, either at a nursery associated with the corresponding school or with a breeder with whom the school collaborates, as is the case for FGF. The prospective guide dogs remain there with their mothers for approximately 9 weeks. During this period, a health check is conducted by a veterinarian, and tasks related to the dogs' vaccination and identification are accomplished.

The puppy raiser

After being weaned, puppies leave the litter and are homed with a foster family. A foster family voluntarily keeps such a dog full time until the beginning of their training period, which is usually 14 to 18 months later (Ennik et al., 2006). Foster families, which are also known as puppy raisers, play a key role in the development of these dogs. In fact, these families care for the dog during a sensitive period. This period involves the development of individuals based on their experiences as a result of the levels of plasticity in their central nervous systems (Freedman et al., 1961; Scott and Fuller, 1965). Opinions vary concerning the exact age boundaries of this sensitive period, but there is consensus regarding the fact that the social development period of puppies ranges between two and thirteen weeks (Freedman et al., 1961; Jokinen et al., 2017; Pluijmakers et al., 2010; Scott and Fuller, 1965). To illustrate this fact, one study demonstrated that good socialization between eight and twelve weeks of age reduces the development of fear in adult dogs (Tiira and Lohi, 2015). Foster families bear the responsibility for socializing puppies and familiarizing them with all types of

external stimuli, both social and environmental. Indeed, during this critical period, these dogs are able to integrate every new form of stimulation and become habituated to them. These families must take the dog everywhere they go, to as many different places as possible, to allow the dog to become used to all kinds of stimuli. The aim of this process is to produce a dog that is comfortable with noises, crowds, public transportation, etc. Later in this thesis, the fact that puppy raisers are authorized by law to take the dogs to every public area will be discussed. To facilitate this process, foster families must live near the guide dog school and must exhibit high levels of availability. In fact, these puppy raisers are usually asked not to leave the dog alone for more than 4 hours per day and to participate in puppy classes with a dog trainer from the guide dog school (2 times per month in the case of FGF). These classes enable the dog trainers to witness the progression of the puppies.

Serving as a puppy raiser is a voluntary task. However, all costs pertaining to the dog are fully covered by the schools (including veterinary care, kibble, leashes, harnesses, collars, bowls, etc.).

Selection of dogs

When the dogs are exactly one year old, FGF administers an emotional reactivity test to evaluate their capacity to react (or to refrain from reacting) to external stimulation, such as sudden noises, the opening of an umbrella, joggers, other dogs, or children. This test allows the foundation to observe the behaviour of the dogs and to determine whether each dog will remain with the programme or whether a dog's

behaviour is inappropriate for this context. In the first case, the dogs enter the training programme when they are approximately 18 months old. The other dogs are rehomed, i.e., they become pet dogs and can be adopted by the foster family, who have first choice, or by someone else if the foster family does not desire to keep the dog.

The selection of breeders (male or female) is also performed during this period. At one year of age, the dogs undergo medical examinations at an affiliated veterinary clinic to conduct a series of tests (hip X-rays, shoulder/elbow scans, eye checks, histiocyte sarcoma indices). These physical tests, alongside the behavioural tests, allow the team to determine which dogs will be kept for breeding. Dogs who are not selected as breeders are neutered.

Training at the guide dog school

Prospective guide dogs participate in a training programme with a dog trainer for 6 to 8 months. The dogs' routines may vary depending on the specific guide dog organization in question. In some cases, dogs remain at the school throughout the entire training period (Chur-Hansen et al., 2015; Kobayashi et al., 2012), while in other cases, dogs return to their foster families each evening or on weekends (Dalibard, 2009). In the case of FGF, dogs return to their foster families during weekends.

Concerning the learning programme, FGF operates in a manner similar to Mira Canada, i.e., on a system that involves progressive learning and gradual exposure to stimuli. The training is thus divided into 4 different blocks. FGF describes these blocks as follows:

- **Block 1 “Pen”**

  Duration: 4 weeks (20 actual working days).

  The indoor pen is the dog's first "working" area.

- **Block 2 “Kennel interior”**

  Duration: 4 to 6 weeks (20 to 30 actual working days).

  Learning in the pen is expanded to encompass the large indoor kennel corridor. Forward movement is gradually introduced, and work with obstacles is taught.

- **Block 3 “Kennel exterior”**

  Duration: 4 weeks (20 real days).

  This block first includes movements outside the kennel (through the contours and course of the site), which allows the trainers to teach the dogs how to navigate curves and an initial approach to crossing the street; the recall of the dogs in the free group is also trained. FGF has constructed a small town known as the "senses itinerary" around the kennel to replicate a safe urban environment (Fig. 3).

- **Block 4 “City”**

  Duration: 8 weeks (40 real days).

  A shopping centre is introduced to simulate working in public and working with elevators, escalators, and a variety of stairs. The dogs are subsequently taken on predefined urban routes. Real-life work begins during this block.

This block allows for multiple routes through a dense city, including metro and public transport. The dog gains experience as it practices, and the instructors complete the courses while wearing blindfolds during this period.



Fig. 3: Senses itinerary of the Frederic Gaillanne Foundation.

At the conclusion of the training programme, all dogs take a guiding ability test. The dogs are tested on an obstacle course and are required to guide the dog trainer, who wears a blindfold. If the dogs succeed, they are deemed suitable to serve as guide dogs and to be chosen to assist a child.

Success and failure factors

The work of a guide dog is very difficult, and it requires a dog who is both physically and behaviourally efficient. These dogs are asked to act against their natural instincts, such as their desire to sense the elements of their environment or to go out to meet other dogs and are instead required to remain focused and listen to their handler. For this reason, not all dogs have the right temperament, and some dogs are therefore excluded from the programme by the dog trainers. The demands of this work result in a high failure rate of approximately 40% (Mengoli et al., 2017), mostly due to behavioural reasons.

Fear-related behaviour, which compromises the dogs' performance and jeopardizes the safety of the handler, is a major reason for the failure of guide dog candidates (Rooney et al., 2016). Distractibility is also a common cause of failure according to Arata et al. (2010). In the evaluations by the trainers, they consider the animal's level of interest, its excitability, and its reactions to sudden movements. The higher a dog's score, the lower the dog's chances of success. Fear/timidity and aggression can also be causes of exclusion (Goddard and Beilharz, 1982).

On the other hand, several studies have been conducted to investigate and predict the factors that cause dogs to pass the guiding ability test (Arata et al., 2010; Batt et al., 2008; Caron-Lormier et al., 2016; Tomkins et al., 2011). Arata et al. (2010) highlighted a behavioural trait that significantly correlates with success on this test, i.e., docility. This trait was measured by considering learning ability, adaptability, stability, and maturity. Sensitivity (auditory, body and moral) is also a predictor of success according to the same authors, as is passivity in the presence of another dog or a noise (Batt et al., 2008). Tomkins et al. (2011) went further and reported more success

among dogs who were given a long rest period during the evening, which can result in more effective learning.

Even if behavioural reasons are the primary causes of failure, physiological or health problems also reduce the number of successful dogs. In fact, some health conditions require the release of potential guide dogs from the program, including hip and elbow dysplasia, epilepsy, atopic dermatitis, and multifocal retinal dysplasia (Jones, 2016b).

What place do guide dogs have in our societies?

Economic consequences of failure

The breeding and development of potential guide dogs cost a great deal of money for the organization that raises them. In fact, each dog costs approximately 25,000 euros (chiensguides.org). This price includes veterinary care, food, training, partnership training, and ongoing partnership training (Table 1).

Table 1. Distribution of the cost of a guide dog.

Breeding; puppy walking	Puppy between the ages of 0 to 12 months: veterinary care, food, puppy classes	3 500 €
Training programme	12 to 18 months: training, veterinary care, food	15 000 €
Locomotion instructions Partnership training	Locomotion instructor, accommodation, and training of the beneficiary throughout the various classes	3 000 €
Ongoing partnership support	Regular meetings at home during the time in which the dyad works together	3 500 €
Total cost of a guide dog		25 000 €

Source: https://www.chiensguides.org/fr/cout-du-chien-guide-50.html

Most of the costs required for the production of potential guide dogs pertain to the training programme. To reduce these economic losses, possible solutions include ways of decreasing the number of failures or the earlier detection, possibly even prior to the beginning of the training programme, of dogs who are not acceptable as guide

dogs (Tomkins et al., 2011). As discussed in the previous section, researchers are focused on this issue. Most such studies have emphasized the characteristics of the perfect guide dog. However, tools to improve the number of successes by focusing on emotional balance in dogs are lacking.

How are guide dogs funded?

The institutions that raise working dogs tend to be associations or foundations. These institutions do not receive direct aid from the state to finance their operations. Their funding is mainly based on donations from companies or private individuals, and they frequently organize events to solicit donations. The downgrading of each dog entails important financial losses, while money can be difficult to obtain.

Guide dog organizations and researchers have the same objective, i.e., to allow all visually impaired people who wish to do so to benefit from the help of a guide dog. However, is the world fit for the inclusion of people with disabilities, especially when an assistance dog is involved?

Explanation of the law



Each country has its own regulations concerning the ability of assistance dogs to access public places. In France, the law n°2005-102 from February 11, 2005 pertaining to equal rights and opportunities for as well as the participation and citizenship of disabled people, stipulates the following principles:

- Authorization and free access for assistance/guide dogs and their owners. Access to transportation, to places open to the public, and to locations that provide a professional, training-focused or educational activity is provided for guide dogs accompanying people who have a "mobility inclusion" card that includes the terms "disability" and "priority" mentioned in article L. 241-3 of the code of social action and their families or the person in charge of their education throughout their entire training period.
- Muzzle exemption for assistance/guide dogs.
- Fines for the impairment of the access of assistance/guide dogs and their owners. The amount of such a fine corresponds to that of a minor offense of the 3rd class, i.e., 68 euros (45 euros if paid in advance, 180 euros if paid late).

The objective of this law is to combat social exclusion. Other countries that cater to guide dogs and assistance dogs have similar regulations. However, despite the existence of such laws, people with an assistance dog are nevertheless refused access to public places, such as supermarkets or cabs. Ignorance of the law or categorical refusal to accept such a dog, for whatever reason, entails that the beneficiaries of such dogs must continue to fight every day to assert their rights. Awareness campaigns exist

to combat these instances of discrimination, such as the following advertisement from the Mira Foundation:

Et si on interdisait les fauteuils roulants ? - YouTube

Guide dogs have become indispensable to people whose autonomy, independence and sense of security have increased considerably due to the efforts of their companion. The help of such dogs demonstrates its value on a daily basis, which is why it is essential to guarantee the welfare of these animals to repay them for all their assistance.

3. The welfare of the guide dog

Definitions of animal welfare

To define the concept of animal welfare, it is essential to employ a multidisciplinary approach, including physiological, ethological, and psychological dimensions (Carenzi and Verga, 2009). A first definition of the notion developed in 1979 by the government of the UK (the Farm Animal Welfare Council) focused on respect for *The 5 Freedoms*:

- Freedom from hunger or thirst via ready access to fresh water and a diet that can sustain full health and vigour;

- Freedom from discomfort by the provision of an appropriate environment including shelter and a comfortable resting area;
- Freedom from pain, injury or disease due to prevention or rapid diagnosis and treatment;
- Freedom to express normal behaviour by providing sufficient space, proper facilities and company of the animal's own kind;
- Freedom from fear and distress by ensuring conditions and treatment that can prevent mental suffering.

A limitation of this definition is that it measures welfare in terms of the absence of discomfort or negative experiences. Broom (1986) noted that the welfare of an individual is its state with regard to its attempts to cope with its environment, thus including a mental element in the definition of welfare.

The French National Agency for Food, Environmental and Occupational Health Safety (Anses) proposed a more in-depth definition of animal welfare in 2018 as follows:

“Animal welfare is the positive mental and physical state associated with the satisfaction of its physiological and behavioural needs and expectations. This state varies according to the animal's perception of the situation.”

According to these definitions, the expression of positive emotions is expected to ensure the animal’s welfare (Mormède et al., 2018). To assess animal welfare,

research has mainly focused on the identification of negative indicators of welfare, such as stress, or abnormal behaviour, such as stereotypies. At present, the search for positive indicators requires consideration of notions that are included in the new definition of animal welfare and to take into account the concepts of positive mental state and the satisfaction of expectations alongside the notion of pleasure (Broom, 2000). Performance and productivity are also used as positive indicators of welfare, particularly in farm animals (Grimard et al., 2019; Veissier et al., 2007).

In the following section, some of these indicators (both positive and negative) are presented.

Physiological indicators of dogs' welfare

Neurohormones, neurotransmitters and neuropeptides

The hypothalamic–pituitary–adrenal (HPA) axis plays an important role in physiological responses to stress. Cortisol is the most common glucocorticoid hormone associated with emotional and physical stress and is released when the HPA axis is activated (Covalesky et al., 1992). The HPA axis can be activated by a challenging situation, thus leading to an acute response to stress (Appleby et al., 2018), and this axis is frequently used in the literature to assess stress in dogs (Beerda et al., 1997; Bennett and Hayssen, 2010; Bergamasco et al., 2010; Coppola et al., 2006; Wojtas et al., 2020). Cortisol can be measured in plasma, saliva, faeces, urine, and hair (Cobb

et al., 2016). Studies have demonstrated that other factors can influence HPA activity, such as age and sex (Sapolsky et al., 2000; Turner et al., 2012). Additionally, interindividual variability exists, indicating that caution should be taken when interpreting the concentration of cortisol (Cobb et al., 2016). For these reasons, it is more useful to evaluate changes from the baseline than merely to compare the rates for one animal with normal rates for the species (Appleby et al., 2018).

Stress can also be evaluated via assessment of the neutrophil/lymphocyte (N/L) ratio. Beerda et al. (1999) demonstrated that an abnormal increase in neutrophils is associated with stress in dogs. Furthermore, exposure to a long period of stress has the effect of reducing the proliferative response of lymphocytes (Beerda et al., 1997; Swan and Hickman, 2014; Widowski et al., 1989). Accordingly, the N/L ratio can be used as a potential indicator of chronic stress (Swan and Hickman, 2014).

In addition to the well-known role it plays in the lactation process, prolactin has been linked to emotional disorders and anxiety-related behaviours in several species, especially mammals (Gutiérrez et al., 2019; Kataria and Kataria, 2011; Landgraf et al., 1999; Yayou et al., 2010). This hormone can therefore be used as an indicator of welfare (Mason and Mendl, 1993).

Neurotransmitters such as serotonin are also associated with emotional functions, behaviours, and moods. It has been demonstrated that a low concentration of serotonin in dogs and other species is linked to impulsive behaviours, anxiety, aggressiveness, and depression (Miyata et al., 2007; Peremans et al., 2005; Vermeire

et al., 2011; Wolff and Leander, 2002). A study by Alberghina et al. (2019) encouraged the use of these neurotransmitters as a new marker to assess the welfare of dogs.

Oxytocin is commonly known as the “hormone of love”. In fact, this hormone plays an important role in social, maternal, and sexual behaviours (Gimpl and Fahrenholz, 2001) and in attachment (Insel and Young, 2001). A review by Chen and Sato (2017) highlighted the role of oxytocin in positive social behaviour, positive emotion, and stress regulation. Furthermore, it has been shown that intranasal administration of oxytocin increases social play (Romero et al., 2015) and promotes positive expectations according to a cognitive bias paradigm in dogs (Kis et al., 2015). This information has led some authors to suggest the use of oxytocin as an indicator of positive welfare (Broom and Zanella, 2004; Rault, 2016). However, the oxytocinergic system is complex, and an appropriate methodological design must be developed before it can be used as a positive indicator of animal welfare (Rault et al., 2017).

Heart rate variability

Heart rate and heart rate variability (HRV) are considered to be indicators that can be used to evaluate the autonomic regulation of cardiac activity to assess stress and emotional states in animals (von Borell et al., 2007). In particular, the use of a polar to measure HRV has been well recognized in the case of horses, and studies have demonstrated a link between HRV and equine welfare (Baragli et al., 2014; Vitale et al., 2013; von Rosenberg et al., 2017). In the context of dogs, HRV has also been used as an indicator in several studies (Bowman et al., 2017; Katayama et al., 2016;

Köster et al., 2018; Kuhne et al., 2014; McGowan et al., 2018). However, it has been demonstrated that the validity of this indicator is higher for dogs in a static position than for moving dogs (Jonckheer-Sheehy et al., 2012; Virtanen et al., 2018). Therefore, some methodological issues must be resolved prior to the interpretation of HRV in dogs.

Behavioural indicators of dogs' welfare

Animal welfare is also commonly assessed via behavioural parameters. In the context of dogs, many stress-related behaviours have been noted in the literature, such as panting (Beerda et al., 1998), autogrooming (Palestrini et al., 2017), turning head (Schilder and Van Der Borg, 2004), low or high levels of activity (Beerda et al., 1997), yawning (Rooney et al., 2007), lip licking (Beerda et al., 1997), whining (Rooney et al., 2007), hypersalivation (Dreschel and Granger, 2005), and aggressiveness (Beerda et al., 1999). Stereotypies are also indicators of poor welfare. Indeed, these behaviours were defined by (Broom, 1983) as repetitive behaviours, which are invariable and without apparent function and which emerge in aversive environments for the animal and frequently have negative effects on the animal's health and productivity. The following are examples of stereotypies in dogs: pacing, spinning, bouncing off the walls, circling, repeatedly walking the perimeter of their kennels, biting at their enclosures, excessive licking of their environments, self-mutilating behaviours and excessive autogrooming (Polgár et al., 2019). Stereotypies are particularly common among captive animals (Mason and Latham, 2004).

As long as we cannot reduce animal welfare to merely an absence of suffering, positive welfare indicators remain important in assessing animal welfare. Behaviours such as playing (Sommerville et al., 2017) and sleeping (Owczarczak-Garstecka and Burman, 2016) have been found to be indicators of positive welfare. Another method that can be used to evaluate welfare and affective states in dogs is the cognitive bias paradigm. This paradigm is an interesting way of indirectly assessing the emotional state of an individual to reveal a positive or negative valence. It was first studied in the context of humans (Mogg and Bradley, 1998), and it is based on the fact that affective states influence cognitive processes, such as judgement, memory and attention (Burman, 2014; Paul et al., 2005). This paradigm stipulates that people in a negative affective state are more attentive to aversive stimuli, recall more bad memories and judge ambiguous stimuli more negatively than people in a positive affective state (Burman, 2014). Experiences with animals encourage the use of this method to assess their welfare (Doyle et al., 2010; Harding et al., 2004; Mendl et al., 2010).

Animal welfare has received increasing amounts of interest since the 1990s and has become an important issue for our society. Therefore, the increasing use of working dogs poses the question of the welfare of these animals. The following section is dedicated to the welfare of guide dogs from a legislative and ethical perspective.

The welfare of guide dogs

Guide dog school regulations

It is essential for laws to exist to supervise guide dog organizations and working dogs to ensure the welfare of these animals. However, such laws differ across countries. In France, several laws exist to regulate professionals who work with pets who are members of domestic species. Some examples are as follows:

The Ministry of Social Affairs and Health drafted the **decree of 20 March 2014 concerning technical criteria for the labelling of assistance dog training centres and guide dog training centres and the creation of a national certificate** (Annex 1). This decree stipulates the following:

- The purpose of the training centre must be to deliver and monitor the dogs it trains to disabled persons free of charge.
- The facilities must comply with regulations concerning health and animal protection. These regulations are defined in the decree of 3 April 2014 (amended by *1* Order from 7 July 2016), which established the rules for animal health and protection with which activities related to pets who are members of domestic species covered by Articles L. 214-6-1, L. 214-6-2 and

L. 214-6-3 1* of the Rural and Maritime Fishing Code must comply (see below) (Annex 2).

- The centre must procure the services of a veterinarian and a doctor.
- The guide dogs must be able to guide their handlers with maximum safety on any route those handlers desire to take while avoiding obstacles and responding to standard commands.
- These dogs must have been selected for good health. They must have undergone a general assessment, for which the guide dog training centre is responsible.
- Foster families voluntarily undertake to socialize, in accordance with data provided by the guide dog training centre, a puppy that is entrusted to them between the ages of 8 weeks and 12 months or longer if necessary.
- It is important for foster families to provide the puppy with stability and balance to ensure its proper psychological development. The socialization of the puppy includes providing it with access to as many places as possible that it will later visit alongside its blind or visually impaired owner, including forms of public transportation, shops, offices, and administrative facilities.
- Criteria required for the foster family:
    - Availability and constant presence during the initial months.
    - A suitable geographical location at a reasonable distance that can facilitate frequent contact with the guide dog training centre.
    - Ease of travel for the designated guardian in the foster family, who is required to take the puppy to the guide dog training centre for visits.

    - A sense of volunteerism, motivation, and willingness to follow the instructions issued by the guide dog training centre.
    - An adequate environment: The puppy must have sufficient space in the home and a dedicated area in which it can be left undisturbed. If the home has a garden, it must be enclosed.
- The presence of children is recommended, provided that an adult supervises them and ensures that their relationship with the puppy is harmonious; similarly, the presence of other animals is not discouraged, except in the case of subjects suffering from behavioural problems.
- Monthly follow-up of the foster family conducted by its referent in the centre, which makes it possible to chart the evolution of the puppy in a follow-up booklet.
- The guide dog training centre makes a decision regarding the downgrading of the puppy if necessary; in this case, the puppy is offered for adoption.
- Monitoring of the visually impaired person-guide dog team is essential to observe and support the person. Regular contact must be ensured during the first trimester, and several home visits must be conducted during the first year.

Decree of 3 April 2014 (amended by *1* Order of 7 July 2016) establishing rules for animal health and protection with which activities related to pets who are members of domestic species covered by Articles L. 214-6-1, L. 214-6-2 and L. 214-6-3 1* of the Rural and Maritime Fishing Code must comply (Annex 2):

- Dogs are to be provided with waterproof and thermally insulated housing to protect them from the weather and excessive climatic conditions, which must be appropriate to their size and must feature a dry sleeping area and be insulated from the ground.
- The minimum space required for the accommodation of dogs is 5m^2 per dog, with a height of 2m. All or part of this accommodation must be sheltered from the weather and sun. This space may be reduced for stays in isolation facilities during treatment of a sick animal.
- The floor of the accommodation must be solid and continuous. The floor of the accommodation and runs must be designed and maintained to mitigate disturbances and health risks and to ensure the welfare of the dogs. Appropriate devices and accessories must be provided to encourage occupation and play.
- Dogs are to be housed as frequently as possible in harmonious social groups, except when it is justified to isolate them for health-related or behavioural reasons.
- Special care is to be taken when dogs are grouped together or when a new dog is introduced to a group. In all such cases, social compatibility within groups is to be monitored regularly.
- The dogs must have daily access to positive interactions with humans and other dogs. Special attention is given to their socialization and familiarization.

- Dogs must be able to move freely without any hindrance or discomfort. They may only be tethered on occasional and in accordance with the decree of 25 October 1982 concerning the breeding, maintenance and detention of animals.
- Dogs, with the exception of animals who are sick or who have been temporarily isolated for health reasons, regardless of their age and the manner in which they are kept, are to be taken outside every day as required so that they can romp and play with one another and interact with humans. An outdoor exercise area of suitable design and size is to be available.

These laws provide a framework for animal-related activities and promote an environment that is conducive to animal welfare. However, some problems related to the context of the development of prospective guide dogs cannot be avoided, such as changes in their social and physical environments.

Changes in the social and physical environments of guide dogs

As discussed in a previous section, potential guide dogs are required to adapt to several changes in their environment (Serpell et al., 2010). They change attachment figures and environments several times. Notably, they live in a foster home for a year, following which they live in a kennel. These disruptions can have negative psychological effects that may potentially jeopardize their success as guide dogs. These animals are chosen because they are close to humans. They develop strong

attachment bonds with particular humans, and the resulting separations could be stressful experiences (Serpell et al., 2010). Over a period of less than 2 years, guide dogs experience three different bonds: with the foster family, with the dog trainer, and with the blind person (or with a third person in cases in which the dog is downgraded and subjected to adoption) (Fallani et al., 2006). Fortunately, a study by Fallani et al. (2006) reported that a breakdown of the relationship with the foster family does not prevent the dog from forming a strong attachment bond with the blind person. This finding demonstrates the resilience of these dogs. However, it is important to anticipate these separations and these environmental changes by gradually habituating the animals to their new environment and target of attachment.

The retirement of guide dogs

Typically, guide dogs are retired when they are approximately 10 years old, i.e., after 8 years of work. However, the exact time of retirement is dependent on each dog, and a final decision is made by the guide dog school (Wenthold and Savage, 2007). Ng and Fine (2019) stipulated in a review that such dogs should reach retirement before their physical or mental health conditions become incompatible with the needs of their work. Retirement is a life transition that can be difficult for these dogs. First, because the dogs are used to physical and cognitive stimulation throughout their entire lives, not working at all can be very challenging for them (Ng and Fine, 2019). Second, retirement can engender another change in the dogs' environment. In fact, the dogs

can remain with their handlers, but this situation could be confusing for them if they are no longer required to respond to the commands of the blind people in question. For this reason, these dogs are typically placed at the home of a family member of the blind person, with their first foster family, or at the home of another individual. The best management strategy for the retirement of guide dogs remains unknown (Bremhorst et al., 2018). Further research is needed to ensure the welfare of these dogs both during and after this transition and to guarantee them the best retirement possible after many years of loyal service.

This introduction highlights the specific developmental context of potential guide dogs and illustrates some potential concerns regarding the dogs' welfare. Against this backdrop, several objectives emerged, which can be used to the proceedings of this PhD thesis.

OBJECTIVES

The main objective of this thesis was to identify factors that could influence the welfare and performance of future guide dogs for blind children.

Specific aspects of this object were defined as follows:

- To focus on the developmental context of the dogs, ranging from birth until delivery to the beneficiary

- To highlight the importance of the role of the foster family in this context

- To develop tools to detect the onset of fears in puppies at an earlier stage

- To understand the attachment of these dogs to humans and the impact of such attachment on their success as guide dogs

- To optimize the training environment to improve the dogs' welfare, emotional balance, and performance

RESULTS

CHAPTER 1: Early evaluation of fearfulness in future guide dogs for blind people

Fanny Menuge, Míriam Marcet-Rius, Muriel Jochem, Orane François, Camille Assali, Camille Chabaud, Eva Teruel, Justine Guillemot and Patrick Pageat

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1.1 Introduction

The proportion of dogs who are involved in a guide dog programme who actually complete the programme and become a guide dog is relatively low. In fact, almost 40% of dogs fail to complete the programme, and fear is the leading cause of such failure. For this reason, it is important to develop tools that allow us to detect the nature and causes of the development of these fears as soon as possible to avoid financial loss. Because dogs live with a foster family from the time they are weaned to the beginning of their education, this sensitive and crucial period for the socialization and familiarization of these dogs occurs when the dogs are surrounded by these foster families. For the first study of this thesis, we decided to focus on young dogs (at the age of 5 months) for the primary purpose of investigating the link between the familiarization of puppies to the environment handled by their puppy raiser and the emergence of fear by conducting an emotional reactivity test.

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Early Evaluation of Fearfulness in Future Guide Dogs for Blind People

Fanny Menuge [1,*], Míriam Marcet-Rius [1], Muriel Jochem [2], Orane François [3], Camille Assali [2], Camille Chabaud [4], Eva Teruel [5], Justine Guillemot [2] and Patrick Pageat [6]

1 Animal Behavior and Welfare Department, Research Institute in Semiochemistry and Applied Ethology (IRSEA), Quartier Salignan, 84400 Apt, France; m.marcet@group-irsea.com
2 Frederic Gaillanne Foundation (FGF), 84800 L'Isle-sur-la-Sorgue, France; muriel.jochem@fondationfg.org (M.J.); camille.assali@fondationfg.org (C.A.); justine.guillemot@fondationfg.org (J.G.)
3 Animal Experimentation Service, IRSEA, Quartier Salignan, 84400 Apt, France; o.francois@group-irsea.com
4 Molecular Biology and Chemical Communication Department, IRSEA, Quartier Salignan, 84400 Apt, France; c.chabaud@group-irsea.com
5 Data Management and Statistics Service, IRSEA, Quartier Salignan, 84400 Apt, France; e.teruel@group-irsea.com
6 Head Researcher in Charge for Innovation, IRSEA, Quartier Salignan, 84400 Apt, France; p.pageat@group-irsea.com
* Correspondence: f.menuge@group-irsea.com; Tel.: +33-490-756-567

Simple Summary: Nearly half of the dogs bred to become guide dogs for blind people fail before the end of their training, the main cause being the presence of fear. Detecting the nature and causes of these fears as early as possible was the main aim of this study. We therefore sought to determine whether the appearance of fear could be explained by insufficient habituation of the puppies. To evaluate fear, we employed an emotional reactivity test (ERT) and a physiological parameter (salivary cortisol). The physiological data supported behavioral data for two of the three parts of the ERT, suggesting that the puppies were able to control their behavioral responses to sound and visual stimuli (SVS). Additionally, the habituation level of the puppies affected both their physiological reactions during the test and their behavioral responses, especially their reactions to SVS. The results suggested that the less accustomed a dog is to a stimulus, the more frightened the dog will be, showing the importance of focusing on early puppy socialization and habituation to improve the numbers of guide dog candidates becoming successful guide dogs.

Abstract: Fear is the leading cause of guide dog failure. Detecting the nature and causes of these fears as early as possible is the first step in preventing their occurrence. The process of habituation is a fundamental part of fear prevention. In this study, 11 puppies, all five months of age, underwent an emotional reactivity test (ERT) composed of 12 scored items, classified into three categories: unknown person (UP), sound and visual stimuli (SVS), and body sensitivity (BS). Salivary cortisol was also measured. Foster families were asked to complete a questionnaire concerning puppies' habituation. The physiological data were correlated with UP ($r = 0.71$) and BS scores ($r = 0.67$), but not with SVS scores ($r = 0.16$), suggesting the ability of these dogs to control themselves when faced with the latter stimulus category. Additionally, the more time a puppy spent alone, the more likely it was to be afraid of SVS ($p = 0.05$). A correlation, albeit moderate, was detected between cortisol and habituation scores ($r = 0.48$). These results give us interesting avenues to explore, particularly regarding the importance of focusing on early puppy socialization and habituation to improve the numbers of guide dog candidates becoming successful guide dogs.

Keywords: emotional reactivity test; guide dog selection; habituation; puppy raiser; salivary cortisol

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1. Introduction

According to the World Health Organization's 2018 figures, nearly 217 million people are visually impaired [1]. In France, 207,000 people live with total or profound visual impairment [2]. Among these people, only 1500 have a guide dog to accompany them during their daily lives [3]. The selection criteria for guide dogs must be strict, requiring a high level of performance from the dog. Indeed, when carrying out their functions, they must be able to control themselves in all circumstances and find solutions when faced with obstacles in order to guarantee the safety of the person they accompany [4,5]. For these reasons, the failure rate of guide dogs is consistently very high worldwide [6,7]. As the cost for one fully trained dog is approximately 25,000 € [8], failures lead to significant financial losses for guide dog organizations. Finding ways to predict the success of a guide dog as early as possible, based on its temperament, in order to help training establishments is therefore an important issue and the subject of many publications [7,9–20]. While some dogs are forced out of the program due to physical ailments, behavioral problems account for a majority of guide dog candidates failures [17,21]. Docility, adaptability, stability [7], and passivity [15] are all behavioral traits that facilitate guide dog success. In contrast, fear-related behavior is one of the main reasons for the downgrading of guide dog candidates, behind distraction, shyness, and aggressivity [5,7,17].

Fear is an emotional state caused by an individual's perception of danger, resulting in an adaptative behavioral response triggered to manage the threat [22]. Fear manifests itself in physiological, behavioral, and emotional ways. An individual's expression of fear is affected by the interaction of genetic and environmental factors [23], such as inherited characteristics [17], the influence of the present environment, and learned experiences during the sensitive period [24]. During this period, the level of plasticity of the central nervous system allows for the development of individuals based on experiences. Even if opinions on the exact age of the sensitive period and its period of time diverge in the literature, it is commonly accepted that the social development period of puppies is from two to thirteen weeks [25–28]. For example, focusing on the socialization period, a study of Tiira and Lohi [29] demonstrated that more socialization experiences between eight and twelve weeks of age were associated with lower fearfulness in adult dogs. Emotional reactivity tests (ERTs) are commonly used to score dogs according to their reaction to a series of external stimuli [14]. They can thus be useful for working dog organizations, especially for animal selection, by eliciting not only behavioral but also physiological responses, such as an increase in plasma and salivary cortisol secretion [30,31]. Little consensus exists regarding the tests used, although standardized tests are being developed in the literature [14,20,31]. To meet their own needs, some institutions are even developing their own tests. This is the case for the Frederic Gaillanne Foundation (FGF—L'Isle-sur-la-Sorgue, France), which trains guide dogs specifically for visually impaired children. The FGF administers this test to one-year-old dogs as a selection test just before the beginning of their training. Guide dogs must be able to behave appropriately in any situation, including frightening stimuli [32]. A dog with excessive fear of busy, noisy, or unusual situations would be unsuitable for guide dog training [32].

It has been shown that genetics could influence the development of the dog's behavior [33]. Besides, these dogs have been selected genetically for their interesting characteristics (e.g., responsive, friendly, clever) that make them potentially good guide dogs [34]. However, habituation and socialization remain primordial in the development of puppies, forming the basis for the prevention of fear [23,24,35,36], and must conform to certain rules to be successful: gradual presentation of the potentially aversive stimulus, regular exposures, and the ability of the animal to freely withdraw from it [37]. To be effective, this process must take place during the sensitive period, in the first months of life [36]. If puppies do not have the opportunity to encounter some types of stimuli during this period, they may develop inappropriate behavioral responses to these stimuli in adulthood [28], which may consist of aggressive or avoidance responses [24]. Notably, a study conducted by Kutsumi et al. [38] showed the benefits of a puppy socialization class for the future tem-

perament of dogs. However, there is no standardized habituation or socialization protocol for future guide dogs. From the age of eight weeks, puppies intended to become guide dogs are usually placed in foster families for approximately one year before beginning their specialized training. It is the foster families' responsibility to instill the first basics of training but, above all, to socialize the puppies and to accustom them to all types of external stimuli, whether social or environmental [39]. The foster family is therefore a crucial step in the development of future guide dogs [40]. In spite of the importance of this issue, there is a lack of research focusing on the early development of the future guide dogs in their foster families.

This study was carried out in collaboration with the FGF. The ERT developed by its members was used here at an earlier age (at five months of age), and a questionnaire for the foster family measured the amount of habituation that the puppies underwent. The aims were to confirm or refute the validity of this test and to detect the presence of certain fears in the puppies at an early stage. Finally, we also investigated the presence of a link between the level of habituation in the foster family and the puppies' reactions to the test through behavioral and physiological indicators.

2. Materials and Methods

This project was approved by the Research Institute in Semiochemistry and Applied Ethology (IRSEA) Ethics Committee (approval number CE_2020_06_ADOB_03).

2.1. Subjects

Eleven 5-month-old dogs from the FGF, including 7 female and 4 male Labernois (Labrador/Bernese mountain dog cross), participated in the study. All subjects were candidate guide dogs and were attributed to a foster family (different for each puppy) at 2 months old. They come from 2 different litters, but they were born to the same breeder.

2.2. Procedure

The experiment took place at the FGF. Foster families were asked to bring their puppies between 8:00 and 8:30 a.m. and pick them up in the afternoon. For each puppy, the entire procedure was performed between 9:00 and 11:00 a.m. (2 puppies per morning) to minimize the impact of circadian influences on cortisol levels [41]. This test is an evaluation procedure generally used by the FGF to evaluate dogs at one year of age. It was developed by members of the foundation specializing in canine behavior and by a veterinary behaviorist, adapted from some other procedures used by previously published tests in dogs or other species [20,31,42–44]. Due to the young age of the dogs, some items from the original evaluation procedure were removed to avoid overloading them emotionally. Confrontations with joggers, other dogs, and children were therefore eliminated, in agreement with the FFG. These items were placed at the end of the test., therefore, removing them had no effect on the rest of the test. As used in this study, the test, which lasts approximately 20 min, consists of 12 items confronting the animal to different forms of stimulation: sound and visual stimuli (SVS), a test of body sensitivity (BS), and an unknown person (UP), as follows:

1. **Indoor part (4 min)—unfamiliar room (3 m × 3 m) (Figure 1a)**

Item 1 (UP): The dog is alone in the unfamiliar room. A UP enters the room without approaching the dog, stands with his back to the door and his arms at his side, and remains neutral. After 10 s, the UP crouches down and makes contact with the dog. If the dog does not come into contact after 10 s, the UP pats his thighs 3 times to attract the dog. If the dog does not approach, the procedure is stopped and the UP moves on the next item.

Item 2 (BS; UP): A UP sits on the bed in the room; presents an artificial hand on a long arm to the dog for 10 s; and then moves to touch the dog on the back, the sides, the legs, and then the head with the artificial hand. His approach is lateral. If the dog does not approach, the handler calls him once. The handler must not use his actual hands; if the dog retreats, the procedure is stopped.

Item 3 (BS; UP): A UP walks up to the dog, talking to him, and lifts him up from the front. Then, he crouches down, opens the dog's mouth to look at his teeth, looks inside the ears, takes a front paw and pulls slightly on it, then pulls the tail slightly. If the dog struggles, the procedure is stopped.

Item 4 (UP): The unknown person offers a rope to pull and strongly encourages the dog to play by shaking the toy. He throws the toy approximately 2 m away. If the puppy does not fetch the rope, the UP picks it up and try again, 3 times.



Figure 1. Photos of the rooms used for items 1 to 4 (**a**) and 5 (**b**).

2. **Indoor part (30 s)—corridor (3 m × 12 m) (Figure 1b)**

Item 5 (UP): A handler (a dog trainer who spent one hour with the puppies just before the test—on a walk for 15 min and then on her desk) holds the dog on a leash and stands with his back to the wall in the middle of a corridor. A UP dressed in a strange outfit (hat, glasses, large cape, cane) knocks at the door. He enters and passes in front of the dog, not stopping until the end of the corridor. The UP then turns around, adopts an exaggerated gait (waving his arms, limping), and then crouches down in front of the dog, presenting his hand. After 10 s, the UP gets up and walks away. During this item the handler must not react or move until the stranger is gone.

3. **Outdoor part (6 min)—senses itinerary: the dog is walked through the course on a 2-m leash by the handler, passing by 8 different points (Figure 2).**

Item 6 (SVS): The handler stops for 1 min in front of a 30 cm high statue representing a dog.

Item 7 (BS): The route continues over a small footbridge. The dog must climb 4 steps (open risers) to cross it. The dog must surmount the obstacle of his own free will. If, despite the handler's encouragement, the dog refuses to climb the steps, the handler then carries the dog and places it at the top of the footbridge.

Item 8 (BS): The floor of the footbridge has a segment that consists of a metal grid. The dog must walk on it for approximately one meter. As for the stairs, if the dog refuses, the handler carries the dog over this obstacle.

Item 9 (SVS): The handler stops at a given point and waits; a UP moves forward with a wheeled suitcase, passes close to the dog (1 m away), moves 4 m away, comes back towards the dog, and walks away.

Item 10 (SVS): The handler stops at a given point. A UP walks 3 m away from the dog with an umbrella, opens and closes it 3 times at 3-s intervals (left, top, right), and then walks away.

Item 11 (SVS): The handler stops at a given point. At this point, a UP located 2 m away activates a horn 3 times at 3-s intervals.

Items 12 (SVS): The handler stops at a given point. At this point, a UP throws metal cans (3 successively at 3 s intervals) next to the dog (1 m away).

For each item, puppies were scored according to the scale established by the FGF. The addition of all those items gave the ERT overall score. UP score, BS score, and SVS score were also calculated by adding up their corresponding items (see Table 1). Scores vary between −1 and 3. The more the dog shows signs of stress, the lower the score will be. In contrast, the more confident the dog is, the higher the score will be (Table 2). All parts of the ERT were filmed and scored *a posteriori* by two independent observers, specialists in canine behavior.



Figure 2. Senses itinerary of the Frederic Gaillanne Foundation (FGF). The locations of the items completed on this course (outdoor part) are indicated by their numbers, from 6 to 12.

Table 1. Scores obtained from the emotional reactivity test (ERT) test associated with the corresponding items.

	UP Score	BS Score	SVS Score	Overall Score	Startle Score	Avoidance Score
Items	1–2–3–4–5	2–3–7–8	6–9–10–11–12	Items 1 to 12	5–6–9–10–11–12	1–2–5–6–7–8–9–10–11–12

Table 2. Emotional reactivity test score grid for each item.

Evaluation Criteria	Score
Tail high, ears high, relaxed dog, the dog is at ease, and rather excited or cheerful. The dog remains active, with exploratory behavior.	3
Neutral position: the posture adopted is relaxed. The dog can mark slightly but quickly recovers. The dog has little or no reaction to the stimulus.	2
The tail is low, the dog shows signs of stress (lip licking, yawning, avoidance).	0
The dog is completely panicked (tries to run away) or threatening behavior (grunt, shows teeth).	−1

For relevant test items, additional score was reported as the startle reactions (items 5–6–9–10–11–12) and the avoidance reactions (items 1–2–5–6–7–8–9–10–11–12), scored on a

scale of 1–5 points. A high score on the startle reaction scale indicates high startle and high score on the avoidance reaction scale indicates strong avoidance.

We obtained, for each dog, 7 scores in total, recapitulated in Table 1.

In order to analyze cortisol levels, saliva samples were collected just before the test (T0), 15 min afterward (T1), and 45 min afterward (T2) by using the Salivette system (Salivette, Sarstedt, Numbrecht, Germany). For the sake of avoiding contamination, the dogs must not have eaten within 1 h before each sample. As soon as the samples were collected, they were placed in a cooler to keep them at a temperature below 4 °C until they were centrifuged (2 min, 1000 rpm). After being centrifuged, the samples were frozen at −20 °C until analysis (Salimetrics® Cortisol Enzyme Immunoassay Kit, Kiel, Germany).

For the habituation scores, a questionnaire was completed by the foster families to assess the frequency with which the puppies were brought to different locations (unknown places, stores, busy parks, walks in the countryside, along roads highly frequented by cars, public transport, noisy places). For each question, they could choose one of five options (less than once a month—less than once a week—about once a week—more than once a week—every day), giving a global score between 7 and 35. Questions about the frequency (less than once a month—less than once a week—about once a week—more than once a week—every day) and duration (less than 1 h—between 1 and 4 h—between 4 and 7 h—more than 7 h) of time the puppy spent alone at home were also asked, giving a global score between 2 and 9 (isolation score).

2.3. Statistical Analysis

The analyses were carried out using SAS 9.4 software Copyright (c) 2002–2012 by SAS Institute Inc., Cary, NC, USA. The significance threshold was fixed at the classical value of 5%.

Interobserver reliability between the 2 independent readers was assessed for each score of the ERT.

Correlations were made between salivary cortisol and ERT scores, between startle and avoidance scores and ERT scores, and between salivary cortisol and foster family questionnaire scores. For this purpose, when normality was verified, the Pearson correlation coefficient was calculated. Otherwise, the Spearman correlation coefficient was used. According to Martin and Bateson [45], r = 0.4–0.7 indicates a moderate correlation (substantial relationship), r = 0.7–0.9 indicates a high correlation (marked relationship), and r = 0.9–1.0 indicates a very high correlation (very dependable relationship).

The associations between the foster family questionnaire score, the ERT score, and the salivary cortisol were also evaluated using linear regressions. The assumptions of normality of model residuals, homoscedasticity, and linearity were verified, and if necessary, the models were simplified to increase power by maximizing the adjusted R^2.

In order to evaluate the time course of salivary cortisol levels, we applied a general linear mixed model for repeated measures after checking the normality of the residuals.

3. Results

The statistical analysis of interobserver reliability revealed strong associations for all the parameters (≥80%). Thus, the average of the data collected by the two readers was taken for the rest of the analysis.

UP, BS, and SVS scores were highly correlated with the ERT overall score (r > 0.8).

A moderate negative correlation was found between salivary cortisol (T1) and the overall score of the ERT (r = −0.54). However, the correlation was higher between salivary cortisol (T1) and BS (r = −0.67) and UP (r = −0.71) scores. A moderate negative correlation was also detected between salivary cortisol (T1) and the habituation score (r = −0.48). Startle behaviors supported the results obtained in the test (see Table 3).

A significant linear regression indicated that the lower the UP score, the more cortisol secreted by the animal ($p = 0.02$). For every five-point increase in the UP score, the average salivary cortisol value at T1 dropped by 0.038 µg/dL. A statistical trend ($p = 0.0547$) was

also observed for the effect of the isolation score on the SVS component of the ERT. For every 1-point increase in the isolation score, the average SVS score dropped by 0.854 points.

Table 3. Table of correlations between emotional reactivity test (ERT) scores with salivary cortisol (T1), startle and avoidance reaction.

	T1	Startle	Avoidance
Unknown person (UP)	**−0.71** **$p = 0.02$**	**−0.6** **$p = 0.05$**	−0.12 $p = 0.07$
Body sensitivity (BS)	**−0.67** **$p = 0.03$**	**−0.61** **$p = 0.05$**	−0.37 $p = 0.26$
Sound and visual stimuli (SVS)	−0.16 $p = 0.66$	**−0.87** **$p < 0.01$**	−0.45 $p = 0.16$
Overall score	−0.54 $p = 0.1$	**−0.79** **$p < 0.01$**	−0.29 $p = 0.37$

In bold: data with a p-value ≤ 0.05.

Concerning the cortisol level, the general linear mixed model for repeated measures revealed no significant difference between before (T0), during (T1), and after (T2) the ERT (GLMM; DF = 2; F = 0.01; $p = 0.9948$) (Table 4).

Table 4. Descriptive statistics of salivary cortisol.

Time	N	Mean	Std Error	Minimum	Maximum
T0	11	0.149	0.016	0.077	0.228
T1	10	0.147	0.016	0.065	0.212
T2	9	0.150	0.024	0.091	0.299

4. Discussion

The aim of this study was to find suitable ways to evaluate puppies at an early stage for the presence of fear, which is the reason for a significant number of failures in guide dog training [32]. The items used to construct this test have been independently validated in the literature [20,31,42–44]. The test confronts the animals with three categories of stimuli: sound and visual stimuli (SVS), body sensitivity (BS), and an unknown person (UP). The primary interest was to confirm or refute the validity of this test for use on future guide dogs, i.e., whether it triggered an emotional response in fearful dogs, thus making it possible to detect these fears and highlight undesirable behavior at an early stage. Initially, statistical analyses revealed that all stimulus categories were involved in the test score, as they were all highly correlated with the overall score. When the behavioral data were cross-tabulated with the physiological data, the latter partially agreed with the former. Indeed, salivary cortisol was only moderately correlated with the overall test score, whereas it was significantly correlated with the scores obtained for UP and BS categories. These results suggest that these two categories have a greater impact on cortisol secretion than SVS. The stage at which puppies most readily approach UPs is before two months of age [36]. Results indicate that after this age, mistrust begins to set in. Additionally, evidence suggests that if a puppy has not had contact with strangers during his early development, he may perceive them as something particularly stressful, as previously described [36]. However, another explanation may account for the low correlation between SVS score and salivary cortisol. In one of their studies, Fallani et al. [46] also demonstrated a mismatch between physiological and behavioral data in guide dogs during stressful episodes. In fact, when separated from their owner, this study showed that guide dogs could restrain their behavioral reaction, but their cardiac activation increased. These dogs must learn from an early age to react as little as possible to such stimuli and thus to control their behavioral response. This may explain why direct observation of behavior may not correspond to the animal's physiological data and may not reflect the dog's actual experience. The use of

physiological parameters is therefore essential to obtain a reliable measurement, especially for guide dogs, as performed in the present study.

The presence and intensity of the startle reaction, which is a brief behavioral response, could, in this case allow more sensitive detection of a fear reaction in the animal. Indeed, the results significantly support the overall score of the test. Startling could therefore be a relevant indicator, as demonstrated in a study conducted by Slabbert and Odendaal [47]. Their research showed that, in police dogs, a significant percentage of puppies that achieved low scores for the startle test did not become successful police dogs. It would therefore be interesting to compare this behavioral response with guide dog training outcomes.

In general, despite the small number of individuals, these results indicate, through the correspondence between behavioral and physiological parameters, that the ERT test influences the emotional state of animals, and thus makes it possible to verify their reactions to different stimuli. These results should be cross-referenced with their entry into the training program (when they are one year old) and their success as a guide dog (at the end of their training) to assess whether this test is an early predictor of whether the dog has the necessary predisposition for this work.

No significant differences were detected among T0, T1, and T2 in salivary cortisol. This could be easily explained by the fact that the puppies were in an unfamiliar environment, separated from their foster families, and in the presence of other dogs, all of which may have increased their cortisol secretion as much as the test itself. Similarly, these same elements explained the fact that the puppies had difficulty returning to a state of homeostasis. This result may suggest the importance of progressive entry into training or prior habituation, as Rooney et al. suggested in one of their studies [48]. This would facilitate dogs' adaptation to this environment, thus promoting their welfare and their learning capacity [49,50]. Cobb et al. [51] have shown that it is difficult to establish a "normal" interval of cortisol secretion. However, according to the mean salivary cortisol concentration of healthy dogs suggested by some authors [52,53], analysis of salivary cortisol did not detect any abnormally high levels. Abnormally high cortisol would indicate that the dogs were stressed by the program. The results suggest that this was not the case for these dogs.

The questionnaire for foster families gave two scores (habituation and isolation scores). Comparing these with other variables allowed the identification of relationships, which could be the subject of more in-depth studies. For example, a moderate linear relationship was detected between cortisol during the test (T1) and the habituation score. The less the dog is accustomed to frequenting different environments, the more cortisol it will secrete. However, the correlation was low; it would thus be interesting to confirm these results by increasing the sensitivity of the questionnaire and the number of individuals assessed. Similarly, a trend was observed for the effect of the isolation score on the SVS component of the ERT. It is possible that puppies spending more time alone were given less exposure to stimuli. When they encountered unfamiliar stimuli, they showed a fearful response. Although this result was not significant, the strong trend encourages us to believe that a replication of this study in a larger sample of animals would confirm these results at a statistically significant level. According to the answers to the questionnaires, the foster families did not leave the dogs alone for more than four consecutive hours, as recommended by the guide dog schools. The difference therefore lay in the frequency with which this happened, i.e., whether it was a common occurrence. These results showed us the importance of the frequency of these episodes, in addition to their duration. The involvement of foster families is therefore very important in the training process, as the more the puppies accompany them in their daily lives, the more the animals will be able to develop appropriate coping strategies. Accustoming the dogs to any new environment from an early age seems vital in their formation and for the development of an emotional balance essential to their future work, and this difficult role falls on foster families.

5. Conclusions

In conclusion, the results showed a correlation between physiological and behavioral data during the test for the UP and BS categories, which shows the good validity of this test for the items belonging to these categories. However, this was not the case for SVS, suggesting a potential ability of the dogs to control their behavior, despite showing physiological signs of stress, when faced with this type of stimulation. Additionally, the results suggested that the less the foster family leaves the puppies alone and the more they accustom the puppies to all kinds of outside stimuli, the less likely they will show physiological signs of stress during ERT.

This study demonstrates the difficulty in providing dogs with an ideal, standardized, and controlled development framework, allowing the acquisition of the necessary basics to promote the emotional balance that is essential for guide dogs. In this context, a plurality of factors could determine the development of fears, and thus the success or failure, of these dogs. These results give us interesting avenues to investigate, particularly on the importance of focusing on the early development period of the puppies, which is a decisive phase influencing their temperament in adulthood. Providing the same environment for the animals would increase the educators' control over the development of the dogs, especially during the sensitive period, thus minimizing interindividual variation and allowing better targeting and correction of the causes of failure. At this age, ERT could also have a use initially in identifying puppies with a tendency to be fearful, but also to target educational work on desensitizing stimuli, leading to a fear response in these puppies. That could allow more dogs to remain in the program and succeed as a guide dog.

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1.3 Discussion

The familiarization of puppies to the environment plays a key role in their development and in their acquisition of the ability to cope with external stimuli in an efficient and balanced manner. In this study, we highlighted the importance of the role played by the foster family in the development of prospective guide dogs. Correlations were found between the dogs' level of familiarization and the behavioural and physiological reactions of dogs to an emotional reactivity test. Testing the puppies at an early stage may allow for the earlier detection of dogs that should be excluded from the programme or those to whom special attention should be given. In addition, standardization and assistance to foster families to facilitate the familiarization and socialization procedures of puppies could be a way of decreasing the number of failures.

In addition to playing this very important role, the foster family also represents the first interspecies relationship experienced by the future guide dog, which is evaluated in the following experiment.

CHAPTER 2: Attachment: A new approach to predict the success of future guide dogs? A pilot study

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2.1 Introduction

This paper is a preliminary study. With respect to the time spent by dogs with their foster family (between 1 year and 1.5 years) and the fact that dogs develop strong relationships with humans, we were interested in exploring the attachment behaviours of the dogs towards the foster family. In fact, these dogs are raised by people who know that the dog is going to leave them. This situation could have an impact on this relationship and thus influence the emotional state of dogs. The first interest of this experiment was to determine whether dogs are allowed to develop special bonds with their foster families. We also evaluated the suitability of using psychometric scales to assess dogs' emotional states.

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Research Article

Attachment: a new approach to predict the success of future guide dogs? a pilot study

Fanny Menuge[a], Estelle Descout[a], Sana Arroub[a], Céline Lafont Lecuelle[a], Emeline Gautier[b], Manuel Mengoli[b]* and Patrick Pageat[a]*

[a]*Research Institute in Semiochemistry and Applied Ethology (IRSEA), Quartier Salignan, 84400 Apt, France*

[b]*Clinical Ethology and Animal Welfare Centre (CECBA), IRSEA, Quartier Salignan, 84400 Apt, France*

***Corresponding author:** Hagar Goldberg, The University of British Columbia (UBC), Vancouver Campus, 2329 West Mall, Vancouver BC, V6T 1Z4, Canada

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Abstract

Guide dogs are a major resource for people with visual impairment. Unfortunately, more than one-third of them are reformed each year because they do not have the required qualities. Their specific training leads them to establish several interspecific attachment bonds, the first one with their puppy raisers, which constitutes an important emotional base for their future cognitive-emotional development and mental stability. Eight guide dogs aged between 1 and 2 years in the training period and their puppy raisers were involved. This is a preliminary study that aimed to investigate attachment behaviours in guide dogs towards their puppy raisers using the Strange Situation Test (SST). The second goal was to assess the suitability of using psychometric scales measuring the emotional state of the animals in this shared-custody context. Two psychometric scales that reflect dogs' impulsivity and sensitivity levels were applied through questionnaires-the Dog Impulsivity Assessment Scale (DIAS) and the Positive and Negative Assessment Scale (PANAS)-completed by the puppy raisers and principal trainers. The SST revealed a preference among the dogs for their puppy raisers over a stranger, as evidenced by expressing more secure base effect behaviour when the puppy raiser was present (Tukey-Kr; DF=13.9; t=3.05; p=0.02) and greater proximity seeking behaviour towards the puppy raiser than towards the stranger (Student's t-test; DF=1; t=2.66; p=0.03). The test also highlighted a strong difficulty in remaining alone, as demonstrated by a longer duration of door proximity behaviour when alone (Tukey-Kr; DF=7; t=5.15; p<0.001). Notably, the results of the questionnaire score analysis revealed a discordance between the perception of educators and puppy raisers, giving uncorrelated scores. This study will be repeated once the dogs have been adopted by visually impaired children to assess the continuity of the attachment bond developed with the foster family over time and determine whether this bond influences the success of the dogs and their ability to create a new bond in the long term.

Keywords: Ainsworth Strange Situation Test; Attachment; Emotional state; Foster family; Human dog relationship

Introduction

Guide dogs accompany people with visual impairments to increase their independence by providing them assistance in their daily tasks [1]. The relationship is based on mutual trust, which is essential for the proper functioning of the dyad [2]. Indeed, the dog must be able to deal with all sorts of unforeseen circumstances that might be encountered in the course of its work. Not reacting when faced with a sudden event, organizing the environment, and showing the way when encountering an obstacle are all indispensable skills that help ensure the safety of the person and the fluidity of his/her movement [3]. Emotional balance and the capacity to cope with emotional distress are therefore the most desired characteristics in guide dogs [4]. These characteristics could play a fundamental role in both the acquisition of relevant skills and in the dog's ability to put these skills into practice every day with the person it accompanies. Unfortunately, a large proportion of dogs are excluded from the guide dog programme [5-7]. In 2016, the percentage of reformed or reoriented dogs was estimated at approximately 40% in France [8], of which 60% occurred for behavioural reasons [9]. Dogs are usually trained by a federated association, foundation or training programme. Early detection of problems or difficulties is therefore desirable to avoid as much financial and time loss as possible due to the high cost of each animal for diet, health care and training, which usually amounts to between 20,000 and 25,000 euros per dog in France [10].

As a result of training, guide dogs establish different emotional bonds with different people during the first two years of life:

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the dog's mother and siblings initially, then the foster family, the educator, and finally the visually impaired person the dog will accompany. Foster families keep dogs from the weaning stage and teach them basic rules in terms of education. They are therefore considered very important in the breeding of future guide dogs [11]. Attachment, as developed, is described as the product of behaviours aimed at seeking and maintaining proximity to a specific person. This is an important concept defended by several authors, including [12-15], who claim that the theory is valid for all mammals. According a secure base effect is the main factor indicating the presence of an attachment bond between two individuals. Accordingly, they constructed the strange situation test (SST - fully described by the authors, 1970) to measure the relationship between a child approximately one year old and his or her significant person, most often the mother. Since then, several researchers have shown that attachment can also exist interspecifically between a dog and its handler, thanks to a revised version of this test, the SST-R [16-20]. The presence of this bond was confirmed by the work and who stipulated that people can act as a secure base for their dogs. This paper is a preliminary study of the largest research area. The goal is to understand the bond developed by the future guide dogs towards their puppy raisers and to assess the appropriateness of using scales measuring the emotional state of the animals through its levels of sensitivity and impulsivity in this particular context, in which several people share phases of dog development. The study was conducted in collaboration with the Frederic Gaillanne Foundation (L'Isle-sur-la-Sorgue, France), which is the first school in Europe to train guide dogs only for children with visual impairment and assistance dogs for children with developmental disabilities.

Materials and Methods

Subjects

Eight dogs, including 5 females and 3 males St. Pierre dogs from the Frederic Gaillanne Foundation participated in the study with their puppy raiser. They were all neutered and had a mean age of 24 months (± 6 months). To be included in this study, they had to live with their foster family for at least 3 months and not have experienced any major change in their routine before the test. They all started their guide dog training 7 months ago. They are at the foundation from Monday to Friday, then they go back to their host families on weekends.

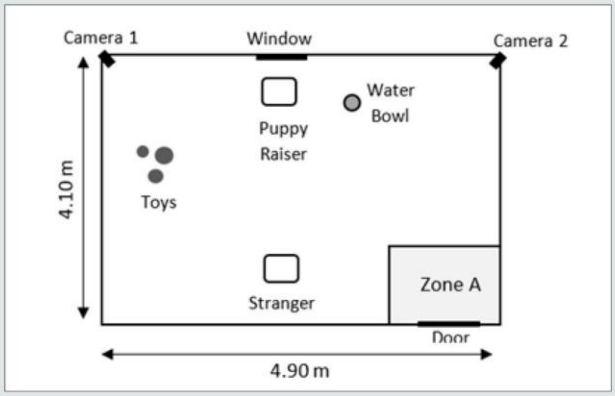

Figure 1: The experimental room was equipped with two chairs, three dog toys, a water bowl and two video cameras. Zone A (1.40 m x 1.30 m) = Door Proximity Area.

Experimental design

The experimental environment was as close as possible to the standards used in the SST-R [21,22]. The room (4.9 m × 4.1 m), which was located at the Clinical Ethology and Animal Welfare Centre (CECBA) of the Research Institute in Semiochemistry and Applied Ethology (IRSEA), was unfamiliar to the dogs. The room was equipped with 2 chairs, 3 toys, 1 water bowl and 2 cameras (Figure 1). All tests were performed in the late morning between 10 a.m. and 11 a.m., and only 1 test was performed per day to avoid any possible olfactory influence.

Procedure

The puppy raiser participating in the experiment had previously received and completed two questionnaires-the Positive and Negative Assessment Scale for dogs (PANAS) [23] and the Dog Impulsivity Assessment Scale (DIAS) [24]-via Google Forms; these scales measure the levels of sensitivity and impulsivity of dogs, respectively. The PANAS consists of 21 items, and the DIAS consists of 18 items. Both questionnaires use a 5-point Likert scale, ranging from "strongly disagree" to "strongly agree". A "don't know/not applicable" option was also possible. The lead educator for each dog was also asked to complete the PANAS and DIAS. The experiment was based on the modified version of the Ainsworth SST adapted to dogs [15,17] and was video recorded. The experimental phase was divided into the episodes described in Table 1. The same woman played the role of the stranger in all scenarios as described in the literature [7,12,16]. All participants signed an authorization to acquire, reproduce and broadcast images.

Table 1: Description of the strange situation procedure (Fallani et al., 2007).

Episode (3 minutes each)	Description
1: PR* and dog	The PR sat quietly, interacting with the dog only if it was looking for attention. The dog was free to explore the room.
2: PR, dog and S*	The stranger entered the room, sat quietly for 1 min, conversed with the PR for the second minute, approached the dog and attempted to stimulate play during the last minute. At the end of this episode, the PR left the room unobtrusively.
3: S and dog (1st separation episode)	The stranger continued to play with the dog if it was willing; if it was inactive or distressed, the stranger attempted to distract it with play or by providing verbal and tactile comfort.
4: PR and dog (1st reunion episode)	The PR entered the room and greeted and/or comforted the dog as usual. The stranger quietly exited the room. The PR had been told that he/she was free to play with the dog throughout the episode. At the end of this episode, the PR left the room.
5: Dog alone (2nd separation episode)	The dog remained alone for 3 min but was constantly observed by the PR and researchers on the monitor in the adjacent room.
6: S and dog	The stranger entered the room and followed the same protocol as in episode 3.
7: PR and dog (2nd reunion episode)	The PR entered the room and greeted the dog as usual. The stranger left the room unobtrusively. At the end of this episode, the experimenter arrived and officially terminated the procedure.

Data collection

All tests were filmed and analysed twice by the same person at 6-month intervals to evaluate intraobserver reliability. Behavioural Observation Research Interactive Software (BORIS) [10] was used with the 5-second interval scan sampling method for the 7 episodes. Based on previous studies [4,12,13], the behaviours described in Table 2 were noted and organized into categories. These behaviours were mutually exclusive, except for "physical contact" and "greeting behaviour", which could be associated with each other. The duration of "greeting behaviour" and "door proximity" was also collected. The sensitivity (PANAS) and impulsivity (DIAS) questionnaires were scored according to the method described by the authors [20,24], yielding scores between 0 and 1. The two scales comprise different factors and the following scores:

Table 2: Behavioural categories recorded in the Strange Situation Test.

Behaviour category	Definition
Secure Base Effect	
Individual play	Any vigorous or galloping gaited behaviour directed towards a toy when clearly not interacting with any participants, including chewing, biting, shaking from side to side, scratching or batting with the paw, chasing rolling balls and tossing using the mouth.
Social play	Any vigorous or galloping gaited behaviour performed when interacting with the PR* or the stranger, including running, jumping, active physical contact, and chasing toys.
Exploration	Activity directed towards physical aspects of the environment, including sniffing, close visual inspection, distal visual inspection, and gentle oral examination such as licking.
Passive behaviour	Sitting, standing or lying down without any obvious orientation towards the physical or social environment.
Physical contact	Being in physical contact with the PR or the stranger.
Proximity Seeking	
Following	Following the person around the room or to the door.
Approach	Approaching while clearly visually oriented to the person.
Oriented to a person	Staring fixedly at the PR or the stranger regardless of whether the behaviour was reciprocated.
Search Behaviour	
Scratching the door	All active behaviour resulting in physical contact with the door, including scratching the door with the paws, jumping on the door, and pulling on the door handle with the forelegs or mouth.
Oriented to the chair	Staring fixedly at the PR's or stranger's empty chair.
Oriented to the door	Staring fixedly at the door either when close to it or from a distance.
Greeting behaviour duration (sec.)	All greeting behaviours towards the entering PR or stranger, such as approaching, tail-wagging, jumping, and physical contact.
Door proximity duration (sec.)	Time spent in the area near the door (zone A, Fig. 1).
Other behaviour	All behaviour not included in this catalogue.

*PR = Puppy Raiser

1. The DIAS includes 4 scores: "behavioural regulation", "aggression/response to novelty", "responsiveness" and "overall score".
2. The PANAS includes 5 scores: "negative activation" and "positive activation", itself composed of "energy and interest", "persistence" and "excitement" scores.

Statistical analysis

The analyses were carried out using SAS 9.4 software Copyright (c) 2002-2012 by SAS Institute Inc., Cary, NC, USA. The significance threshold was classically fixed at 5%.

Evaluation of attachment

Intraobserver reliability between the 2 observations separated by a 6-month interval was assessed for each parameter ("secure base effect", "comfort seeking", "proximity seeking", "search behaviour", "greeting behaviour" and "door proximity"). Pearson correlation coefficients were calculated if the hypothesis of normality of the parameters being compared was verified; otherwise, Spearman correlation coefficients were calculated. The behaviours "physical contact", "proximity seeking", and "greeting behaviour" were analysed using a 2 x 2 comparison between the puppy raiser and the stranger. For this purpose, after checking the normality conditions, Student's t-test for matched samples was carried out using the t-test procedure. The behaviours "secure base effect" and "search behaviour" were compared between episodes 2 and 4 and episodes 3 and 6, respectively. The "door proximity" behaviour was analysed under 3 conditions: when the dog was alone (episode 5), when the dog was in the presence of the puppy raiser (the average of episodes 2, 4, and 7), and when the dog was in the presence of only the stranger (the average of episodes 3 and 6). To do this, the assumption of normality was tested using the residual diagnostics plots and the UNIVARIATE procedure. Comparisons between conditions were performed with a general linear model (GLM) using the MIXED procedure to consider repeated measurements. The best variance-covariance matrix was selected by minimizing the AICC and BIC of the model. When a significant difference was observed, multiple comparisons were made using the Tukey-Kramer test using the LSMEANS statement.

Evaluation of psychometric scales

Regarding the DIAS and PANAS questionnaires, 2 x 2 correlations were made between the responses of the puppy raisers and educators for the overall scores and each of the factors. For this purpose, when normality was verified using the UNIVARIATE procedure on SAS software, the Pearson correlation coefficient was calculated using the CORR procedure. Normality was not verified for the "aggression/response to novelty" factors or the overall DIAS score, and the Spearman correlation coefficient using the CORR procedure was preferred. According to Martin and Bateson (2007), r = 0.4-0.7 indicates a moderate correlation (substantial relationship), r = 0.7-0.9 indicates a high correlation (marked relationship) and r = 0.9-1.0 indicates a very high correlation (very dependable relationship). Finally, the association was evaluated by computing the square of the correlation coefficient.

Results

Strange Situation Test

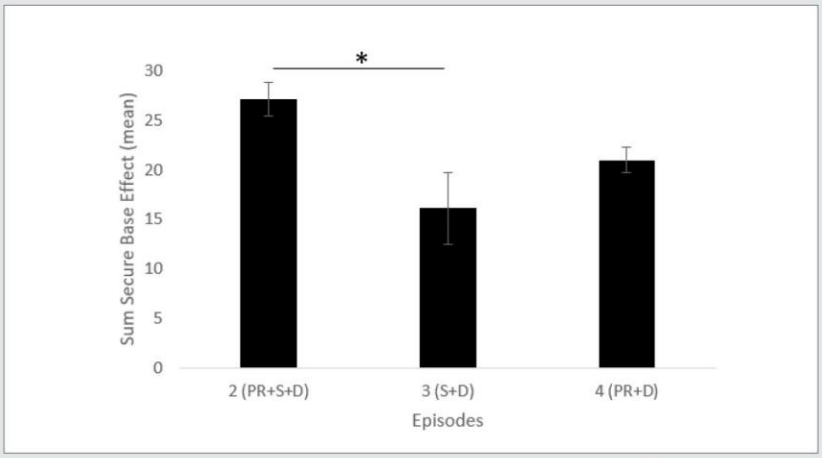

Figure 2: Episode effect on secure base effect. The mean ± standard error. S = Stranger; PR = Puppy Raiser; D = Dog. *p < 0.05.

The statistical analysis of intraobserver reliability revealed a strong association (≥ 80%) between the observations for each parameter, with a minimum correlation of 0.89 (significance test; p<0.001). The results obtained for the "secure base effect" highlighted significant differences between episodes (GLMM; DF=2; F=4.66; p=0.03), with behaviours expressed more often when the puppy raiser was present with the stranger than when the dog was alone with the stranger (Tukey-Kr; DF=13.9; t=3.05;

p=0.02) (Figure 2). "Proximity seeking" behaviours were also more common in the presence of the puppy raiser than in the presence of the stranger (Student's t-test; DF=1; t=2.66; p=0.03). To support these results, significant differences were found between episodes for "search behaviour" (GLMM; DF=3; F=11.00; p=0.01) and "door proximity" (GLMM; DF=2; F=11.98; p<0.001). These behaviours were significantly less common in the presence of the puppy raiser and when the dog was in the presence of the stranger than when the dog was alone see Figure 3. Contrary to findings reported in the literature [15], data obtained for "greeting behaviour" were not significantly different across episodes (Student's t-test; DF=1; t=0.61; p=0.56), and a tendency was found for "physical contact" towards the stranger (Student's t-test; DF=1; t=-2.14; p=0.07).

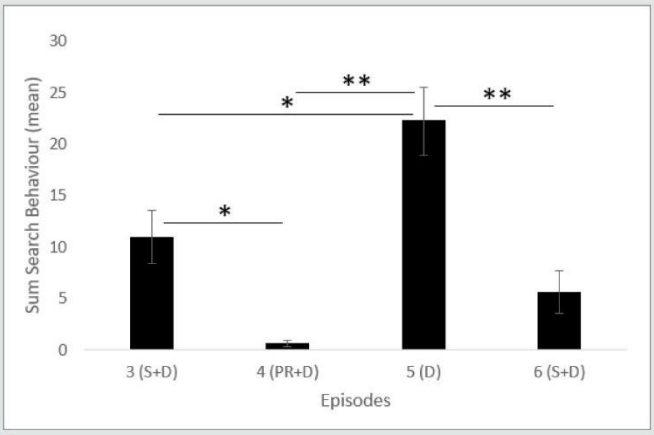

Figure 3: Episode effect on search behaviour. The mean ± standard error. S = Stranger; PR = Puppy Raiser; D = dog. *p < 0.05; **p < 0.01.

Psychometric scales

On the psychometric scales, no significant positive correlation was found between the responses of puppy raisers and educators. However, moderate negative correlations were detected for the reactivity score (r = -0.69; p = 0.06) and aggressiveness/response to novelty (Tables 3,4).

Table 3: Table of descriptive data and correlations between the results of the scores on the DIAS completed by the puppy raisers (PR) and the educators (ED).

	Mean ± Std error		Median		Min-Max		Correlation coefficient
	PR	ED	PR	ED	PR	ED	
Behavioural regulation	0.36 ± 0.02	0.46 ± 0.06	0.36	0.41	0.26-0.46	0.28-0.84	0.06
Aggression/response to novelty	0.31 ± 0.03	0.39 ± 0.03	0.28	0.40	0.24-0.44	0.24-0.48	**-0.44**
Responsiveness	0.73 ± 0.04	0.62 ± 0.03	0.74	0.60	0.56-0.88	0.50-0.80	**-0.69**
Overall questionnaire score	0.49 ± 0.02	0.52 ± 0.04	0.48	0.49	0.38-0.58	0.41-0.80	0.36

Table 4: Table of descriptive data and correlations between the results of the scores on the PANAS completed by the puppy raisers (PR) and the educators (ED).

	Mean ± Std error		Median		Min-Max		Correlation coefficient
	PR	ED	PR	ED	PR	ED	
Negative activation	0.34 ± 0.03	0.53 ± 0.05	0.32	0.53	0.24-0.48	0.36-0.76	-0.21
Positive activation	0.59 ± 0.04	0.53 ± 0.07	0.56	0.49	0.42-0.74	0.33-0.85	-0.03
Energy and interest	0.82 ± 0.06	0.60 ± 0.06	0.88	0.58	0.53-0.95	0.30-0.90	0.14
Persistence	0.39 ± 0.03	0.43 ± 0.10	0.40	0.33	0.25-0.50	0.20-0.90	0.05
Excitement	0.55 ± 0.08	0.53 ± 0.11	0.55	0.55	0.30-0.90	0.10-1.00	0.17

Discussion and Conclusion

The objectives of this preliminary study were to investigate attachment behaviours in guide dogs and determine whether the impulsivity and sensitivity scales could be reliable indicators of the emotional state of these animals in this context of shared-custody dogs. The attachment relationships between dogs and their puppy raisers were assessed using the modified version of the Ainsworth SST-R. In this study, no inappropriate or pathological attachment was observed in the dogs, who expressed a preference for their puppy raisers. Regarding the psychometric scales, both the educators and the foster family were asked to complete the questionnaires to compare scores, but unexpectedly, no correlation was found, asking about the most reliable person to answer these questionnaires.

In the results obtained for the attachment test, comparisons of "secure base effect" behaviours according to episode revealed a higher occurrence of these behaviours in episode 2 (stranger and puppy raiser) than in episode 3 (stranger). This indicates that in the presence of an unknown person, puppy raisers facilitate their puppies' coping strategies, defined in psychology as a set of cognitive and behavioural efforts that individuals deploy in response to environmental variations they assess to be threatening. The presence of their attachment figure allows dogs to focus on the environment and interact with it more serenely. No significant differences in the "secure base effect" were found between episodes in which the dog was alone with the stranger (episode 3) and when it was alone with its puppy raiser (episode 4), unlike the results found and for both pet and working dogs (search and rescue dogs). In the present study, the presence of a stranger without established attachment with the dog did not prevent the dog from interacting confidently with the environment. Due to their training background, these dogs are subject to strong socialization and to high human contact from an early age and are not alone more than 4 hours per day. Subjects in the study [2] had a less disturbed routine, spending a good part of their days with only their owner(s) or alone at home, which could explain this discrepancy in results. Episodes 2 (stranger and puppy raiser) and 4 (puppy raiser) were also similar, as expected. Indeed, as long as the puppy raiser was present, the company of a stranger did not have a significant impact on the behaviours, indicating a secure base effect.

Despite a similar secure base effect in episodes 3 and 4, the dogs exhibited more searching behaviours, including "door orientation", when the puppy raiser was not in the room (episode 3). And he has shown that with secure attachment, searching behaviours are more common in the absence of the owner. In this study, a preference for the puppy raiser should be noted, and the preference may result in a functional attachment. These behaviours also occurred more often when the subjects were alone in the room than when in the presence of only the stranger. This observation may show that a person, even if unknown, is able to reduce the expression of certain behaviours expected during a separation from the owner. Some difficulty in remaining alone may be expected if attachment is too strong, as mentioned above. The conclusions are similar for time spent near the door; human presence was preferred over being alone, resulting in a large amount of time spent in the area near the door when the dog was alone.

In accordance with what is found in the literature [7,14] , more "proximity seeking" behaviour was exhibited towards the puppy raiser than towards the stranger during the test. These behaviours are representative of a visual search, looking for someone. This could represent the previously mentioned coping strategy, facilitating the return to exploration or other interactions with the environment (a secure base effect) after searching for the attachment figure. Physical contact, which is associated with an attachment bond [15,22], was not significantly higher for the puppy raiser and even showed a reverse trend during the test. In the absence of their puppy raiser, the dogs tended to interact with the unknown person through physical contact while performing searching behaviours (orientation towards the door, proximity to the door) simultaneously. The dogs moved between the door and the person, so physical contact could be interpreted here as a sign of seeking comfort and therefore be emotionally palliative, as previously discussed and is on one of their study. Finally, the times spent greeting the puppy raiser and the stranger when they entered the room were also comparable, contrary to expectations and the results, which showed that dogs generally spend more time greeting and in physical contact with their owners. The training received by these dogs and breed selection (St. Pierre) aim to foster close relationships between these animals and humans and to encourage the dogs to spontaneously seek contact, even with unknown people, which could justify these results.

One of the most important steps in the breeding of a guide dog is the period during which it lives with its foster family [15], which is why this study focused on the relationship that dogs can establish with their primary raisers. The foster family represents a particular situation in which the puppy raiser is not the real owner of the animal. Foster families, unlike dogs, are warned of the temporary nature of the relationship and tend to develop an avoidant type of attachment as a form of resistance in contrast to the animal, which develops attachment as any other puppy would with its new family. Accordingly, the first bonds of attachment that the animal establishes with humans-the puppy raiser in this case-may impact the mental balance of the dog and the implementation of coping strategies adapted to its work as a guide dog, allowing it to effectively manage daily challenges and respond adequately to unforeseen circumstances. Thus, the foster family is considered a very important element in the breeding of future guide dogs [12]. In this pilot study, the attachment between the dogs and their puppy raiser seemed balanced and functional, as the dogs recognized their puppy raiser as the person caring for them (feeding them, playing, etc.). The dogs also seemed to manage the strange episodes in the presence of an unknown person.

These dogs, generally benefiting from good socialization, would have greater interest in foreign persons than do pets, which agrees with the results of previous studies [5,16]. Desire for human contact makes it easier for these dogs to compensate and bridge the gap with

another human when their puppy raiser is absent. They frequently experience separations from their puppy raiser (training period with the educator at school) and interact with different people. It is important to note that these changing environments may raise questions about their well-being and the consequences it may have on the quality of their work. Several works [18,17] have shown that good welfare is linked to abilities and training performance in guide dogs. For this reason, it would be interesting to assess, in a future study, the impact of these multiple separations on the welfare of the dogs and thus on their ability to perform as guide dogs.

Puppy raisers and educators also completed psychometric scales to assess levels of impulsivity (DIAS) and sensibility (PANAS). A good emotional balance is fundamental to obtaining a good guide dog [11]. The aim here was therefore to determine whether these scales could be relevant tools and good indicators of the emotional state and so of the success of these dogs. No statistical positive correlation was found between the scores given by the educators and the puppy raisers in this context. In particular, "reactivity" and "aggressiveness/response to novelty" showed a moderate negative correlation. The reactivity score was higher in the answers of foster families answer, and the aggressiveness score was in those of the trainer. Several factors could explain these results. Indeed, even if the dogs live with the foster families, dog trainers are more experienced in evaluating dogs and could tend to be more demanding towards them because certain behavioural traits must be exhibited before a deadline in order for a dog to receive its certification. Another reason could be that the dogs act differently depending on their environment. They could learn that the foster family's home is a more permissive environment than the guide dog school. The limits given by the educator may be more specific, clearer and more comprehensible than those given by foster families. Finally, dogs are not exposed to the same stimulations in the same way. In fact, dog trainers are used to anticipating dogs' reactions to external stimulation, which may reduce the reactivity of the dog, but puppy raisers have less experience in this domain.

In this context, it is difficult to use these psychometric scales on guide dogs in an ongoing training program. It would be interesting to use a larger sample to determine whether there is a relationship between the results of these scales (from puppy raisers or dog trainers) and a dog's success, and whether the type of relationship established with the puppy raiser enables a lasting and complementary guide dog/child dyad. These results could be useful for identifying dogs with undesirable behaviours as soon as possible, to exclude them from the training program to avoid financial losses, or to focus the education on problematic behaviours.

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Ethics Approval Statement:

This project was approved by the Research Institute in Semiochemistry and Applied Ethology (IRSEA) Ethics Committee (approval number CE_2020_01_ADOB01).

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2.3 Discussion

The results of the strange situation test showed that, even in this context, dogs develop an attachment to their foster families. The dogs exhibited better coping strategies in the presence of the puppy raiser, who acted as a secure base effect. However, unlike pet dogs, these dogs used the stranger to receive some degree of comfort via physical contact when they were alone with the stranger, thereby highlighting the difficulty of being alone. This behaviour could be explained easily by their training and the suggestion that educators make to the foster families by encouraging them bring the dog everywhere they go. Dogs are not familiar with being alone, which could explain these results. Unfortunately, we could not take advantage of the psychometric scales because there was no correlation between the answers of educators and puppy raisers, who exhibited disagreement.

The existence of an attachment bond raises questions concerning the quality of this attachment and the impact that can have on puppies' success as guide dogs.

CHAPTER 3: Could the success of guide dogs be influenced by the bond they developed with their puppy raisers?

Fanny Menuge, Míriam Marcet-Rius, Eva Teruel, Emeline Gautier and Patrick Pageat

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3.1 Introduction

To pursue our constant aim of decreasing the number of failures, we desired to deepen our understanding of the attachment bonds that emerge between foster families and prospective guide dogs. As the dogs' first interspecies relationships, these bonds constitute the foundation on which the dog's future relationships are constructed. The importance of the infant-caregiver bond to the development of the individual has been demonstrated repeatedly, as have similarities between these two species in terms of their expressions of attachment. We therefore wondered what kind of attachment develops between dogs and their foster families and whether this attachment could be associated with the dogs' success or failure. In accordance with recent publications, we used Ainsworth's strange situation test to classify dogs according to their attachment pattern, i.e., secure or insecure. Additionally, the Pet Attachment Questionnaire was employed to obtain scores for avoidance and anxiety from the puppy raiser regarding the dog.

COULD THE SUCCESS OF GUIDE DOGS BE INFLUENCED BY THE BOND THEY DEVELOPED WITH THEIR PUPPY RAISERS?

Fanny Menuge[1], Míriam Marcet-Rius[1], Eva Teruel[1], Emeline Gautier[1], Patrick Pageat[1]

[1] Research Institute in Semiochemistry and Applied Ethology (IRSEA), Quartier Salignan, 84400 Apt, France;

f.menuge@group-irsea.com

The authors declare no conflict of interest.

Puppy raisers have a decisive role in the development of guide dogs (Koda, 2001). It is the animal's first interspecific attachment bond, which could constitute an important emotional base for their mental stability, essential for these dogs (Naderi et al., 2001). The purpose of this study was to evaluate the influence of this bond on their success, i.e., if the dogs were graduated or not as guide dogs.

Sixteen potential guide dogs and their puppy raisers participated in the study. The revised version of the Ainsworth Strange Situation Test (SST-R) was used to assess the attachment type of the dog toward its puppy raiser, i.e., secure or insecure. The Pet Attachment Questionnaire was also completed by puppy raisers, giving an avoidant score and an anxious score. Finally, the success or failure of each dog was noted. Parameters were analysed using logistical regressions.

The observed trend suggested that the higher the puppy raiser avoidant score was, the more likely the dog was to succeed (DF = 1; X^2 = 3.23; p = 0.07). Also, descriptive data revealed that 50% of the insecure dogs failed compared to 25% of the dogs classified as secure.

The strong imbalance between groups (4 insecure vs 12 secure; 5 failure vs 11 success) added to the small sample size did not allow to conclude at a statistical level. Nevertheless, descriptive data suggested that attachment could be a relevant indicator, but additional studies are needed to verify the link between attachment and success of guide dogs.

3.3 Discussion

For this experiment, the number of individuals was too low to allow us to observe significant results, but we could nevertheless observe interesting descriptive results. The experiment revealed that a high avoidance score from the puppy raiser tended to be linked to the success of the dog. The fact that the puppy raiser was more distant could be beneficial to guide dogs, which allowed them to cope better with separation and to develop good emotional balance. In this way, dogs could be free to express their behaviours and the skills they acquired during training. In this study, we also observed from the strange situation test that most prospective guide dogs developed a secure attachment bond with their puppy raiser, indicating that a relationship that is not excessively founded on contact requests from the puppy raiser could be positive and could thus allow the dogs to be more secure. Finally, we also noted that dogs who developed a secure attachment style had a greater chance of being successful than did those who developed an insecure attachment style. Given these results, this study could provide a way of exploring the link between the quality of attachment and the welfare and skill development of these animals, with the aim to decrease the number of failure.

Most prospective guide dogs develop a secure attachment style with their foster family. For the following study, we focused on the potential impact of a discontinuity in this link during the training period.

CHAPTER 4: Repeated separations between a future guide dog and its foster family modify stress-related indicators and affect dog's focus

Fanny Menuge, Míriam Marcet-Rius, Camille Chabaud, Eva Teruel, Cécile Berthelot, Galice Kalonji, Cécile Bienboire-Frosini, Tiago Mendonça, Eric Lascar and Patrick Pageat

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4.1 Introduction

Collaboration with the Frederic Gaillanne Foundation enabled us to evaluate the efficiency of their housing management for dogs involved in an ongoing training programme. Dogs remained at the school from Monday to Friday to received their education and returned on weekends to their foster family, with whom the dogs had established an attachment bond. This system is easier to manage for the guide dog organization, and dogs are allowed to maintain a link with a family environment. Nevertheless, this schedule requires repeated separations from the foster family as well as weekly changes in environment and routine. By reference to behavioural and physiological parameters, this study aimed to evaluate the consequences of these changes on the welfare and performance of these animals.

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Repeated separations between a future guide dog and its foster family modify stress-related indicators and affect dog's focus

F. Menuge [a,*], M. Marcet-Rius [a], C. Chabaud [b], E. Teruel [c], C. Berthelot [a], G. Kalonji [a], C. Bienboire-Frosini [b], T. Mendonça [d], E. Lascar [e], P. Pageat [f]

[a] Animal Behaviour and Welfare Department, Research Institute in Semiochemistry and Applied Ethology (IRSEA), Quartier Salignan, 84400 Apt, France
[b] Molecular Biology and Chemical Communication Department, IRSEA, Quartier Salignan, 84400 Apt, France
[c] Data Management and Statistics Service, IRSEA, Quartier Salignan, 84400 Apt, France
[d] Centre for Clinical Ethology and Animal Welfare (CECBA), Quartier Salignan, 84400 Apt, France
[e] Frederic Gaillanne Foundation (FGF), 150 chemin de la Tour de Sabran, 84800 L'Isle-sur-la-Sorgue, France
[f] Research and Education Board, IRSEA, Quartier Salignan, 84400 Apt, France

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ABSTRACT

In some guide dog organisations, future guide dogs for blind individuals are required to undergo separation from their foster family from Monday to Friday as part of their training. These separations and the constantly changing environment may induce stress, thus impacting the welfare of these dogs and their performance. The aim of this study was therefore to evaluate this stress through physiological and behavioural measures. The results showed a significant increase in salivary cortisol levels at the time of separation (GLMM; DF = 2; F = 10.31; $p < 0.0001$). Additionally, the dogs were more passive on Friday than on other days (GLMM; DF = 2; F = 7.53; p = 0.0090), and "head movements" were expressed less frequently on Fridays (GLMM; DF = 2; F = 5.12; p = 0.0141). Performance increased across weeks, despite a lower "focused" score on Mondays (GLMM; DF = 2; F = 4.39; p = 0.0243). These results showed both adaptation to life in the kennel and that the dogs need to readapt to the situation each week. The increase in serotonin levels during the 3 weeks of testing (GLMM; DF = 2; F = 4.87; p = 0.0224) may also indicate that the dogs can adapt to the kennel environment. Therefore, this study questions the relevance of noncontinuous training programmes. In future research, it would be interesting to compare these results with those of a group of dogs staying at school on weekends.

1. Introduction

Animal welfare has now become an important issue in society. According to its definition, welfare must consider the physical and mental state of the individual (Carenzi and Verga, 2009) and recognise the importance of subjective experience (Lund et al., 2006). While improving animal welfare has an intrinsic primary goal, it also attracts interest due to its role in increasing productivity, particularly that of farm animals (Waiblinger et al., 2006), and the performance of working animals (Rooney et al., 2009; Vincent and Leahy, 1997). In the latter category, guide dogs for blind individuals are examples of animals that must excel in terms of learning ability but also in terms of emotional balance and being able to cope with emotional distress (Naderi et al., 2001). Indeed, their main purpose is to accompany a visually impaired person everywhere they go (Mariti et al., 2013). Calm, initiative and control when facing attractions are therefore essential characteristics for being a good guide dog and guaranteeing the safety of the visually impaired person in any situation (Koda et al., 2011).

To ensure the combination of these qualities, dogs are selected on genetic and behavioural criteria (Serpell and Hsu, 2001; Svartberg, 2006). Unfortunately, approximately 40% of the dogs intended for this work do not become guide dogs, considering also the reforms due to physical ailments or genetic diseases (Mengoli et al., 2017). However, most of the failure are due to behavioural reasons, as distractibility, fearfulness, shyness or aggressivity (Arata et al., 2010; Goddard and Beilharz, 1982). Many studies have examined the factors affecting the failure and success of these dogs (Batt et al., 2008; Bray et al., 2017; Caron-Lormier et al., 2016), but very few consider the context of their development and training. Indeed, these animals follow an atypical course, since foster families take care of the puppies from the time of

* Corresponding author.
E-mail address: f.menuge@group-irsea.com (F. Menuge).

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weaning until the dogs are approximately 1 year old. Therefore, the foster family represents a key element in a puppy's development (Dalibard, 2009; Koda, 2001; Menuge et al., 2021). At around this age, the dog will begin training at a guide dog school with a canine educator for approximately 6–8 months (Alterisio et al., 2019; Arata et al., 2010). The organisation of this period is managed differently according to the institution and country. In some cases, the dogs leave their foster family permanently to stay in the kennel at the training school (Arata et al., 2010; Chur-Hansen et al., 2015), and in other cases, the dogs obtain their education during the week and return to their foster family on the weekends (Dalibard, 2009). In the latter case, the dogs continue to experience a family environment. However, it also subjects the animals to repeated separations from their main attachment figure. Even if they express it in a more controlled way than pet dogs do (decreasing unnecessary excitability is part of their training), guide dogs develop a strong attachment bond with their owner or foster family (Fallani et al., 2006), while foster families tend to develop an avoidant attachment style to a dog from which they have to separate after one year (Oliva et al., 2019). According to Fallani et al. (2006), the breakdown of the bond with the foster family does not have a negative impact on learning ability or the capacity to re-establish an equilibrate bond with humans. However, weekly separations, accompanied by a change in environment, could have an influence on the animal's well-being, particularly by inducing stress. This stress, even on an on-off basis, could impact the performance of the dogs, especially on Mondays, the day of separation. Given the importance of providing additional keys to organisations raising guide dogs to enable them to reduce their failure rate, it is necessary to consider how the performance of dogs could be optimised. Therefore, the aim of this study was to assess the impact of repeated separations on the welfare and performance of guide dogs during training. Physiological and behavioural data representative of the emotional state of the dogs were compared between the day of separation and other days of the week. This research was carried out in collaboration with the Frederic Gaillanne Foundation (FGF—L'Isle--sur-la-Sorgue, France), whose guide dogs return to their foster families on weekends.

2. Materials and methods

The study protocol was approved by the French Ministry of Research (APAFIS#24626-2020031113263522 v2).

2.1. Animals

Nine healthy potential guide dogs (7 males and 2 females) in the training programme of the FGF participated in this study. The dogs were either St-Pierre, Labernois (Labrador/Bernese Mountain dog cross) or Labrador. All dogs followed the same course before integrating the training program. They were homed on their foster family after the weaning, at 2 months, and followed puppy classes at the FGF 1 or 2 times a month. At 1 year old, they passed a behavioural test to evaluate their reaction face to external stimulations, as noise, or a strange person. Some of them could be reformed at this moment and when their behaviour was not appropriate, they could not integrate the training program.

During this study, they were all between 12 and 24 months old and started their training at the school 6 weeks prior to this study. All dogs underwent their training programme at the school during the week and returned to their foster family on the weekend. They ate 2 times a day (morning and evening), and the dog school provided food (kibble) to the foster family.

2.2. Experimental design

Physiological and behavioural data were collected over a period of 3 consecutive weeks (see Table 1).

Table 1
Summary of data collection days for each parameter.

	Monday	Wednesday	Friday
Saliva	T1: after the separation from foster family (between 8:15 a.m. and 9:00 a.m.). T2: after training session (between 9:00 a.m. and 12:00 p.m.). T3: after a period of rest (between 1:30 p.m. and 2:00 p.m.).		T1: between 8:15 a.m. and 9:00 a.m. T2: after training session (between 9:00 a.m. and 12:00 p.m.). T3: after a period of rest (between 1:30 p.m. and 2:00 p.m.).
Blood sampling		x	
Weight	x	x	x
Behaviour at rest	x	x	x
Behaviour during training	x		x
Performance	x	x	x

Salivary cortisol: Saliva was collected by using the Salivette® system (Salivette®, Sarstedt, Numbrecht, Germany) specifically dedicated to the determination of cortisol in saliva. The study design was repeated on Monday and Friday morning to control for the natural influence of the circadian rhythm on cortisol secretion at the time of the experiment. 20 min is the time that takes to the cortisol to be secreted in saliva (Dreschel and Granger, 2009; Hernandez et al., 2014), so samples were carry out to suits this timing. To prevent sample contamination, the dogs did not eat for 1 h prior to sampling. On Monday, the foster families fed the dog more than an hour before arriving at the foundation, and on Friday, the dogs were fed after the saliva sample was taken. If the presence of blood was visually observed in the saliva samples, the samples were discarded. Immediately after the samples were collected, they were stored at 4 °C until they were centrifuged (2 min, 1000 rpm, 4 °C). After centrifugation, the samples were frozen at −20 °C until the day of the cortisol assay, which was performed using a Salimetrics® Cortisol Enzyme Immunoassay Kit (Kiel, Germany) according to the manufacturer's guidelines.

Blood sampling: Up to 15 ml of blood was collected from the cephalic vein on the forelimb using a 21 G needle to assess the level of free oxytocin, prolactin, serotonin, and the neutrophil/lymphocyte ratio (N/L ratio); the blood was collected by a veterinarian on Wednesdays mornings, once a week for welfare considerations. For the hormonal analyses, blood was collected into prechilled EDTA-aprotinin tubes (BD® tubes, Elvetec, Pusignan, France) for plasma oxytocin determination and into tubes with a gel separator (Vacuette®, Greiner Bio-One, Alcyon, Paris, France) for serum prolactin and serotonin determination. The tubes were immediately stored in an ice box where they remained at 4 °C until centrifugation (1800 g, 12 min at 4 °C). Plasma or serum was recovered and stored at − 20 °C until the analyses. The free plasma oxytocin concentration was determined with the commercially available Oxytocin ELISA kit from Cayman Chemical (Arbor Inn, MA, USA) after solid-phase extraction on C18 columns (Hypersep 1 g, Thermo Fisher Scientific, Illkirch, France) following the procedure described in Oliva et al. (2019). Serum prolactin was assayed with a Prolactin (Canine) ELISA kit (Demeditec, Kiel, Germany) following the manufacturer's instructions. Serum serotonin was assayed with the commercially available Serotonin ELISA kit from Enzo Life Science (Villeurbanne, France), which was previously validated for this species (Chabaud et al., 2018). For the N/L ratio, a complete blood cell count was carried out with an automated LaserCyte from IDEXX (Westbrook, MA, USA) on the day of blood sampling using EDTA tubes provided by the manufacturer (IDEXX VetCollect Tubes). The total white blood cell count, including neutrophil and lymphocyte counts, was recorded, and the N/L ratio was calculated from these data.

Weight: Dogs were weighed at the same time each day.

Behaviours: Dog behaviours related to stress or positive mental state were reported during a period of rest and during their morning training session (Table 2). Concerning resting behaviours, dogs were videotape for 45 min on Mondays, Wednesdays and Fridays on their boxes between 12:00 p.m. and 1:00 p.m. For the videotape, dogs were 3 per boxes (8 m^2 each) and were placed in the same box for the 3 weeks of testing. All behaviours were analysed by 2 independent observers using Boris software (Friard and Gamba, 2016). Concerning behaviours in training session, they were observed by direct observation by the same observer throughout the study. Behaviours were defined as state events (with duration) or point events (frequency of occurrence).

Performance: A visual analogue scale developed by the experimenter was completed by the dog trainer professional after each dog's morning training session, scoring the dogs between 0 and 10 on eight criteria (focused, stressed, agitated, attentive, effective, distracted, posture and an overall note from the education session).

Table 2
Ethogram of dog behaviour assessed during periods of rest and during training session.

Behaviour	Description	References
	Behaviours during period of rest	
Locomotion (SE)	Walking, pacing, or running around without exploring the environment or playing.	Fallani et al. (2007)
Panting (SE)	Rapid shallow breathing (mouth open).	Beerda et al. (1998)
Grooming (SE)	The action of cleaning the body surface by licking, nibbling, picking, rubbing, etc., directed at the animal's body (self-grooming).	Beerda et al. (1998); Palestrini et al. (2017)
Exploration (SE)	Activity directed towards physical aspects of the environment.	Fallani et al. (2007); Palestrini et al. (2017)
Social play (SE)	Any vigorous behaviour performed when interacting with another dog, including running, jumping and active physical contact.	Fallani et al. (2007)
Passivity (SE)	Lying down with the head on ground without any obvious orientation towards the physical or social environment.	Palestrini et al. (2017)
Head movement (PE)	Characteristic movement sidewards and downwards.	Schilder and Van Der Borg (2004)
Withdrawal (PE)	Avoiding interaction with another dog by either moving away, very clearly turning away or looking away.	Palestrini et al. (2017)
Barking (PE)	Barking.	Beerda et al. (1998); Stellato et al. (2016)
Whining (PE)	Throaty, low-frequency sound.	Beerda et al. (1998); Stellato et al. (2016)
Yawning (PE)	Wide opening of mouth.	Beerda et al. (1998); Palestrini et al. (2017)
Lip licking (PE)	Portion of the tongue is shown and moved along the upper lip.	Beerda et al. (1998); Palestrini et al. (2017)
	Behaviours during training session	
Lip licking (PE)	Portion of the tongue is shown and moved along the upper lip.	Beerda et al. (1998); Palestrini et al. (2017)
Yawning (PE)	Wide opening of mouth.	Beerda et al. (1998); Palestrini et al. (2017)
Head movement (PE)	Characteristic movement sidewards and downwards.	Schilder and Van Der Borg (2004)
Body shaking (PE)	Body shaken.	Beerda et al. (1998); Stellato et al. (2016)
Panting (SE)	Rapid shallow breathing (mouth open).	Beerda et al. (1998)

SE = State event; PE = Point event.

2.3. Statistical methods

Data analysis was conducted with SAS 9.4 software (Copyright (c) 2002–2012 by SAS Institute Inc., Cary, NC, USA). The significance threshold was fixed at 5%.

For salivary cortisol, the effects of week, day and time were studied. For oxytocin, serotonin, prolactin and the N/L ratio, the effect of week was studied. For weight, rest and training behaviour and performance, the effects of week and day were evaluated. As all these factors were measured several times in the same dogs, mixed models were used to analyse the data and the dog was considered a random effect.

For continuous data (cortisol, oxytocin, serotonin, prolactin, N/L ratio, behaviours (state events) and performance scores), general linear mixed models (GLMMs) were constructed with raw data when the assumption of model residue normality was verified. When this assumption was violated, a Box-Cox transformation was applied to the data, and the GLMM was constructed with the transformed data. For discrete data (point events), mixed Poisson regressions were applied when the data were appropriately dispersed according to the Pearson chi-square/DF value. When overdispersion was detected, a negative binomial mixed model was preferred because this sort of model allows a dispersion scale parameter to account for overdispersion problems.

For the remaining behaviours, the interobserver reliability between two independent readers was assessed for each parameter by computing the square of the Pearson correlation coefficient. Few dogs were observed exploring, withdrawing, barking, whining, or grooming. These variables were not analysable quantitatively and were transformed into binary variables. Then, mixed logistic regression analyses were performed.

For each model, the best covariance structure was selected by minimising the corrected Akaike information criterion (cAIC) and Bayesian information criterion (BIC), and multiple comparisons were computed using the Tukey-Kramer adjustment.

3. Results

3.1. Salivary cortisol

For salivary cortisol, day effect (GLMM; DF=1; F=8.45; $p = 0.0043$), time effect (GLMM; DF=2; F=4.54; $p = 0.0124$) and day*time interaction was significant (GLMM; DF=2; F=10.31; $p < 0.0001$). The level of salivary cortisol was significantly higher on Monday than on Friday, and

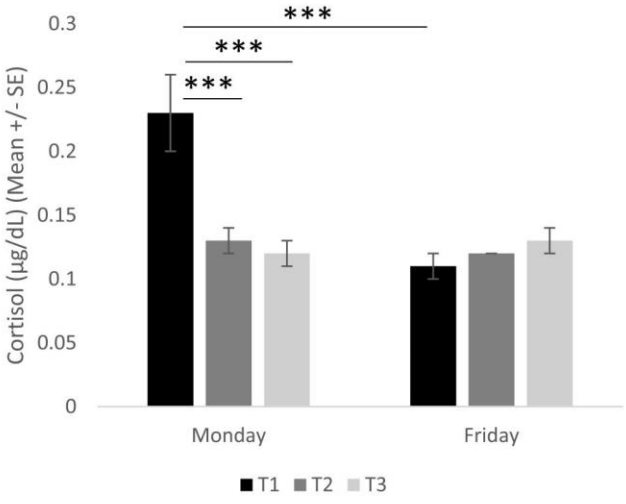

Fig. 1. Mean salivary cortisol concentration on Monday and Friday over the three weeks. T1 = after the separation from puppy raiser (or equivalent time for Friday); T2 = after their training session; T3 = after a period of rest. *** = $p < 0.001$.

particularly on T1 (separation time) (Fig. 1). Week effect was no significant (GLMM; DF=2; F=1.65; p = 0.1961).

3.2. Blood analysis

For the level of serotonin, a significant week effect was present (GLMM; DF=2; F=4.87; p = 0.0200). The level of serotonin was significantly lower at week 1 than at week 2 (Tukey-Kr; DF=16; t = −2.14; p = 0.0481) and week 3 (Tukey-Kr; DF=16; t = −3.04; p = 0.0079) (Fig. 2a).

For the level of prolactin, atypical values were found for one dog for each of the 3 weeks. This dog was excluded from the statistical analysis for this parameter. The week effect was significant (GLMM; DF=2; F=6.05; p = 0.0100). The level of prolactin was lower at week 3 than at week 1 (Tukey-Kr; DF=15.4; t = 2.75; p = 0.0146) and week 2 (Tukey-KR; DF=13.7; t = 3.44; p = 0.0041) (Fig. 2b).

No significant difference was observed among the three weeks for oxytocin (GLMM; DF=2; F=0.18; p = 0.8342) or the N/L ratio (GLMM; DF=2; F= 0.88; p = 0.4347) (Figs. 2c and 2d).

3.3. Weight

For weight, the day effect was significant (GLMM; DF=2; F=10.11; p = 0.0100). The average weight (μ) of the dogs over the 3 weeks was higher on Monday than on Wednesday (μ_{Monday} = 33.78 ± 0.85 kg vs. $\mu_{Wednesday}$ = 33.43 ± 0.83 kg; Tukey-Kr; DF=14.2; t = 4.60; p = 0.0004) and Friday (μ_{Friday} = 33.43 ± 0.82 kg; Tukey-Kr; DF=14.5; t = 3.97; p = 0.0013). Week effect was no significant (GLMM; DF=2; F=1.41; p = 0.2771).

3.4. Behaviour at rest

The statistical analysis of interobserver reliability revealed strong associations for all the parameters (>90%). Thus, the average of the data collected by the two readers was used for the rest of the analysis.

Panting, play behaviour and withdrawal were not expressed enough to the statistical analysis.

For the time spent in "passivity", the effect of day was significant (GLMM; DF=2; F=7.53; p = 0.0100). The time spent in "passivity" was higher on Friday than on Monday (Tukey-Kr; DF=14.3; t = −3.61; p = 0.0027) and Wednesday (Tukey-Kr; DF=9.43; t = −2.32; p = 0.0443). Conversely, the effect of day was significant for "head movement" (GLMM; DF=2; F=5.12; p = 0.0100). "Head movement" was expressed less on Friday than on Wednesday (Tukey-Kr; DF=24; t = 3.20; p = 0.0039), and trend to be expressed less on Friday than on Monday (Tukey-Kr; DF=24; t = 1.90; p = 0.0699) (Table 3). For the other parameters, no significant difference was found (Table 3).

3.5. Behaviour in training

No significant difference was found between the days of the week for any behaviour during training, although "panting", tended to be more expressed on Monday (μ_{Monday} = 266.93 ± 65.21 s) than on Friday (μ_{Friday} = 148.56 ± 43.30 s) (GLMM; DF=1; F=3.93; p = 0.0827).

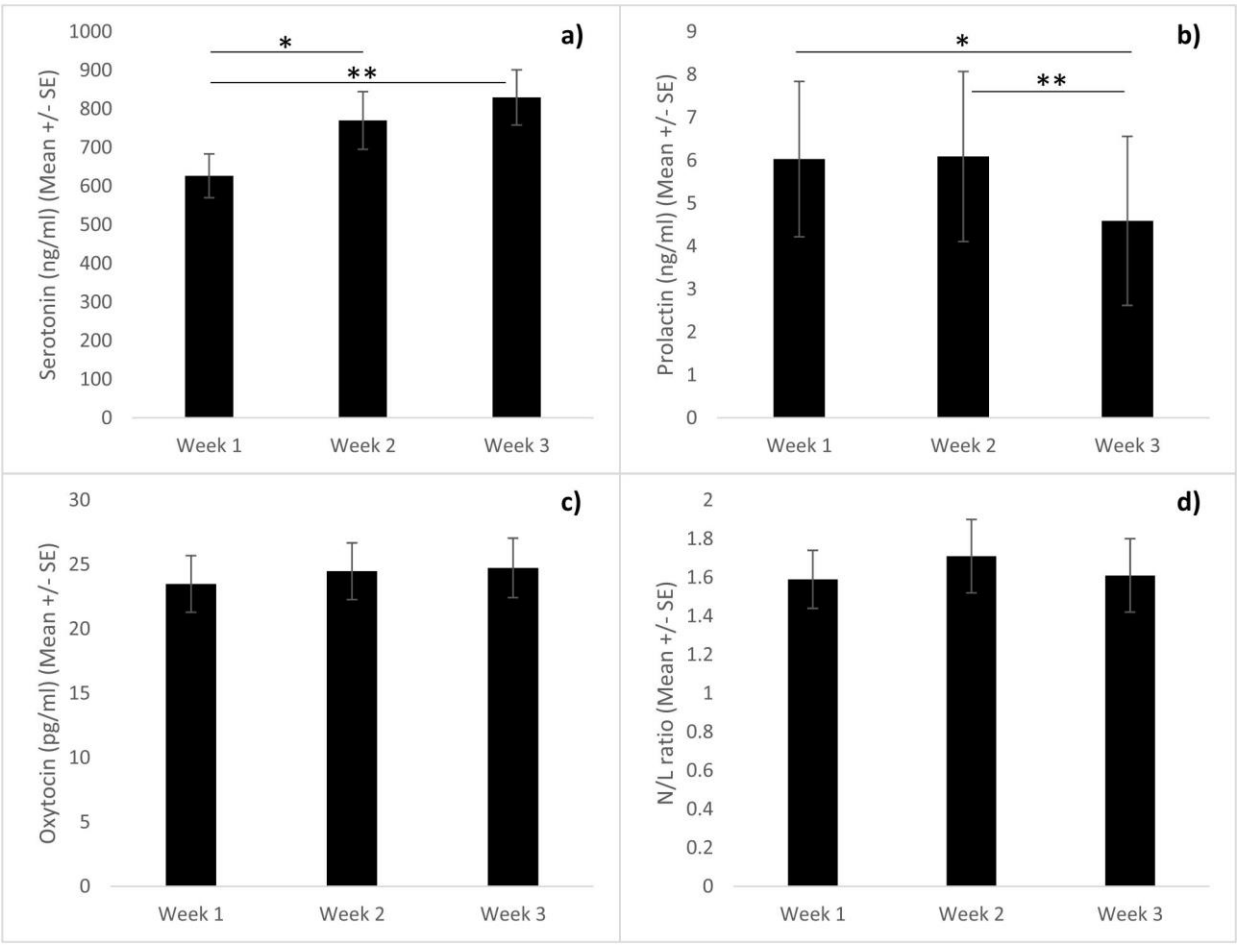

Fig. 2. Mean concentration of serotonin (a), prolactin (b), oxytocin (c) and neutrophil / lymphocyte ratio (N/L ratio) (d) over the 3 weeks of testing. ** = p < 0.01; *= p < 0.05.

Table 3
Descriptive data concerning behaviours at rest.

N = 27	Monday	Wednesday	Friday
n (%) or mean ± standard error			
Locomotion (SE)	16.18 ± 4.22	15.41 ± 3.43	8.86 ± 2.80
Passivity (SE)	2208.58 ± 88.33[a]	2290.96 ± 60.53[a]	2440.49 ± 53.61[b]
Head movement (PE)	25.44 ± 3.62[a]	29.15 ± 3.92[t]	15.43 ± 2.58[b t]
Yawning (PE)	1.41 ± 0.31	0.48 ± 0.23	1.54 ± 0.34
Lip licking (PE)	3.80 ± 1.13	2.52 ± 0.59	3.94 ± 0.94
Grooming[c]	15 (55.56)	14 (51.85)	19 (70.37)
Exploration[c]	6 (22.22)	7 (25.93)	6 (22.22)
Whining[c]	5 (18.52)	8 (29.63)	5 (18.52)

SE = State event; PE = Point event. [t]Trend to be different.
[a] vs
[b] Significantly different at a statistical level.
[c] Variables expressed in binary data (*n* = number of individuals having exhibited the behaviour).

3.6. Performance

A day effect was observed for "focused" (GLMM; DF=2; F=4.39; p = 0.0243) and "agitated" (GLMM; DF=2; F=6.18; p = 0.0151). A trend was also observed for "posture" (GLMM; DF=2; F=3.31; p = 0.0787), "attentive" (GLMM; DF=2; F=3.46; p = 0.0562) and "general note" (GLMM; DF=2; F=3.40; p = 0.0554) (Table 4, Fig. 3a).

A week effect was also observed for "focused" (GLMM; DF=2; F=10.97; p = 0.0008), "attentive" (GLMM; DF=2; F=5.00; p = 0.0158), "efficient" (GLMM; DF=2; F=9.00; p = 0.0014), "posture" (GLMM; DF=2; F=6.48; p = 0.0082), and "general note" (GLMM; DF=2; F=5.27; p = 0.0137) (Table 4, Fig. 3b). For the other criteria, no significant difference was observed.

Table 4
Performance criteria for which multiple comparisons yielded significant results.

Criteria	Week (W) or Day	Statistical results
Focused	W1 < W2	**Tukey-Kr; DF= 17.3; t = −3.71; p = 0.0017**
	W1 < W3	**Tukey-Kr; DF= 17.3; t = −4.33;**
	W2 = W3	**p = 0.0004**
	Monday < Wednesday	Tukey-Kr; DF= 17.3; t = −0.61; p = 0.5472
	Monday = Friday	**Tukey-Kr; DF= 24; t = −3.02;**
	Wednesday = Friday	**p = 0.0059**
		Tukey-Kr; DF= 24; t = −0.89; p = 0.3803
		Tukey-Kr; DF= 24; t = 1.55; p = 0.1337
Agitated	Monday < Wednesday	**Tukey-Kr; DF= 12.1; t = −2.88; p = 0.0138**
	Friday < Wednesday	**Tukey-Kr; DF= 11.8; t = 2.66;**
	Monday = Friday	**p = 0.0212**
		Tukey-Kr; DF= 12; t = 1.29; p = 0.2214
Attentive	W1 < W2	**Tukey-Kr; DF= 24; t = −2.89; p = 0.0080**
	W1 < W3	**Tukey-Kr; DF= 23.9; t = −2.87;**
	W2 = W3	**p = 0.0084**
		Tukey-Kr; DF= 24; t = 0.37; p = 0.7161
Efficient	W1 < W2	**Tukey-Kr; DF= 21.7; t = −3.65; p = 0.0014**
	W1 < W3	**Tukey-Kr; DF= 22.5; t = −4.03;**
	W2 = W3	**p = 0.0005**
		Tukey-Kr; DF= 21.7; t = 0.24; p = 0.8131
Posture	W1 < W2	**Tukey-Kr; DF= 16.8; t = −3.31; p = 0.0042**
	W1 < W3	**Tukey-Kr; DF= 16.8; t = −2.88;**
	W2 = W3	**p = 0.0104**
		Tukey-Kr; DF= 16.8; t = 0.42; p = 0.6765
General note	W1 < W2	**Tukey-Kr; DF= 22; t = −2.55; p = 0.0184**
	W1 < W3	**Tukey-Kr; DF= 23.9; t = −3.23;**
	W2 = W3	**p = 0.0036**
		Tukey-Kr; DF= 22; t = −0.34; p = 0.7340

Note: in bolt = significant results.

4. Discussion

The aim of this study was to determine whether it is appropriate to allow guide dogs in training to return to their foster families every weekend. This practice might allow dogs to "decompress" and return to a family environment. However, it also requires them to experience separation every Monday morning from their main attachment figure. These separations, added to the weekly environmental changes involved in this process, could induce stress in the dogs and thus have a negative impact on their welfare and learning abilities, especially on Monday, the day of separation. To investigate this issue, in the present study, physiological and behavioural parameters were assessed.

First, measurement of the salivary cortisol concentration at key moments made it possible to confirm that the hypothalamo-pituitary-adrenal axis was more activated in dogs on Monday morning at the time of separation from their foster family, leading to an increase in cortisol secretion compared to other times (during a training session or during a rest period in the kennel). It also showed that the dogs secreted more cortisol on Monday than on Friday morning at the same time. The latter result is important since it allows us to rule out the hypothesis that this higher value may be due to the effect of the circadian rhythm on the secretion of this hormone (Giannetto et al., 2014; Kolevská et al., 2003). Separation from the foster family therefore leads to an increase in the level of cortisol, a hormone known to be secreted during stressful situations (Beerda et al., 1998; Chmelíková et al., 2020). In all cases, the cortisol concentrations observed in this study were within the reference range for cortisol concentration in the saliva of dogs (Cobb et al., 2016).

The weight of the dogs revealed a low but significant difference between days. They were heavier on Mondays than on other days of the week. The schools provide the foster families with the dogs' kibble and tell them the prescribed amount. The dogs therefore do not experience a change in diet every week. This difference in weight may be due to different routines, treats given more frequently by the foster families on weekends, or more intense physical and mental exercise during the week, resulting in fat loss. The dogs did not lose weight over the course of the weeks of training. The impact of weight fluctuation on human health is well represented in the literature (Arnold et al., 2010; Lissner et al., 1991; Oh et al., 2019), but there is a lack of studies on the impact of weight fluctuation on dogs. It is thus important to control the animals' diet more effectively and to ensure that they benefit from daily physical activity to prevent them from developing health issues. However, the minor difference in weight in this case raises the question of the real impact of these variations on the dog's health.

Neuro-hormonal parameters were also assessed. A decrease in serotonin levels or an increase in prolactin levels have been suggested to indicate chronic stress or even depression in animals (Alberghina et al., 2019). In this study, an increase in serotonin and a decrease in prolactin were observed. Furthermore, contrary to the results of another study (Oliva et al., 2019), the prolactin level of these dogs was not observed to be higher than that of pet dogs but was in line with the normal concentration reported in the literature (Gutiérrez et al., 2019; Mengoli et al., 2021). The level of oxytocin and the N/L ratio did not vary over the course of the study. The plasma oxytocin concentration appears to be in line with what has been observed in assistance dogs (MacLean et al., 2017; Mengoli et al., 2021; Oliva et al., 2019). The stability of the values over the three weeks of testing may also indicate the dogs' ability to cope with their environment (Marcet Rius et al., 2018). Concerning the N/L ratio, the data corresponded to the values detected in healthy dogs (Hodgson et al., 2018; Rejec et al., 2017). According to those results, the dogs seem to be able to adapt to their living environment without their welfare being compromised. The shelter environment is very stressful for a dog. However, some parameters, such as human interaction, could compensate for the poor welfare associated with the kennel

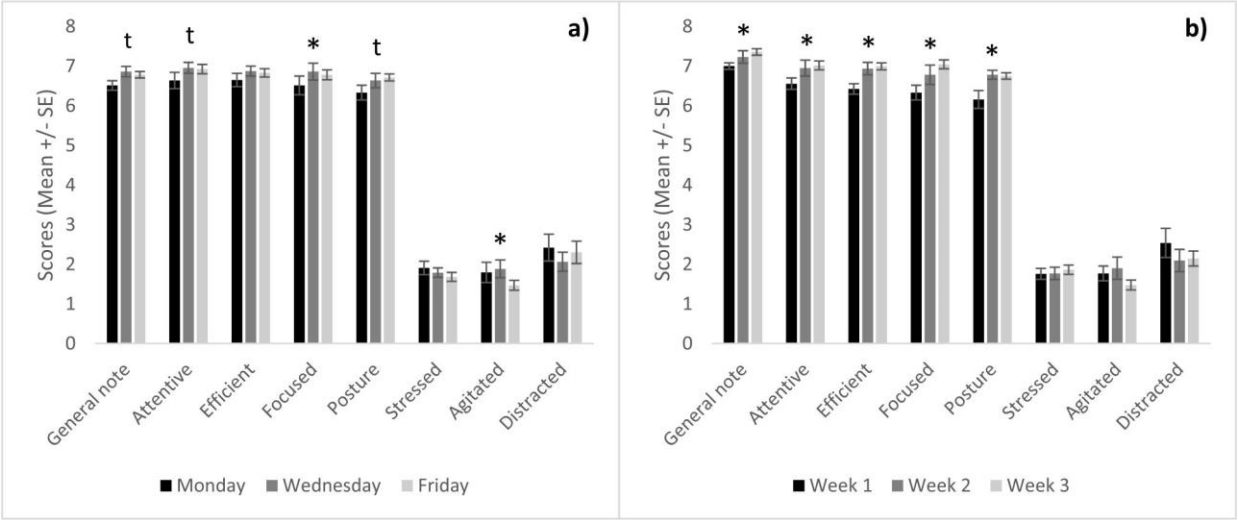

Fig. 3. Mean of score performance per day (a) and per week (b). *= significant difference. ^{t} = Trend (see Table 4 for more details).

environment, as Coppola et al. (2006) demonstrated. In the case of guide dog schools, dogs receive much stimulation through their training, which could greatly differentiate the living conditions of these dogs from those of shelter dogs.

The behavioural parameters studied also confirmed our hypothesis. During the daily rest period spent in the box between 12:00 p.m. and 1:00 p.m., which we selected for the study, the dogs were more passive on Friday than on other days. In addition, less head movement was observed. These results seem to indicate that after several days at school, the animals were calmer, less agitated and less alert and therefore possibly less stressed. Thus, the results suggest that dogs are able to adapt to this environment during the week.

Behaviours representative of a certain level of stress were also assessed during the training sessions. No significant difference was observed among days of the week. The first hypothesis to explain these results is that the dogs did not show signs of stress during the training session because they were not stressed. Analysis of salivary cortisol corroborates this hypothesis since the dogs did not secrete more cortisol during the training session than in their box. Only "panting" behaviour had a trend towards significance, with behaviour that was emitted more on Mondays than on Fridays. Panting is a sign that can be associated with stress (Beerda et al., 1997) or anticipation of a possible reward (Ng et al., 2014; Palestrini et al., 2017). It is therefore difficult to make conclusions based on this behaviour alone.

Finally, this project sought to determine whether these repeated separations had an impact on the animal's performance. The dogs were scored on different criteria after a training session by their trainer on Mondays, Wednesdays, and Fridays during the three weeks of testing. The results allowed the dogs' progress to be noted over the course of both days and weeks. However, it seems that on Mondays, the dogs are less able to learn new things and that they have decreased attention on this weekday. Based on the data collected, their performance does not decrease, but it also does not improve. Monday therefore seems to be a day of recontextualisation for the dogs rather than a truly productive day in the acquisition of new learning, and Wednesday could be the best day for learning. The exception for "Agitated" could be due to the fact that Wednesday was the day of the blood sampling, which could explain that the dogs were more agitated this day. In future research, it would be very interesting to compare the performance and speed of progress in the dogs returning to their foster families on weekends with those of a group that did not return to their foster family on weekends. Dogs that did not have a change in their environment would not need to readapt and would therefore be more willing to resume their education in better condition.

5. Conclusions

This study is part of a larger project, the aim of which is to optimise guide dog training programmes through improvement of guide dog welfare. It is essential to provide guide dog schools with solutions that enable them to reduce the number of dogs that are not handed over to visually impaired people, and this will notably improve training programmes. The welfare of dogs is the priority of these schools, and the link between animal welfare and performance is described in the literature (Rooney et al., 2009; Vincent and Leahy, 1997). Perhaps by keeping dogs at school continuously in an environment and with a routine appropriate to their needs, the welfare of dogs will improve. Therefore, this research provides an avenue to explore, and it would be interesting to compare the results of this study with those obtained from a group of dogs staying at school on weekends. It is still very important that these dogs, who are asked to do much work, are given rest periods. That is why weekends at school should be used for relaxation and not for training.

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Conflict of interest statement

The authors declare no conflicts of interest.

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4.3 Discussion

By comparing the day of separation from the foster family with a control day, i.e., Friday, we confirmed that the changing environment had an impact on the physiology and behaviours of the dogs. Dogs require time to reaccommodate themselves to the kennel each week, which impacts training sessions and potentially the dogs' welfare. However, investigation of long-term indicators of stress suggested that guide dog school did not seem to be a stressful environment. Therefore, questions concerning the efficacy of this housing management are raised by these results.

For the next step of this thesis, the Frederic Gaillanne Foundation provided us with the opportunity to perform the same experiment with dogs who remained at the school throughout the entire training programme for purposes of comparison.

CHAPTER 5: Home or boarding school for the weekend: Which is best for guide dogs involved in an ongoing training programme?

Fanny Menuge, Patrick Pageat, Camille Chabaud, Eva Teruel, Bérénice Ambielle, Galice Kalonji, Cécile Bienboire-Frosini, Tiago Mendonça, Anthony Poupée and Míriam Marcet-Rius

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5.1 Introduction

Regarding our previous results, we investigated this situation more deeply by comparing two management styles. We employed the same experimental design to study a group of dogs who remained at the school during weekends for purposes of comparison with the data collected during the previous study (i.e., the study in which the dogs returned to their foster families each weekend). Both groups participated in the experiment at two different times of the year, but the groups were associated with the same organization and completed the same training sessions with the same educator.

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Home or boarding school for the weekend: which is best for future guide dogs involved in an ongoing training program?

Fanny Menuge[1*], Patrick Pageat[2], Camille Chabaud[3], Eva Teruel[4], Bérénice Ambielle[1], Galice Kalonji[1], Cécile Bienboire-Frosini[3], Tiago Mendonça[5], Anthony Poupée[6], Míriam Marcet-Rius[1]

[1] *Animal Behavior and Welfare Department, Research Institute in Semiochemistry and Applied Ethology (IRSEA), Quartier Salignan, 84400 Apt, France; berenice.ambielle@yahoo.fr; g.kalonji@group-irsea.com; m.marcet@group-irsea.com*
[2] *Research and Education Board, IRSEA, Quartier Salignan, 84400 Apt, France; p.pageat@group-irsea.com*
[3] *Molecular Biology and Chemical Communication Department, IRSEA, Quartier Salignan, 84400 Apt, France; c.chabaud@group-irsea.com; c.frosini@group-irsea.com*
[4] *Data Management and Statistics Service, IRSEA, Quartier Salignan, 84400 Apt, France; e.teruel@group-irsea.com*
[5] *Centre for Clinical Ethology and Animal Welfare (CECBA), Quartier Salignan, 84400 Apt, France; t.mendonca@group-irsea.com*
[6] *Frederic Gaillanne Foundation (FGF), 150 chemin de la Tour de Sabran, 84800 L'Isle-sur-la-Sorgue, France; anthony.poupee@fondationfg.org*

Abstract: The impact of the housing management approach for future guide dogs during the training program on dogs' welfare is still unknown. During the training period, dogs either go back with their foster family every weekend, or they stay at school. The aim of this study was to compare these two management styles on animals' welfare and performance. Behavioral and physiological parameters were assessed on eighteen dogs over a period of 3 weeks; 9 returned to their foster families on weekends (FF group), and 9 remained at school (Sc group). Results showed that dogs staying at school expressed less stress behavior at rest (GLMM; DF=1; F =10.11; p=0.0018). A visual analogue scale completed by the dog trainer indicated that they were more focused during training sessions (GLMM; DF=1; F=5.42; p=0.0326). Also, dogs were well accustomed to life in the school kennel, with the neutrophil/lymphocyte ratio and levels of serotonin, prolactin, and oxytocin in line with normal rates in both groups. These results suggest that school environment has no negative impact on the dogs and avoiding repeated separations with the foster family could reduce stress and increase their welfare. This should prompt consideration of the housing environment of future guide dogs.

Key Words: animal welfare; assistance dogs; emotional balance; foster family; guide dog school; performance.

* *Corresponding Author*: f.menuge@group-irsea.com; Tel.: +33-490-756-567; IRSEA, Quartier Salignan, 84400 Apt, France

Introduction

Consideration of animal welfare is increasing. Furthermore, since research has shown that welfare could be linked to animal performance, interest in this discipline has grown (Rooney et al., 2009; Waiblinger et al., 2006). Guide dogs are essential for people who choose this type of assistance over a white cane (Mariti et al., 2014), but breeding and training future guide dogs is challenging for institutions. It is expensive (25 000 € per dog) (Mengoli et al., 2017), and many dogs are eliminated from the program due to undesirable traits, such as fearfulness, aggressiveness, or distractibility (Arata et al., 2010; Goddard & Beilharz, 1982). Studies demonstrated that dogs'

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early life experiences can shape their adult behavior. For example, Tiira & Lohi (2015) showed that a good socialization between the age of eight and twelve weeks reduce fearfulness in adult dogs. It is fundamental to understand why dogs develop these kinds of behaviors and if it is possible to reduce the failure rate by adapting the development program of the dogs, with a better understanding and consideration of the animal's needs. Unfortunately, to the best we know, there is a shortage of information about this topic in the literature.

Foster families care for dogs from weaning to the beginning of training, when the dogs are approximately 1 or 2 years old. Since they raise the dogs and care for them during a decisive phase of their development (habituation and socialization), their effects on the dogs are important and could have an impact on the dogs' development and their success as guide dogs (Koda, 2001; Menuge et al., 2021). During the training period, which lasts between 6 and 8 months (Alterisio et al., 2019; Arata et al., 2010), dogs' routines could be different depending on the specific guide dog organization. In some cases, dogs stay at the school for the whole training period (Chur-Hansen et al., 2015), or they return to their foster families every night or every weekend (Dalibard, 2009). These different management types could influence animal stress, welfare, and performance. Stay at school all the time could allow dogs to keep a same routine and stay focused easily. Nevertheless, they would also loose connection with a family environment. In the other options dogs keep a link with family environment but it also could induce separation-related behaviors in dogs. A study evaluated the stress and welfare of dogs who go back with their puppy raisers on weekends. It was observed that on Mondays, which is the day of separation with the foster family, dogs were more stressed and less receptive to new learning than on the other days of the week (Menuge et al., 2021). The objective of this research was to go further these results, and to evaluate if it is better for the dogs to go back with their foster family every weekend or to stay at school, by comparing these two types of management styles. We assumed that it would be more beneficial for dogs to stay at the school during the entire training period to avoid exposure to weekly environmental changes. Indicators of stress, welfare and the performance of dogs participating in a program in which they either returned with their puppy raiser on weekends or stayed at school were assessed to evaluate which organization type was associated with better outcomes in the dogs.

Materials and methods

This project was approved by the French Ministry of Research (APAFIS#24626-202003111326352 2v2).

Animals

Eighteen potential guide dogs (5 females and 13 males, all neutered) involved in an ongoing training program at the Frederic Gaillanne Foundation (FGF, L'Isle-sur-la-Sorgue, France) participated in this study, in two different groups. The first group (9 dogs–2 females and 7 males) returned to their foster family from Friday evening to Monday morning (FF group), and the second group (9 dogs–3 females and 6 males) stayed at school throughout the training period (Sc group). The guide dogs in training were Labernese (a crossbreed between a Bernese Mountain dog and Labrador), St–Pierre (at least third-generation Labernese) and Labrador. The FF group was composed of 2 Labernese, 6 St–Pierre and 1 Labrador, and the Sc group was composed of 3 Labernese, 5 St–Pierre and 1 Labrador. Before the beginning of the training period, all the dogs lived with their foster families. They were all between 12 and 24 months old at the time of the study and began their training program at school 6 weeks before the start of the experiment. Concerning housing conditions at school, 3 dogs were placed per box. Each box was made up of an interior part of $8m^2$ and an exterior part of $7m^2$, leaving them a total space of $15m^2$. The facility

also had 3 outdoor parks of 100m^2 each to which the dogs had regular access during the day. A bucket of water was available to them ad libitum. They were fed twice a day (morning and evening), and the guide dog organization supplied kibble to the puppy raisers. The school routines during the week were the same for the 2 groups–all dogs followed a preestablish educational program–but their routines were different during weekends. Dogs in the FF group were occupied differently depending on the routines of their foster families. For dogs in the Sc group, an employee of the FGF came 3 × 2 hours per day on Saturday and Sunday to feed, walk and play with the dogs (no training sessions were conducted on weekends).

Experimental design

Data from the FF group was obtained from a recent paper previously published (Menuge et al., 2021). For this group, study was undertaken from 16th of November 2020 to 4th of December 2020, 6 weeks after the beginning of the training period. The same study design was used for the Sc group to ensure comparability, from 5th of July 2021 to 23rd of July 2021, also 6 weeks after the beginning of the training period. During these 6 weeks, dogs were already implied in the housing management of their corresponding groups: Dogs from FF group came back to their foster family on weekends during this period, and dogs from Sc group stayed at school. For the trial, behavioral and physiological parameters were collected during 3 consecutive weeks. The same design as Menuge et al. (2021) was used for the present study, and the parameters were collected on the same hours:

Behaviors: Dog behaviors related to stress, or a positive mental state were reported during a period of rest (Table 1). Dogs were videotaped for 45 min on Mondays, Wednesdays, and Fridays in their kennels between 12:00 p.m. and 1:00 p.m. All behaviors were analyzed using continuous sampling by 2 independent observers using Boris software (Friard & Gamba, 2016).

Table 1. Dogs' behaviors related to stress or a positive mental state assessed during periods of rest on Mondays, Wednesday, and Friday during 45 minutes.

Behavior	Description	References
Locomotion (SE)	Walking, pacing, or running around without exploring the environment or playing.	(Fallani et al., 2007)
Panting (SE)	Rapid shallow breathing (mouth open).	(Beerda et al., 1998)
Social play (SE)	Any vigorous behavior performed when interacting with another dog, including running, jumping and active physical contact.	(Fallani et al., 2007)
Relaxation behavior (SE)	Lying down with the head on ground without any obvious orientation towards the physical or social environment.	(Palestrini et al., 2017)
Head movement (PE)	Characteristic movement sidewards and downwards.	(Schilder & Van Der Borg, 2004)
Withdrawal (PE)	Avoiding interaction with another dog by either moving away, very clearly turning away or looking away.	(Palestrini et al., 2017)

Barking (PE)	"Rough" sound often repeated in quick succession.	(Tod et al., 2005)
Whining (PE)	Throaty, low-frequency sound.	(Beerda et al., 1998; Stellato et al., 2016)
Yawning (PE)	Wide opening of mouth.	(Beerda et al., 1998; Palestrini et al., 2017)
Lip licking (PE)	Portion of the tongue is shown and moved along the upper lip.	(Beerda et al., 1998; Palestrini et al., 2017)
Total stress behaviors	Total of behaviors expressed as point events.	(Palestrini et al., 2017)

SE = State event (duration in second); PE = Point event (frequency).

Performance: A visual analogue scale developed by the experimenter was completed by the professional dog trainer after each dog's morning training session; the dogs received scores ranging from 0 to 10 for eight criteria (focus, stress, agitation, attentiveness, effectiveness, distraction, posture, and a general note from the education session). The same dog trainer completed the visual analogue scale for both groups.

Salivary cortisol levels: Saliva was collected by using a Salivette® system (Salivette®, Sarstedt, Numbrecht, Germany), which is specifically targeted for the determination of cortisol in saliva. The study protocol was repeated on Monday and Friday morning to control for the natural influence of the circadian rhythm on cortisol secretion at the time of the experiment. According to the literature, it takes twenty minutes for cortisol to be secreted in saliva (Dreschel & Granger, 2009; Hernandez et al., 2014). Therefore, sampling was carried out according to this timing: twenty minutes after the time of separation (or at the corresponding hour for non-separation day or group, i.e., between 8:15 a.m. and 9:00 a.m.), after the morning training session (between 9:00 a.m. and 12:00 p.m., and after a period of rest (between 1:30 p.m. and 2:00 p.m.). To prevent sample contamination, the dogs were not allowed to eat for one hour prior to sampling. If the presence of blood was visually observed in the saliva samples, the samples were discarded to prevent contamination with blood cortisol. Immediately after the samples were collected, they were stored at 4°C until they were centrifuged (2 min, 1000 rpm, 4°C). After centrifugation, the samples were frozen at -20°C until the day of the cortisol assay, which was performed using a Salimetrics® Cortisol Enzyme Immunoassay Kit (Kiel, Germany) according to the manufacturer's guidelines.

Blood sampling: Up to 15 ml of blood was collected from the cephalic vein on the forelimb using a 21 G needle to assess the levels of free oxytocin, prolactin, and serotonin and the neutrophil/lymphocyte (N/L) ratio; blood was collected by a veterinarian for welfare monitoring on Wednesday mornings, between 9:00 a.m. and 11:00 a.m. For hormonal analyses, blood was collected into prechilled EDTA-aprotinin tubes (BD® tubes, Elvetec, Pusignan, France) for plasma oxytocin determination and into tubes with a gel separator (Vacuette®, Greiner Bio–One, Alcyon, Paris, France) for serum prolactin and serotonin de-termination. The tubes were immediately stored in an ice box, where they remained at 4°C until centrifugation (1800 g, 12 min at 4°C). Plasma or serum was recovered and stored at -20°C until analysis. The free plasma oxytocin concentration was determined with the commercially available Oxytocin ELISA kit from Cayman Chemical (Arbor Inn, MA, USA) after solid-phase extraction in C18 columns (Hypersep 1 g, Thermo Fisher Scientific, Illkirch, France) following the procedure described in Oliva et al. (2019). Serum prolactin was assayed with a prolactin (canine) ELISA kit (Demeditec, Kiel, Germany) following the manufacturer's instructions. Serum serotonin was assayed with the commercially

available Serotonin ELISA kit from Enzo Life Science (Villeurbanne, France), which has been previously validated in dogs (Chabaud et al., 2018). For the N/L ratio, a complete blood cell count was carried out with an automated LaserCyte from IDEXX (Westbrook, MA, USA) on the day of blood sampling using EDTA tubes provided by the manufacturer (IDEXX VetCollect Tubes). The total white blood cell count, including neutrophil and lymphocyte counts, was recorded, and the N/L ratio was calculated from these data.

Weight: Dogs were weighed at the same time each day, on the morning.

Statistical analysis

Data analysis was performed with SAS 9.4 software (Copyright (c) 2002-2012 by SAS Institute Inc., Cary, NC, USA). The significance threshold was fixed at 5%.

For all parameters, the effect of the educational regime (FF vs. Sc group) was studied for the mean of the 3 weeks and for each individual week of testing, with the inclusion of group, week and the group*week interaction in the models. For salivary cortisol levels, the effect of day was also investigated.

Given that the data were repeated on the same dogs, the dog was assumed to be a random effect using mixed models. For continuous variables (salivary cortisol, prolactin, serotonin, and oxytocin levels; the N/L ratio; state events, and performance scores), general linear mixed models (GLMMs) were applied. The condition of normality of residuals was checked graphically. When this assumption was not met, the GLMM was performed on the transformed data obtained from a Box-Cox transformation.

For discrete data (point events), mixed Poisson regression was applied when the data were appropriately dispersed according to the Pearson chi-square/DF value. When over-dispersion was detected, a negative binomial mixed model was preferred because this sort of model allows a dispersion scale parameter to account for overdispersion problems. For behaviors at rest, the square of the Pearson correlation coefficient was computed to evaluate the interobserver association between the two independent video readers. Few dogs were observed whining, barking, panting, playing, or withdrawing. It was impossible to analyze these behaviors with methods for quantitative data. These data were transformed into binary variables and mixed logistic regression analyses were achieved for whining and barking. Panting, playing, and withdrawal remained not analyzable, even after the binary transformation.

For each model, the best covariance structure was chosen by minimizing the corrected Akaike information criterion (cAIC) and Bayesian information criterion (BIC), and the Tukey-Kramer adjustment was employed for multiple comparisons. When possible, the model was simplified by removing nonsignificant effects step by step as long as the cAIC and BIC criteria decreased.

Results

Behaviors at rest

The interobserver reliability indicated strong associations for all the parameters (>90%). Therefore, the average of the data gathered by the two observers was applied for the remaining analysis.

The Sc group spent more time performing "relaxation behaviors" (GLMM; Num DF=1; Den DF=17.8; F=12.00; p=0.0028), expressed less "yawning" (GLMM; Num DF=1; Den DF=68; F=8.10; p=0.0058), less "head movement" (GLMM; Num DF=1; Den DF=68; F=6.66; p=0.0120), and less "total stress behaviors" (GLMM; Num DF=1; Den DF=132; F =10.11; p=0.0018) than the FF group (Table 2). For the other parameters, no significant differences were found.

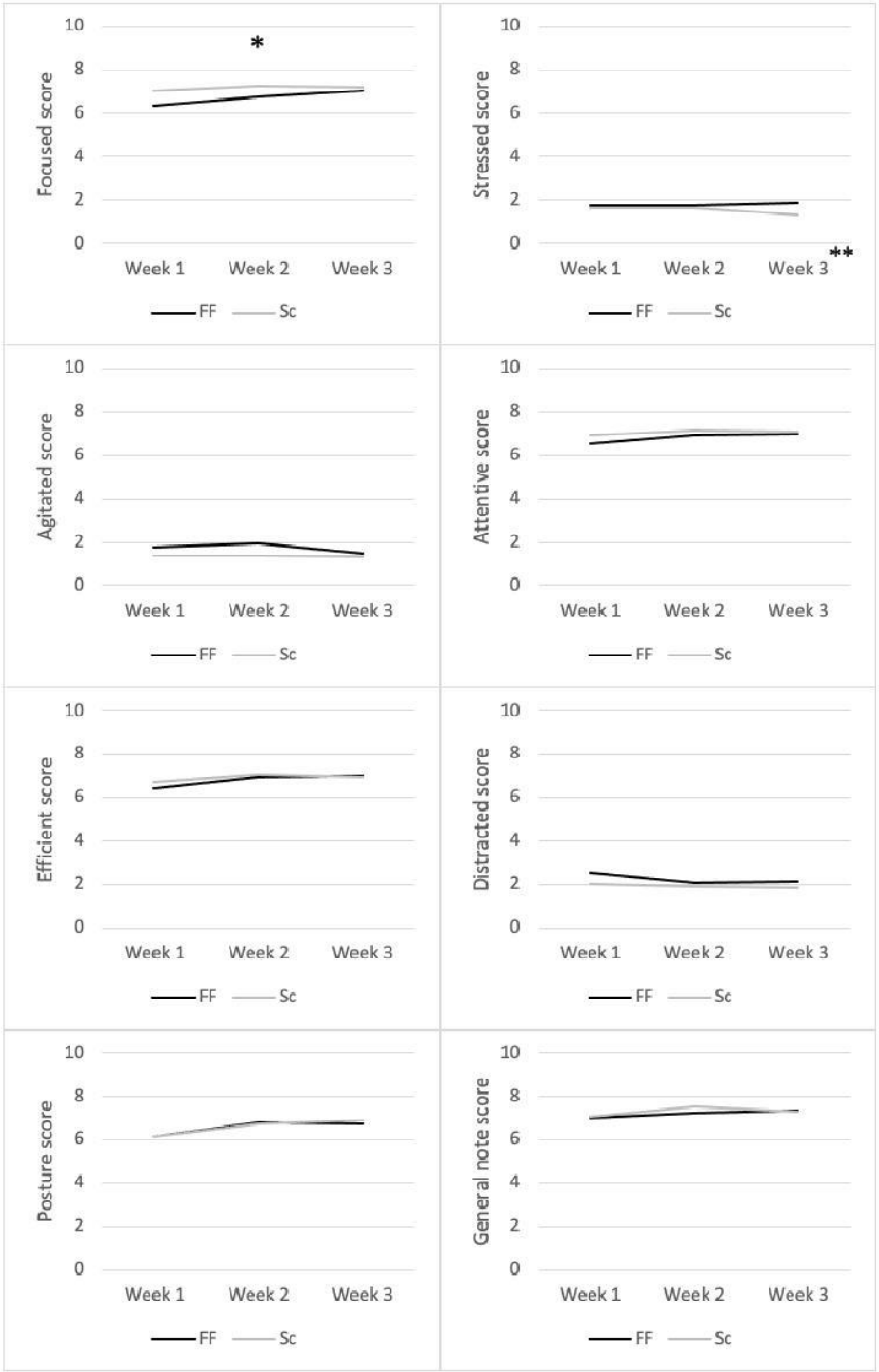

Figure 1. Performance scored by the dog trainer for each criteria. * = $p < 0.05$; ** = $p < 0.01$. FF = Foster Family group; Sc = School group.
* above the curve = presence of a significant result on the general linear mixed model (mean of the three weeks).
* next to the y-axis legend = multiple comparison with significant result.

Table 2. Mean of behaviors expressed per observed day during periods of rest over the three weeks of testing.

N = 81	Foster Family group	School group
mean ± standard error or n (%)		
Locomotion (SE)	13.48 ± 2.05	5.22 ± 0.79
Relaxation Behavior (SE)	2313.34 ± 40.85[a]	2582.79 ± 18.27[b]
Head movement (PE)	23.34 ± 2.06[a]	9.23 ± 1.57[b]
Barking*	15 (18.52)	7 (8.64)
Whining*	18 (22.22)	2 (2.47)
Yawning (PE)	1.14 ± 0.18[a]	0.17 ± 0.06[b]
Lip licking (PE)	3.42 ± 0.53	2.40 ± 0.46
Total stress behavior (PE)	39.03 ± 4.93[a]	12.62 ± 2.02[b]

SE = State event (duration in second); PE = Point event (frequency).
* Variables expressed in binary data (n = number of individuals having exhibited the behavior all observations considered).
[a vs b] Significantly different at a statistical level.

Performance

A group effect was detected for "focused" (GLMM; Num DF=1; Den DF=16.9; F=5.42; p=0.0326) and only for the 3rd week of testing for "stressed" (Tukey-Kramer; DF=21.4; t=2.96; p=0.0074). The Sc group was more focused and less stressed than the FF group. No significant differences were detected for the other criteria (Figure 1).

Salivary cortisol levels

Generally, Sc group secreted more salivary cortisol than FF group (GLMM; Num DF=1; Den DF=16.1; F=6.72; p=0.0196). Moreover, a significant difference was observed between Monday and Friday in the FF group (GLMM; Num DF=1; Den DF=136; F=8.45; p=0.0043) but not in the Sc group (GLMM; Num DF=1; Den DF=135; F=1.57; p=0.2129) (Figure 2).

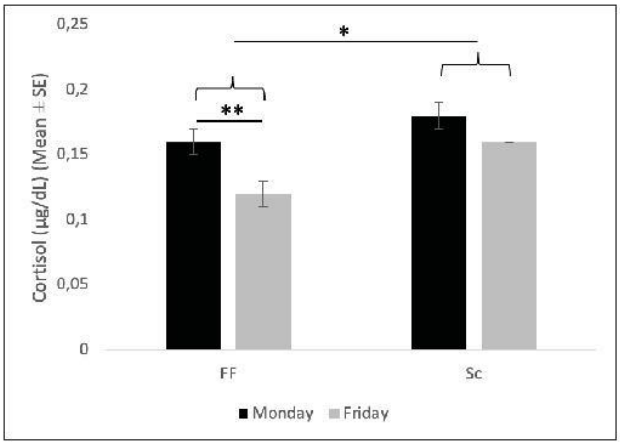

Figure 2. Mean salivary cortisol concentration in the foster family group (FF) and school group (Sc) on Monday and Friday of the 3 weeks of testing. * = $p < 0.05$; ** = $p < 0.01$.

Blood analyses

For prolactin levels, atypical values were observed for two dogs for the 3 weeks of testing (one dog from the FF group and one from the Sc group). These dogs were not included in the statistical analysis for this parameter. Four other samples in the Sc group were below the detection limit of the kit (0.4 ng/ml), suggesting very low or no prolactin secretion in these dogs (one dog over the three weeks of testing and one dog during the second week). An extrapolation of the standard curve using the plate reader software (BioTek Gen5 Data Analysis Software) was performed so that a concentration of prolactin of 0.091 ng/ml was detected for one dog, but not for the others, suggesting the absence or lower levels of prolactin. According to the canine prolactin ELISA kit, normal values are found between non-detectable and 21 ng/ml. Therefore, we replaced these data with 0 to be considered in the statistical analysis.

Although not statistically significant, the serotonin concentrations had a non-significant tendency to be higher in the Sc group compared to the FF group on the first week of testing (Tukey–Kramer; DF=35.2; t=-1.82; p=0.0780) (Table 3). Groups were not different at a statistical level for the concentration of oxytocin (GLMM; Num DF=1; Den DF=16.1; F=2.76; p=0.1162), prolactin (GLMM; Num DF=1; Den DF=14; F=1.62; p=0.2237) or N/L ratio (GLMM; Num DF=1; Den DF=16; F= 2.96; p=0.1044).

Table 3. Mean plasma oxytocin, serum serotonin, and serum prolactin concentrations and neutrophil/lymphocyte ratios (N/L ratios) in dogs in the foster family group (FF group) and school group (Sc group) of the 3 weeks of testing.

Mean ± standard error	**FF group**	**Sc group**
Serotonin (ng/ml)	742.08 ± 41.32 *(n = 27)*	751.43 ± 20.89 *(n = 27)*
Oxytocin (pg/ml)	24.24 ± 1.25 *(n = 27)*	20.08 ± 1.10 *(n = 27)*
Prolactin (ng/ml)	5.57 ± 1.07 *(n = 24)*	3.30 ± 0.52 *(n = 24)*
N/L ratio	2.14 ± 0.50 *(n = 27)*	1.35 ± 0.12 *(n = 27)*

Weight

No significant difference in weight was observed between the Sc (32.61 ± 0.38 kg) and FF (33.55 ± 0.48) groups (GLMM; Num DF=1; Den DF=16; F=0.23; p=0.6347).

Discussion

This project intended to evaluate the different effects between two housing management approaches on the welfare and the performance of potential guide dogs involved in an ongoing training program.

First, behaviors were assessed three times a week during periods of rest to evaluate the capacity of the dogs to relax when they were not solicited by the dog trainer. The comparison between these two groups showed that the dogs in the Sc group expressed fewer stress-related behaviors in general and spent more time in relaxed positions than those in the FF group. These results suggest

that it might be easier for dogs who stay at school all the time to understand the routine of the training sessions and to appropriately adapt their behavior in this context. In fact, when dogs go back to their foster families from Fri-day evening to Monday morning, they seemed to have to readapt themselves to the training environment every week (Menuge et al., 2021), which did not seem to be the case for the dogs that stayed at school.

Regarding the performance of the dogs from the dog trainer's point of view, the dogs in the Sc group were more focused than the dogs in the FF group. This could be the result of a more stable environment, in which dogs could have better anticipated the routines of the training sessions. Additionally, the dogs in the Sc group were less stressed on the third week of the study period than the dogs in the FF group, supporting this hypothesis. Even though differences in the other criteria were nonsignificant, it is interesting to highlight that according to the descriptive data, the Sc group was probably more emotionally balanced than the FF group. In fact, they tended to have lower agitation and distraction scores and higher attentive, efficient, and general note scores.

Salivary cortisol detection allowed us to evaluate the activation of the hypothalamus-pituitary-adrenal axis at key moments in a non-invasive way. For all the samples combined, the cortisol concentration was higher in the Sc group than in the FF group, with a medium-sized effect ($d = 0.375$) (Valentine & Cooper, 2003) and with results in both groups within the normal values described in the literature (Cobb et al., 2016; Colussi et al., 2018). This data wonders on the presence of a real im-pact of this difference on the welfare of these dogs. Regarding the comparison between days, a variation in salivary cortisol concentration was observed in the FF group, with an increase in secretion on Monday (day of separation) compared to Friday (basal value), while data remained stable for the Sc group. Therefore, the stress of the dogs could be tempered by staying at school during weekends.

The neutrophil/lymphocyte (N/L) ratio was evaluated to assess the level of long-term stress (Swan & Hickman, 2014). It was measured each week on Wednesday. Groups were not statistically different, and the results did not show values representative of the presence of chronic stress. Indeed, values were lower than those found by Hodgson et al. (2018) in healthy dogs in both groups. Furthermore, although the difference was nonsignificant, the N/L ratio in the Sc group was lower than that in the FF group (1.35 vs. 2.14, respectively).

Neurohormonal parameters reflecting the emotional states of the dogs were also investigated on Wednesdays. Levels of oxytocin, prolactin and serotonin were assessed. Considering the mean of the three weeks for each group, no significant difference was found, with results in line with published works (Gutiérrez et al., 2019; Mengoli et al., 2021). However, during the first week, the dogs in the Sc group tended to secrete more serotonin than the dogs in the FF group. An increased level of serotonin can be induced by social exposure to other dogs, which has been shown to have positive effects on canine welfare (Alberghina et al., 2019; Rooney et al., 2009). However, this difference probably only has a small effect on the animal behavior and welfare, given the weak effect size ($d = 0.05$). Furthermore, groups were analyzed at two different periods of the year, and we cannot exclude the fact that it could have an impact on these results. However, dogs spent most of their time inside the building, where the temperature was controlled, limiting the impact of external temperature on dogs' hormonal secretion. It is valuable to mention that dogs in guide dog school are subjected to many stimulants, differentiating them from shelter dogs. The latter live in a challenging environment, even if human interaction has a positive impact on their welfare (Coppola et al., 2006; Hennessy et al., 2020).

Finally, the weight of the dogs unveiled no significant difference between groups. Therefore, this result suggest that the housing management approach did not have an impact on the weight of the dogs. All dogs were carefully and frequently checked by staff at the guide dog school, and their weights were balanced and adjusted according to the needs of each dog.

Conclusion

Staying at school during the entire training period does not seem to be detrimental for guide dogs, confirming the observations of a previous study (Menuge et al., 2021), and avoiding repeated separations could reduce stress and therefore increase the welfare of these dogs. Further research would be interesting with a larger number of dogs to compare deeply both housing management styles.

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Casa o scuola per il fine settimana: quale soluzione è la migliore per i futuri cani guida coinvolti in un programma di addestramento continuo?

Fanny Menuge[1], Patrick Pageat[2], Camille Chabaud[3], Eva Teruel[4], Bérénice Ambielle[1], Galice Kalonji[1], Cécile Bienboire-Frosini[3], Tiago Mendonça[5], Anthony Poupée[6], Míriam Marcet-Rius[1]

[1] *Animal Behavior and Welfare Department, Research Institute in Semiochemistry and Applied Ethology (IRSEA), Quartier Salignan, 84400 Apt, France; berenice.ambielle@yahoo.fr; g.kalonji@group-irsea.com; m.marcet@group-irsea.com*

[2] *Research and Education Board, IRSEA, Quartier Salignan, 84400 Apt, France; p.pageat@group-irsea.com*

[3] *Molecular Biology and Chemical Communication Department, IRSEA, Quartier Salignan, 84400 Apt, France; c.chabaud@group-irsea.com; c.frosini@group-irsea.com*

[4] *Data Management and Statistics Service, IRSEA, Quartier Salignan, 84400 Apt, France; e.teruel@group-irsea.com*

[5] *Centre for Clinical Ethology and Animal Welfare (CECBA), Quartier Salignan, 84400 Apt, France; t.mendonca@group-irsea.com*

[6] *Frederic Gaillanne Foundation (FGF), 150 chemin de la Tour de Sabran, 84800 L'Isle-sur-la-Sorgue, France; anthony.poupee@fondationfg.org*

Sintesi

L'impatto sul benessere della sistemazione dei futuri cani guida durante il programma di addestramento è ancora sconosciuto. Durante il periodo di addestramento, i cani tornano con la famiglia adottiva ogni fine settimana o rimangono a scuola. Lo scopo di questo studio è stato quello di confrontare questi due stili di gestione sul benessere e le prestazioni degli animali. I parametri comportamentali e fisiologici sono stati valutati su diciotto cani per un periodo di 3 settimane; 9 sono tornati alle famiglie affidatarie nei fine settimana (gruppo FF) e 9 sono rimasti a scuola (gruppo Sc). I risultati hanno mostrato che i cani che frequentavano la scuola esprimevano un comportamento meno stressante a riposo (GLMM; DF=1; F=10,11; p=0,0018). Una scala analogica visiva completata dall'addestratore cinofilo ha indicato che erano più concentrati durante le sessioni di addestramento (GLMM; DF=1; F=5,42; p=0,0326). Inoltre, i cani erano ben abituati alla vita nel canile della scuola, con il rapporto neutrofili/linfociti e livelli di serotonina, prolattina e ossitocina in linea con i tassi normali in entrambi i gruppi. Questi risultati suggeriscono che l'ambiente della scuola di addestramento non ha alcun impatto negativo sui cani ed evitare ripetute separazioni con la famiglia affidataria potrebbe ridurre lo stress e aumentare il loro benessere. Ciò dovrebbe indurre a considerare l'ambiente abitativo dei futuri cani guida.

5.3 Discussion

Dogs who remained at school throughout the entire training period exhibited fewer stress-related behaviours and were more focused during their training sessions. In fact, they were less easily distracted, and they understood more quickly the instructions of the dog trainer. In addition to the resulting disruptions in their routine, when dogs return to their foster families on weekends, they are not supervised by a dog trainer or a professional. One hypothesis to explain these results could be that some foster families are more permissive and tolerant, and dogs in such a situation must readapt their behaviour at school to the professional, which could also be stressful. With respect to the previous study, blood analyses did not demonstrate the presence of chronic stress in both groups, thus showing that the guide dog school environment is not detrimental to the welfare of the animals.

These findings contribute to the discussion concerning better ways of managing and organizing the housing of dogs during the training period, with the aim to reduce failure rate. To deepen this research, the correlation between housing management and the success of the dogs could be investigated.

GENERAL DISCUSSION

The growing interest in animal welfare in our societies has driven research to develop a better understanding of animals' needs, including the welfare of working dogs. Throughout this thesis, the question at hand has asked how the developmental context of the dogs can be improved, with the dual purpose of improving their welfare and reducing their failure rate.

In France and elsewhere, the failure rate of potential guide dogs is very high, despite the education provided by professionals. As the total cost of a dog is 25,000 euros, the financial loss caused by these failures represents a tremendous loss of income for training associations. Reducing the failure rate or detecting the dogs that are not fit for the job as early as possible can allow these guide dog schools to optimize their capacities. For this thesis, we were therefore interested in the context in which these dogs developed, ranging from birth to the end of their training period. As a reminder, after they are weaned, the puppies are given to a foster family who care for them for 1 to 1.5 years until they begin their training programme at the guide dog school, which lasts 6 to 8 months (Alterisio et al., 2019; Arata et al., 2010). During this training period, several possibilities emerge depending on the organization of each school: (1) the dogs live at school 7 days per week; (2) the dogs return to their foster family each weekend or (3) the dogs return to their foster family each evening.

The need to study the current situation of guide dog schools alongside the issues already mentioned leads us to propose the following research plan, which consists of three main investigations:

- The familiarization to the environment, which is a crucial process in the development of puppies, including with respect to shaping their fears, are relegated to foster families, i.e., to nonprofessionals.
- The dogs are required to live with different people in different environments, which involves several transfers of the attachment bond.
- What form of housing organization during the training period is best suited to promoting the welfare of the dogs and their performance during training?

In the first study, entitled “Early evaluation of fearfulness in future guide dogs for blind people”, we started by investigating the impact of foster families on the development of fearful behaviour in puppies, which is a primary cause of downgrading (Rooney et al., 2016). In order for the puppies to develop into emotionally stable dogs who can safely guide a blind or visually impaired person, it is essential that they be raised in an environment that is conducive to the development of such balance. The familiarization to the environment and socialization received by the dogs during the sensitive period are therefore decisive. As the sensitive period occurs during the dog’s first few months of life (Freedman et al., 1961; Jokinen et al., 2017; Scott and Fuller, 1965), foster families play an important role in this process. These families are encouraged to take the dogs everywhere they go as much as possible rather than leaving it alone in their home. The aim of this process is to familiarise the dog with all types of places, people, sounds, population densities, etc., so that it can feel at ease in all situations. The foster families thus seem to play a crucial role, if not the most important role, in the dog's future and success as a guide dog.

In this study, the familiarization to the environment allowed by the foster family, the reactions of 5-month-old puppies to an emotional reactivity test, and the level of cortisol secreted during the test were assessed. The results suggested that the less often dogs are taken to various public places, the more cortisol is secreted during the test. In addition, the more time the puppies spend alone at home, the more signs of stress they exhibit in response to auditory and visual stimuli. This study is important because it highlights the need for the close involvement of foster families in the emotional development process of these animals.

Therefore, the study underlines the need to establish some preselective criteria for recruiting foster families, as such criteria seem to be essential, even if the task of developing them is difficult. A couple with two children living in a house with a garden do not necessarily constitute a better foster family than a person living alone in a flat. The decisive factor is the work that must be done with the dog. A high degree of availability is therefore essential. Although a preliminary interview makes it easier to come to know and select people, the reality is always different; thus, the task of familiarizing foster families with the investment that this special mission implies is essential. It is interesting to note that families who renew their applications and have already fostered several dogs have a better chance of raising a dog that will be able to guide a blind or visually impaired person (Mai et al., 2021). The experience of the foster family is therefore a factor in the dog's success.

Foster families are usually monitored by the guide dog school team. Puppy classes are regularly organized, allowing the dog trainers to follow the evolution of the dogs and to provide advice and answers to any questions the volunteer families may

have. Some schools may also organize home visits to obtain a more concrete view of the environment in which the dog is developing. This support may seem to be sufficient for dogs and families that do not face any particular problems, but it may be insufficient for dogs that are more resistant. Foster families, although they are very willing and motivated, each take a different approach to the animal and have a different way of treating it. Preparing a puppy to become a guide dog is a very special task. Closer follow-up by professionals could be helpful to provide better supervision to foster families during this process and to prepare the puppies in a more efficient and controlled manner.

The focus of a second study entitled "Attachment: A new approach to predict the success of future guide dogs? A pilot study" is on the bond that develops between the foster family and the dog. Because of their daily interactions with the animal, we wondered about the possibility of using foster families to detect behavioural problems that are incompatible with the work of a guide dog objectively. The aim of this process is to develop these behaviours as early as possible or to downgrade the dog to avoid unnecessary investment. The foster families as well as the dog trainers therefore completed two questionnaires that have been validated in the literature, i.e., the Dog Impulsivity Assessment Scale (DIAS-Wright et al., 2011) and the Positive And Negative Assessment Scale (PANAS-Sheppard and Mills, 2002), which facilitated our evaluation of the dogs' impulsivity and sensitivity levels, respectively. The results highlight a mismatch between the responses of foster families and those of dog trainers. This result prevents us from considering the potential use of these scales, as

it is difficult to determine who is more qualified to judge the animal's behaviour, the family who lives with the animal on a daily basis or the dog trainer who has an expert perspective.

However, during this period of his or her life, the dog can establish an emotional bond with the foster family specifically. The Ainsworth Strange Situation Test used in this study confirms the existence of an attachment bond between the foster family and the potential guide dogs after the beginning of the training programme at the school. During this period, the dogs included in the study remained at the school during the week and resided with their foster families during the weekend. However, the test was conducted during the two-week holiday period, and so the dogs remained at their foster families' homes for at least five days. This situation shows that despite the instability of the caregiver figure, the dog continues to have a special bond with the person who has cared for him for almost a year. During this test, some results differed from those found in the context of pet dogs. Indeed, during episodes of separation from the foster family, in the presence of a stranger, the guide dogs in training sought comfort from the latter, thus highlighted the difficulty they experienced with remaining alone and their need for human presence, unlike pet dogs. Foster families are explicitly asked to take their dogs with them wherever they go. Dogs who are involved in the guide dog programme are therefore less familiar with being left alone than are pet dogs, which could explain these results.

The training period involves a weekly transfer of the attachment bond exhibited by these dogs between the foster family and the dog trainer. For this reason, it would have been interesting to perform the strange situation test a second time to focus on

the dog trainer instead of the foster family to compare the dog's reactions to these two potential targets of attachment. However, this approach would have caused problems concerning the validity of the results, as the surprising and unfamiliar nature of the test would not have been possible to duplicate during the second iteration, thus biasing the results.

In the third study, entitled "Could the success of guide dogs be influenced by the bonds they develop with their puppy raisers?" we desired to advance our understanding of the attachment bond between the dog and its foster family. For this purpose, we changed the way we interpreted the dog's reactions during the strange situation test. In the previous study, the reading method employed was a scan based on an ethogram that had been preestablished in the literature (Mariti et al., 2013; Palestrini et al., 2005; Prato-Previde et al., 2003; Topal et al., 1998). Statistical analyses allowed us to compare these behaviours across the test episodes. It could then be seen, for example, that the dogs exhibited more behaviours that were representative of a security base effect during the episodes in which the foster family was present. However, this reading did not allow us to determine the nature or valence of this bond. In humans, this test highlights the attachment style that emerges between a child of approximately one year of age and his or her primary attachment figure. This test was adapted to the context of human/dog relationships by Topal et al. (1998) as described above. More recently, researchers have gone further and adapted the manner in which this test is analysed to render it closer to the original version developed by Ainsworth et al. (1979). Thus, by making a holistic reading method of the

dog's behaviour, it is possible to categorize the dog according to its attachment style: secure, insecure-avoidant, insecure-ambivalent, or insecure-disorganized (Schöberl et al., 2016; Solomon et al., 2019; Thielke and Udell, 2019). In this experiment, most of the dogs (75%) developed a secure attachment to their foster family. A secure bond could promote the emotional balance of the animals, which is essential to the development of a good guide dog. To confirm this finding, we wanted to determine whether there was a link between the dog's attachment style and its success as a guide dog. The small number of individuals in the sample did not allow us to draw conclusions on a statistical level. However, the descriptive data encouraged us to take this variable into consideration with respect to the success of the dogs. Indeed, half of the dogs with an insecure attachment failed their training, compared to a 25% failure rate for secure dogs. This question of the link between attachment and success therefore warrants further investigation by future research projects. In addition to the fact that a secure attachment could be a favourable element with respect to the emotional stability of the animals and therefore facilitate their learning process during training sessions, other consequences could result from such an attachment. In fact, in humans, the caregiving environment of children is associated with their attachment style in adulthood (Fraley and Roisman, 2019; Sundin et al., 2002). Research has shown that the attachment system of dogs is relatively similar to that of humans. Therefore, we can imagine that the first interspecies bond in which the dog participates could influence its ability to establish future bonds, particularly the bond that he will develop with the blind person whom he is to accompany. On certain occasions, dogs obtain their guiding certificate but are subsequently downgraded after experiencing a few months with a beneficiary

(Batt et al., 2010). It is possible that this bond plays a role in such a phenomenon, a possibility which should be investigated by future studies.

In addition to providing a basic educational foundation, foster families may therefore play a more profound role in the emotional development of dogs. In the same study, another interesting result was uncovered. The attachment style that the foster family itself developed regarding the dog could also have an impact on the dog's success. The higher the Pet Attachment Score related to avoidance was (Zilcha-Mano et al., 2011), the better the dog's chances of success tended to be. This finding may highlight a difference in attachment needs between the two species and serve as a warning against anthropomorphism. It is possible that a more avoidant style of attachment from owners may facilitate the development of a secure attachment style and emotional stability in dogs. A review of Tereno et al. (2019) explain that, in human species, early attachment intervention programme improve the quality of attachment of the child. Intervening with foster families could therefore have positive effects and help them to have the right behaviour towards the dogs. In particular, it could be suggested to foster families that they should engage in more distant behaviour, for example, by not responding to the animal's requests systematically but on the contrary that they should initiate contact. In this manner, dogs can learn to remain alone and stay calm, even when they are in the presence of humans.

Since the existence of a bond between potential guide dogs and their foster families was confirmed, we wondered about the impact of the repeated separations to

which the dogs are subjected when attending some schools during the training period. In the fourth study, "Repeated separations between a future guide dog and its foster family modify stress-related indicators and affect dog's focus", we therefore assessed the stress, welfare and performance of the dogs during the course of the week and compared Monday (the day of separation) with Friday (a control day). The data collected through physiological and behavioural parameters confirmed our hypothesis, indicating that dogs had higher cortisol secretion and were less calm and more alert on Monday. In terms of performance, dog trainers felt that training sessions were more difficult on Mondays, and this feeling was confirmed by the results, which indicated that dogs were less focused on those days. Several factors may explain this finding. Separation from their foster family, their change of environment, and their reunion with other dogs entail a high level of activation in the dog, which makes them less cognitively available during training sessions. The dog trainer must therefore make the dogs more amenable to learning and repeat instruction from the previous days before the dogs are able to receive new learning. This situation results in a loss of efficiency and potential additional stress for the dogs, who are required to readapt quickly. This readjustment may be more or less easy depending on the dogs in question, as each dog is treated differently by its foster family. Some foster families are more tolerant than others, resulting in interindividual variability. If we take the example of walking on a leash, dogs that have a foster family that is uncompromising with respect to this criterion have not developed bad habits in this area over the weekend, thus making readaptation easier for both the dog trainer and the dog. The ways in which the dogs are housed during these 6-8 months of training must therefore also be taken into

account from an educational perspective but especially from that of the animals' welfare, particularly since their welfare may have an impact on their performance (Rooney et al., 2009; Vincent and Leahy, 1997).

As a follow-up to the results of this study, we wanted to go further and investigate whether the dogs' welfare and performance could be improved if they were to remain at the school 7 days per week. Therefore, the Frederic Gaillanne Foundation made arrangements for the dogs to remain at the school on weekends throughout an entire training period, and the comparison between these two methods was the subject of our fifth and final study: "Home or boarding school for the weekend: Which is best for guide dogs involved in an ongoing training programme?". The environment, dog trainers and training methods were similar for both groups of dogs. The factor that differentiated the groups was housing management. Although biases are inevitable, especially in the context of a field study, this approach helped mitigate such issues significantly. The results showed differences between groups, indicating that dogs in the group that remained at the school were less agitated, less stressed, and more focused, leading to the conclusion that preserving some degree of continuity in the animal's environment could increase the chance of dogs' success. However, it is a popular belief that kennel life is less pleasant for the dog than a home environment, thereby justifying the dogs' return to their foster families on weekends. Physiological data as well as the behavioural observations just discussed have revealed important information in this regard. The dogs' levels of oxytocin, prolactin, neutrophil/lymphocyte ratio and serotonin were within species norms for both groups. Serotonin secretion was

even higher in dogs who remained at the school for the duration of the training period during the first week of the test. This arrangement is therefore not detrimental to the welfare of the animals; on the contrary, such an approach could promote their welfare. However, comparison between groups, particularly with respect to physiological data, can be biased by the fact that the groups were tested sequentially rather than concurrently, thereby inducing potential seasonal bias.

The studies conducted to support this thesis had the same aim: to highlight aspects of the development and training of potential guide dogs that could potentially be improved. This project is important because it has the dual purpose of improving the welfare of these dogs and thereby reducing the failure rate. Optimizing the breeding programme and the context in which the dogs develop would allow the training schools to limit the financial losses resulting from the high cost of training each dog (25,000 euros-Mengoli et al., 2017).

The involvement of foster families in this system has been adopted by guide dog schools worldwide. The point of the inclusion of such families is to save money in the context of housing management prior to the beginning of the training programme. This principle is based on a system of collective solidarity, which is helpful in developing the appropriate associations but also allows the dogs to become familiar with a family environment. Schools have therefore chosen to involve the general public in the training of guide dogs rather than to employ professionals during this period of puppy development. However, despite the undeniable advantages of this system, it also has

some drawbacks. Foster families are not dog training professionals, but they must nevertheless serve as key factors during the sensitive period of potential guide dogs. The bond these families develop with the dogs also has an impact on the latter's emotional balance and success. This claim supports the idea that more frequent and regular support from the schools could be beneficial in this context by facilitating improved supervision of the puppies' development. Finally, the housing management of dogs differs across guide dog schools. Our studies lend support to an approach that maintains the same environment for the dog, avoiding weekly changes in the environment. Although a study by Gunter et al. (2019) showed that temporary fostering in volunteers' homes could reduce the stress experienced by shelter dogs, the life conditions experienced by potential guide dogs differ. Indeed, potential guide dogs receive a great deal more mental and physical stimulations than shelter dogs, and the environment and care they receive meet their needs, which differ from those of shelter dogs. Furthermore, the same authors also noted that the living conditions in the kennels have an impact on the dogs' stress and welfare (Gunter et al., 2021, 2019).

CONCLUSIONS AND PERSPECTIVES

Conclusions

This PhD project suggests new implications concerning the developmental context of future guide dogs for blind people. This research draws the following main conclusions:

1. To breed successful guide dogs is a complex process that involves many factors.

    a. The processes of familiarization and socialization are important with respect to the development of fears, which are the first cause of downgrading. The greater the extent to which the foster families take the dog everywhere they go and the less they leave it alone, the less likely it is that the dog will be afraid when confronted with auditory and visual stimulations.
    b. The attachment style developed by dogs towards their foster families can have an impact on the dogs' success. Secure dogs have a greater chance of succeeding than do insecure dogs.
    c. A situation in which the attachment between the foster families and the dogs is more distant can be beneficial with respect to the emotional states of the animals.

d. Keeping dogs at school throughout the entire training period has a positive impact on the welfare of dogs as well as on their performance during training sessions.

2. Foster families bear a certain degree of responsibility for the development of prospective guide dogs. Indeed, they play a role in several factors associated with the success of such dogs, as shown above.

3. Animal welfare is associated with the success factors of potential guide dogs throughout the breeding process:

    a. This finding highlights the importance of respecting the welfare of such dogs as a way of improving their performance.
    b. A good emotional balance is essential for work as a guide dog.
    c. The stability of the dogs' environment during the training period contributes to their welfare.

4. Some strategies are suggested by these results to optimize the programme by decreasing the failure rate of dogs or by detecting dogs that should be downgraded earlier to avoid financial losses:

    a. More regular and personalized monitoring of foster families, including more home visits, would be helpful.

b. Later placement with foster families, i.e., when the dogs are approximately five months old, could also be considered to ensure that the sensitive period of the dogs is supervised by professionals.
c. Awareness could be raised among foster families concerning the best emotional distance to employ with the dog.
d. Keeping dogs at school 7 days per week throughout the entire training period can improve the dogs' welfare and performance during training sessions. In addition, information concerning the fact that the kennel environment exhibited by guide dog schools is not detrimental to the welfare of these dogs could be provided by this work.
e. The use of psychometric scales to evaluate the temperaments of potential guide dogs with the aim of predicting their ability to achieve success was not relevant to our work.

Perspectives

This work suggests certain perspectives for future studies or experiments:

1. The long-term effect of the dogs' attachment style towards their foster families was not evaluated at any point in this work. A longitudinal study could be helpful to determine whether the attachment style of guide dogs exhibits continuity over time.

2. It could be interesting to conduct an experiment involving a group of dogs who lack foster families. A building could be used to meet all the needs of the puppies, which could feature supervision by dog training professionals. Foster families could play a secondary role by way of a temporary fostering of the dogs in the homes of volunteers.
3. Dog-appeasing pheromone (DAP) is a synthetic analogue of pheromones that are naturally secreted by mammals. Several studies have demonstrated the effectiveness of this product with respect to reducing some stress-related behaviours in dogs (Denenberg and Landsberg, 2008; Gaultier et al., 2005; Tod et al., 2005). We observed that potential guide dogs experience an atypical developmental context. The use of DAP at key moments of their lives, such as during transfer from the breeder to the foster family, separation from the foster family, or the beginning of the training period, could help these dogs better cope with these moments. Testing this hypothesis could be relevant in the future to the task of improving the welfare of guide dogs and of working dogs in general.

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ANNEX

In annex are placed 2 articles in which I appear as co-author.

The first one, entitled “The impact of equine-assisted therapy on equine behavioral and physiological responses”, correspond to a study in which I participated during my master internship.

The second one, entitled “A feline semiochemical composition influences the cat’s toileting location choice”, is related to a project for which I worked in parallel with my PhD project, during the last year.

animals

MDPI

Article

The Impact of Equine-Assisted Therapy on Equine Behavioral and Physiological Responses

Tiago Mendonça [1,*], Cécile Bienboire-Frosini [1], Fanny Menuge [1], Julien Leclercq [2], Céline Lafont-Lecuelle [3], Sana Arroub [3] and Patrick Pageat [4]

1 Behavioral and Physiological Mechanisms of Adaptation Department, Research Institute in Semiochemistry and Applied Ethology, IRSEA, 84400 Apt, France
2 Animal Experimentation Department, IRSEA, 84400 Apt, France
3 Statistical Analysis Department, IRSEA, 84400 Apt, France
4 Semiochemicals' Identification and Analogs' Design Department, IRSEA, 84400 Apt, France
* Correspondence: t.mendonca@group-irsea.com; Tel.: +33-490-755-700

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Simple Summary: Equine-assisted therapies (EATs) are often applied to patients with different types of either mental or physical conditions. The increasing application of EATs has induced interest in the scientific community about equine well-being during these therapies. This study aimed to investigate whether equine-assisted therapy (EAT) creates negative or positive emotions in horses and the influence of patients' therapy expectations (one group of patients had physical and psychological expectations, and one group of patients had only psychological expectations) on horses' behavioral and physiological responses. We observed 58 pairs (patient–horse) during EAT sessions and when the horses were at rest. Then, we compared the horses' behavioral and physiological responses between the different periods of therapy and among the groups of patients. Our results suggested that the EAT in this study was neither a negative nor a positive event. EATs with patients who had both physical and psychological expectations were more challenging for horses than those with patients who had only psychological expectations. Further research on EAT should focus on providing horses with positive stimulation and reinforcement to understand whether a positive association with EAT can be created.

Abstract: Equine-assisted therapies (EATs) have been widely used in the treatment of patients with mental or physical conditions. However, studies on the influence of equine-assisted therapy (EAT) on equine welfare are very recent, and the need for further research is often highlighted. The aim of this study was to investigate whether EAT creates negative or positive emotions in horses, and the influence of patients' expectations (one group of patients had physical and psychological expectations and one group of patients had only psychological expectations) on horses' emotional responses. Fifty-eight pairs (patient–horse) were involved in this study. Behaviors and heart rate variability (HRV) data were collected during a resting phase, a preparation phase in which the patients brushed and saddled the horse, and a working phase. Behaviors and HRV were compared between phases and among the groups of patients. Our results suggested that the EAT in this study was neither a negative nor a positive event. EATs with patients who had both physical and psychological expectations were more challenging for horses than those with patients who had only psychological expectations. Further research should focus on providing horses with positive stimulation and reinforcement to understand whether a positive association with EAT can be achieved.

Keywords: behavior; emotions; horse; equine-assisted therapy; heart rate variability; welfare

1. Introduction

Animal-assisted interventions (AAIs) is a term that is commonly used to describe any modality using animals to improve the physical, mental, and social conditions of humans [1]. These interventions have proven effects in applications for autism spectrum disorder, Alzheimer's disease, behavioral problems, and emotional well-being [1–3]. Other medical issues in which this therapy has been applied include Parkinson's disease [4], heart failure [5], cerebral palsy [6], and neurological impairments and applications for children with developmental delays [7].

AAIs that involve equines are usually defined as equine-assisted therapies (EATs) or equine-assisted activities (EAAs) [8]. EATs have been used to improve social, emotional, and physical domains in patients suffering from anxiety, depression, autism spectrum disorder, multiple sclerosis, and spinal cord injury or to improve balance, muscle symmetry, coordination, and posture [6,8,9].

Animal welfare has become a major concern in all activities that involve animals, including animal production [10], sports [11], work [12] and even pet ownership [13]. The awareness of the welfare of the animals used in EATs is a focus of interest to the scientific community along with its applications [14]. The most commonly used physiological indicators of animal welfare focus on the negative impact of an activity on animal welfare, assuming the absence of a negative impact to be associated with well-being or "positive welfare" [15]. However, the definition of positive welfare includes not only the absence of negative experiences or feelings, but also the presence of positive experiences or feelings [16]. This means that the study of animal welfare should include both negative and positive indicators.

Equine-assisted therapy (EAT) activities that involve horseback riding appear to be no more stressful for horses than recreational horseback riding activities [14,17]. Additionally, in a previous study, interactions between patients experiencing post-traumatic stress disorder and horses assisting in their therapy were found to be equally stressful as the interactions between healthy humans and horses [18]. The authors of this study suggested that horses respond more to physical cues than to emotional cues from humans [18].

Many studies of EATs that have investigated behavioral and/or physiological aspects have suggested that EAT activities do not produce negative outcomes, such as stress, in horses [18,19]. Considering the absence of negative outcomes, some authors have suggested that EAT may produce positive outcomes for horses [14]. However, one study that investigated both negative (plasmatic cortisol concentrations and heart rate variability) and positive (plasmatic oxytocin concentrations) outcomes of EAT found no difference between the parameters [20]. Nevertheless, there are relatively few studies on EATs, and only one investigated positive outcomes of these activities.

Some authors have demonstrated that heart rate variability (HRV) is a suitable tool to study equine emotional responses in animals involved in EAT [21]. HRV analysis the autonomic nervous system (ANS) activity [22]. The frequency domain analysis of HRV studies the sympathetic (SNS) and parasympathetic (PNS) components of the ANS [23]. This analysis includes parameters, such as low frequency (LF), high frequency (HF), and very low frequency (VLF); these are influenced by the respiratory rate, which varies according to species; therefore, the frequency band widths should be adapted in this type of analysis [24,25].

Each of the described HRV parameters reflects different aspects of ANS activity [25]. Some authors have suggested that LF may represent sympathetic activity [22,26]. HF is commonly considered a marker of the cardiac vagal tone, and consequently, the parasympathetic system [22,23,27]. The LF/HF ratio reflects the SNS–PNS balance [22,23,27]. The VLF component of HRV has been associated with a slow recovery of the ANS balance after a mental or physical effort [28]. The analysis of the ANS activity provides an objective tool to study both positive and negative outcomes of EATs [16,29].

The emotional states are best assessed by a combination of behavioral and physiological measures [30]. Physiological and behavioral indicators should include both positive and negative outcomes for the assessment of equine emotional states and welfare [16]. Some behaviors have been associated with negative emotions [14,19], while others have been associated with positive

emotions [31]. Thus, these behaviors may be useful as negative and positive indicators of equine emotions in association with physiological indicators.

The aim of this study was to investigate whether EAT creates negative or positive emotions in horses and the influence of patients' therapy expectations on horses' emotional responses.

2. Materials and Methods

This project was approved by the Research Institute in Semiochemistry and Applied Ethology (IRSEA) Ethics Committee (C2EA125) and the French Ministry of Research (APAFIS Process number 11949).

General and specific information about the patients and the horses was collected through a questionnaire survey. Patients' medical conditions and expectations were collected with the agreement of the patients and/or their families. Nevertheless, to respect patient confidentiality, the diagnoses and expectations of individual patients were not reported; instead, data were classified into different categories.

This study was conducted in Avignon, France, between March and July 2018.

2.1. Population

This study included nine horses (10 ± 3 years old) and 51 patients (18 ± 11 years old) who participated in EATs.

The animals were recruited from two different equestrian facilities. Housing conditions were the same in both centers. Horses lived in paddocks that they shared with other conspecifics. The population included four females and five geldings (six grade horses, one Mérens, one Halfinger, and one Carmargue). These horses have been involved in EAT for: 9 years; 8 years; 6 years; 4 years; two horses for 3 years; 2 years; 1 year; and 6 months.

The patients involved in this study have been diagnosed with different medical conditions (see Table 1). From the patients' and therapists' perspectives, the expectations for improvement related to these therapies could be divided into two categories: psychological (PSY) or both physical and psychological (PHY + PSY) expectations.

Table 1. Disorders diagnosed in the included patients.

PSY	PHY + PSY
Autism spectrum disorder	Head injury with locomotor disability
Attachment disorder	Development deficit with locomotor disability
Schizophrenia	Pediatric spastic triplegia
Intellectual disability	Pitt–Hopkins syndrome
Anxiety	Post-traumatic physical rehabilitation
Attention deficit disorder	Trisomy 21 (Down syndrome)
Hyperactivity syndrome	
Depression	
Phobias with hallucination	

psychological (PSY) or both physical and psychological (PHY + PSY) expectations.

The statistical unit considered for this study was the pair (patient + horse). Some of the included patients had therapy with different horses on different days. Fifty-eight pairs of patient–horse were involved in this study.

2.2. Study Design and Data Collection

This investigation included a resting phase and two phases of EATs (preparation and working phases).

The resting phase consisted of the collection of HRV data in the normal living and husbandry conditions of the horses.

The preparation phase consisted of controlled interactions between the patient and the horse, such as petting, brushing, saddling, cleaning the horses' feet, and feeding or watering the horses. These interactions differed according to the patients' needs and abilities, and were selected by the therapists. Preparative sessions were performed near the paddocks.

The working phase followed the preparation phase. The patients could either ride or perform in-hand training with the horses. In-hand training included the following: the approach and the initial contact with the horse, making the horse move forward or backward, leading the horse to cross obstacles or pass parallel bars on the ground, and lunging the horse in a round pen. The therapists made the decision of whether the patient should ride or perform in-hand training. Working sessions were performed in riding arenas of variable size.

Each phase lasted between 20–30 minutes, and took place between 09:00–13:00 and 14:00–16:00 during the day.

Behavior data were collected for both the preparation and working phases by one observer through direct observation. The considered behaviors included "ears pinned back" (EPB), "head lateral movement" (HLM), "snort", and "defecation" (see Table 2) [14,31]. These parameters were assessed in terms of frequency.

Table 2. Ethogram used for behavioral observation [14,31].

Behavior	Definition	Emotions
Ears pinned back (EPB)	Backward placement of the ears	Stress, irritation, or frustration; negative emotions
Head lateral movement (HLM)	Turning the head to one side or the other when no command is provided	Stress, irritation, or frustration; negative emotions
Snort	Short, powerful exhalation from the nostrils	Relaxation; positive emotions
Defecation	Elimination of excrement	Stress, irritation, or frustration; negative emotions

HRV data were collected during all the phases with Polar V800 Equine® equipment (Kemple, Finland). Then, the collected data were transformed with Kubios® software (Kuopio, Finland), and only normal R-R intervals were considered for the HRV analysis. The described data included time domain (heart rate) and frequency domain analysis (see Table 3). Frequency band thresholds were established within each frequency interval using the following parameters: LF power = 0.005–0.07 Hz; HF power = 0.07–0.5 Hz; VLF power = 0.001–0.005 [25].

Table 3. Adapted description of heart rate variability (HRV) variables from Stucke et al., 2015 and Usui and Nishida, 2017 [23,28].

Variable	Definition	Interpretation
HR (beats per minute)	Heart rate mean	Overall variability of cardiac activity
VLF (%)	Very low frequency	Slow recovery component of the HRV (vagal tone)
LF/HF ratio	Low frequency/high frequency ratio	Representation of the balance between the sympathetic and parasympathetic nervous systems

2.3. Statistical Analysis

Statistical analysis was carried out using SAS 9.4 software Copyright © 2002-2012 by (SAS Institute Inc., Cary, NC, USA). The significance threshold was classically fixed at 5%.

2.3.1. Data

The duration of each phase differed according to the patient's responses to the therapy. For horse behaviors, the proportion of the frequency over the duration of the given phase was calculated to standardize this variable among different phases. Finally, the frequency of each behavior per minute (freq/min) was considered for the statistical analysis.

HRV data were analyzed as raw data to compare the differences between the two EAT phases and between the two categories of patients' therapy expectations. Then, the differences (Δ) between the preparation and resting phases and between the working and resting phases were calculated to study the evolution of these parameters from resting to the two different EAT situations. These parameters were defined as ΔHR, ΔVLF, and ΔLF/HF ratio.

2.3.2. Statistical procedures

For HR and the LF/HF ratio, the assumption of normality of the data was tested using residual diagnostics plots and the UNIVARIATE procedure. Comparisons between phases and between patients' expectations were performed with the general linear mixed model using the MIXED procedure (two-factor analysis). When significant differences were found, multiple comparisons were analyzed with Tukey's test using the LSMEANS statement.

For EPB, HLM, and VLF, the assumption of normality of the data was tested with the above-described procedure, although the assumption was not verified. Then, box-cox transformation was performed. The assumption of normality of the data was retested using residual diagnostics plots and the UNIVARIATE procedure. Comparisons between phases and between patients' expectations were performed with the general linear mixed model using the MIXED procedure. When significant differences were found, multiple comparisons were analyzed with Tukey's test using the LSMEANS statement.

For the differences in the cardiac parameters (ΔHR; ΔVLF; ΔLF/HF ratio), the assumption of normality of the data was tested with the above-described procedure, although the assumption was not verified. Then, the data were analyzed with the Wilcoxon Signed-rank's test (for paired samples).

For snort and defecation frequencies, data were transformed into binary variables, because the animals did not perform this behavior very often. In this case, the following code was adopted: 1 – the behavior was observed; 0 – the behavior was not observed. Comparisons between phases and patients' expectations were performed with the generalized linear mixed model using the GLIMMIX procedure. When significant differences were found, multiple comparisons were analyzed with Tukey's test using the LSMEANS statement.

3. Results

A few patients could not participate in one of the phases, as participation was not recommended due to their medical state, which lead to an absence of data. The lack of data concerned three patients in the preparation phase and one patient in the working phase.

One patient was highly disturbed by the presence of the observers during the preparation phase, so the therapists decided to remove the observers from the patient's visual field. Therefore, it was not possible to observe the horse's behavior during this phase.

One session occurred during feeding time. This caused a distraction for the horse, which was looking at the animal keepers feeding the animals, significantly increasing the HLM parameter. Therefore, the data concerning this parameter in the preparation phase were removed from the analysis.

In one working phase, a side walker was required to ensure the patient's safety. This unexpected situation clearly modified the horse's behavior. As some behavioral or HRV parameters were influenced by this condition, the authors decided to remove them from the statistical analysis (EPB, HLM, LF/HF ratio, and ΔLF/HF ratio).

Due to technical issues related to the heart rate monitor, the HRV data of two horses in the working phase were not recorded.

3.1. Behavior

There was no significant difference in EPB between the different phases, the two groups of patients' therapy expectations, or the interaction of phase*patients' therapy expectations (phases: DF = 1; F = 0.40; $p = 0.53$; patients' therapy expectations: DF = 1; F = 0.13; $p = 0.72$; interaction of phase*patients' therapy expectations: DF = 1; F = 1.64; $p = 0.21$).

Significant differences were observed for HLM in the interactions between phases and patients' therapy expectations (interaction of phase*patients' therapy expectations: DF = 1; F = 6.60; $p = 0.01$). More precisely, in the preparation phase, horses performed on average two times more HLMs with the PSY group than with the PHY+PSY group. Additionally, horses involved in therapies with the PSY group performed on average two times more HLMs in the preparation phase than in the working phase (see Figure 1).

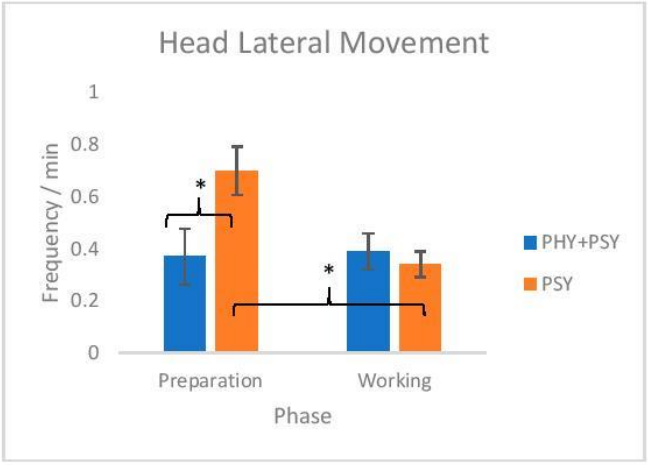

Figure 1. Equine-assisted therapies effect on Head Lateral Movement (freq./min.): effect of phase*patients' therapy expectations. Mean ± standard error. * $p < 0.05$.

There was no significant difference in snort concerning either the different phases or the patients' therapy expectations (phases: DF = 1; F = 0.05; $p = 0.82$; patients' therapy expectations: DF = 1; F = 0.05; $p = 0.82$; interaction of phase*patients' therapy expectations: DF = 1; F = 0.00; $p = 0.99$).

Defecation was only observed 11 times over a total of 111 EAT observations (preparation + working). For that reason, statistical analysis with a model was not applicable for this behavior.

3.2. HRV

HRV results concerning the interaction of phase*patients' therapy expectations were not significant for any of the studied parameters (HR: DF = 1; F = 1.46; $p = 0.23$; VLF: DF = 1; F = 1.27; $p = 0.27$; LF/HF ratio: DF = 1; F = 0.56; $p = 0.46$).

3.2.1. Phase Effect

Significant differences in HR, VLF, and the LF/HF ratio were observed between phases (HR: DF = 1; F = 76.67; $p < 0.001$; VLF: DF = 1; F = 4.59; $p = 0.04$; LF/HF ratio: DF = 1; F = 9.80; $p < 0.01$). More precisely, an increase of 10 beats per minute (bpm) on average in the horses' HR between the preparation and working phases was observed. VLF and the LF/HF ratio were significantly higher in the working phase than in the preparation phase (see Table 4).

Table 4. EAT effect on HRV: effect of phase.

Parameter	Phase	Mean ± SE	Median	Minimum	Maximum
HR (bpm)	Preparation	44.22 ± 0.95 ***	42.00	34.00	67.00
	Working	55.98 ± 1.62 ***	54.00	37.00	95.00
VLF (%)	Preparation	0.70 ± 0.19 *	0.30	0.03	7.44
	Working	0.74 ± 0.09 *	0.57	0.01	3.13
LF/HF Ratio	Preparation	2.71 ± 0.41 **	1.92	0.08	16.12
	Working	4.84 ± 0.57 **	3.39	0.22	15.13
ΔHR	Preparation	−0.46 ± 1.08	−3.00 ***	−11.00	31.00
	Working	11.40 ± 1.61	9.00 ***	−6.00	56.00
ΔVLF	Preparation	0.16 ± 0.21	−0.02 *	−1.08	7.19
	Working	0.21 ± 0.10	0.23 *	−0.98	2.21
ΔLF/HF Ratio	Preparation	−0.01 ± 0.46	−0.58 ***	−5.42	14.60
	Working	2.35 ± 0.58	1.52 ***	−6.64	17.07

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Significant differences in the ΔHR, ΔVLF, and ΔLF/HF ratio values were observed between phases (ΔHR: preparation: median = −3.000 VS working: median = 9.000; S = 570; $p < 0.001$; VLF: preparation: median = −0.020 VS working: median = 0.230; S = 264; $p = 0.01$; LF/HF ratio: preparation: median = −0.577 VS working: median = 1.516; S = 400; $p < 0.001$). More precisely, HR, VLF and the LF/HF ratio inversely changed from the resting phase to the other phases (see Table 4).

3.2.2. Effect of Patients' Therapy Expectations

Significant differences were observed in the LF/HF ratio between the two groups of patients' therapy expectations (patients' therapy expectations: DF = 1; F = 5.83; $p = 0.02$). More precisely, the LF/HF ratio was higher in the PHY+PSY group than in the PSY group (see Figure 2). There were no significant differences in HR and VLF between the different groups of patients' therapy expectations (HR: DF = 1; F = 1.74; $p = 0.19$; VLF: DF = 1; F = 0.42; $p = 0.52$).

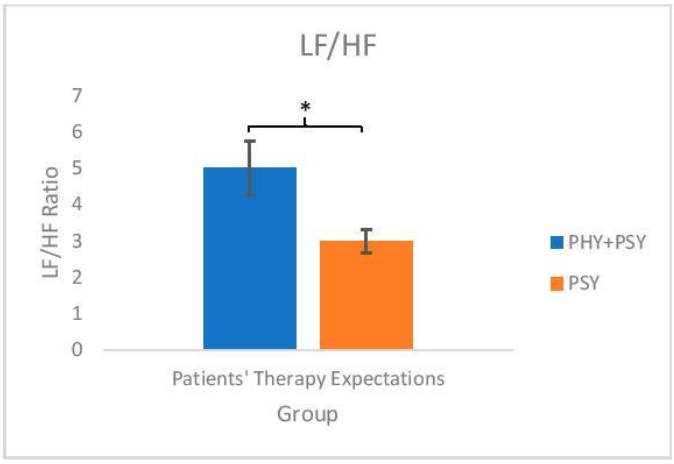

Figure 2. Effect of EAT on the LF/HF ratio: effect of patients' therapy expectations. Mean ± standard error. * $p < 0.05$.

4. Discussion

The results obtained with the different parameters showed that EAT may induce some behavioral and physiological changes in horses. Nevertheless, the results obtained regarding HRV, in particular LF/HF, were within the normal range in both phases [21]. Thus, these responses could not be linked to

any stress-related response in the present study. Therefore, our results are in line with previous studies that have suggested that EAT does not increase stress [14,19,20].

4.1. Behavior

Considering the studied behaviors, the HLM frequency was lower in the PHY+PSY group than in the PSY group during preparation, suggesting that horses searched more for visual information [14] with the PSY group than with the PHY+PSY group. This result could be explained by the curiosity of horses about unusual types of interactions. In addition, a decrease in the HLM frequency from the preparation to the working phase was observed in the PSY group, suggesting that interactions required more visual contact for the horses during the preparation phase than during the working phase. Nevertheless, the observed frequencies of all the behaviors in this study were very low. In general, in this study, horses performed EPB and HLM less than one time per minute. Snort was only performed in approximately half of the observations. Defecation was observed in 11 out of 111 observations.

EPB and defecation, as well as HLM during riding sessions, are behaviors that are commonly associated with negative emotions [14,19]. Snort was previously considered a behavior related to arousal [32–34], but recently, this behavior has been associated with positive situations [31]. The relationship between each of these behaviors and either a positive or negative situation or emotions is interesting, because such relationships may indicate that the EAT activities in the present study were neither positive nor negative for the horses, as none of the behaviors were frequently expressed by the animals.

4.2. HRV

In terms of the HR, an increase of approximately 10 bpm between the preparation and the working phases was observed. VLF was less than 1% in the preparation phase, and a small increase from the preparation to the working phase was observed, although VLF was still less than 1% after the increase. Concerning the LF/HF ratio, an increase of approximately 1.8 times between the preparation phase and the working phase was observed. Additionally, the LF/HF ratio of the horses involved in EAT with the PHY+PSY group was approximately 1.7 times higher than that of the horses involved in EAT with the PSY group.

Regarding the Δ values, a decrease in HR of 3 bpm from the resting phase to the preparation phase and an increase of 9 bpm from the resting phase to the working phase were observed. Moreover, the VLF showed a small decrease (0.02) from the resting phase to the preparation phase, and a higher increase (0.23) from the resting phase to the working phase. Finally, a decrease in the LF/HF ratio from the resting phase to the preparation phase and an increase in the LF/HF ratio from the resting phase to the working phase were observed. Nevertheless, values of LF/HF appear to indicate physiological changes, and usual LF/HF values in EAT horses could be considered to range between 2–5 [21]. Besides the observed variability, the results of the present study concerning the LF/HF ratio were all within the above-described range.

Horses involved in other equestrian activities may show an increase in HR that can exceed 100 bpm during working/training sessions [35,36]. The EAT activities in this study were performed at a low intensity level, which explains the low levels of the HR changes observed in this study. Declining trends in the VLF component of HRV have been related to slow recovery of the ANS balance, and have also been associated with heart disorders [28]. However, in our study, we observed a short decrease in VLF in the preparation phase, which was immediately balanced during the working phase. An increase in the LF/HF ratio represents an increase in sympathetic activity, while a decrease in the LF/HF ratio represents an increase in parasympathetic activity [26,28]. In our study, an increase in this ratio was observed in the working phase, which could be associated with attempts by the horses to cope with the situation. In addition, patients in the PHY+PSY group induced higher LF/HF ratios than the patients in the PSY group. This suggests that therapies with patients in the PSY group were less challenging to

the horses' emotional state than therapies with patients in the PHY+PSY group. However, neither of the groups represented a major stressor to the horses.

4.3. General Discussion

The benefits of EAT for people with mental and physical disabilities have been the subject of many investigations, which have demonstrated improvements in the muscle activity, social skills, and reductions in the anxiety of patients suffering from different medical conditions [6,8]. As EATs are getting popular, the welfare of horses involved in EATs has become a subject of increasing interest in the scientific community [14,19–21,37].

EAT activities are usually performed at low levels of physical activity [21], which was also observed in our study. Some authors have described HRV as a useful tool to study the emotional balance of horses involved in EAT and assess the horse–human partnership [21]. Indeed, our results support the use of HRV as an interesting tool to study equine perceptions of EAT because this analysis allows the study of the animal's response toward a stimulus and its capacity to subsequently recover.

Investigations about EAT have suggested that these activities are minimally stressful for horses and could produce positive outcomes or emotions in horses [14,18,19]. Nevertheless, a recent study suggested that EAT does not increase equine well-being [20]. The conclusions of that study relied on HRV and the plasmatic concentrations of cortisol and oxytocin before, during, and after EAT sessions. The assessment of oxytocin concentrations in equine plasma through enzyme immunoassay requires an extraction phase, as described by Bienboire-Frosini et al. [38]. This procedure was not performed in the previously cited study [20]. However, the results of HRV in that study were in line with the results obtained in the present study. The results of the present study concerning both behavior and HRV suggested that horses experienced neither negative nor positive emotions during EAT activities. In addition, horses rapidly balanced their ANS after these sessions. Hence, we suggest that the horses involved in EAT perceive EAT activities as neutral rather than negative or positive.

The positive or negative indicators of emotions could be considered one limitation in this study. Snort has often been described as an indicator of negative emotions [32–34]; however, recently, some authors have described this behavior as a positive indicator [31]. HLM was also described as an indicator of negative emotions [14], although this behavior could also be related to the natural curiosity of horses. These findings highlight the need for further research on the use of behavioral indicators to study equine emotions, especially positive emotions. Nevertheless, in the present study, HRV was also assessed to complement the behavioral assessment. This tool has been commonly used to study stress or negative emotions, but positive emotions could also be studied through this analysis, because positive emotions create modifications in the activity of the sympathetic and parasympathetic systems [16,29].

Another limitation to this study was the variability of the activities performed in each phase. Activities such as brushing and cleaning the feet, as well as riding the horse or performing in-hand work, may have induced different reactions. The variability in the horses' reactions to the different situations within each phase was not investigated in the present study, as the aim was to assess the overall reaction of horses to the preparation and working phases, and to assess whether EATs were perceived as positive or negative by the horses. Additionally, each group of patients in this study included patients with different illnesses, who may interact differently with the horses, producing negative or positive outcomes.

Previous studies on EAT have focused on the negative outcomes that EAT activities could represent for horses [14,19,20]. The results of these investigations suggested that there are no negative outcomes related to EAT activities for the studied animals. In addition, our results suggested that there were neither negative nor positive outcomes related to EAT for horses. Moreover, as described above, there were significant differences between patients in the PSY and the PSY+PHY groups, but these differences should be interpreted carefully, as the observed variables were within normal ranges [21]. These findings are of great importance, because they show that even if an event is not perceived as

negative or does not induce a negative outcome for horses, a positive association with the event or a positive outcome should not be expected.

5. Conclusions

In conclusion, EAT was neither a negative nor a positive event for the horses in this study, as demonstrated by the horses' behavior and HRV parameters. Behavioral and HRV changes were higher in horses involved in EATs with patients who had both physical and psychological therapy expectations than in horses involved in EATs with patients who had only psychological therapy expectations.

HRV is useful for measuring ANS activity, and thus assessing equine emotional balance during EAT.

Further research focusing on the positive aspects of these therapies for horses is required. This future research should focus on improving equine welfare in EAT, e.g., providing horses with positive stimulation and reinforcement, thus producing positive outcomes for the horses involved in EAT.

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Article

A Feline Semiochemical Composition Influences the Cat's Toileting Location Choice

Naïma Kasbaoui [1,*], Míriam Marcet-Rius [1], Cécile Bienboire-Frosini [2], Fanny Menuge [1], Philippe Monneret [3], Estelle Descout [4], Alessandro Cozzi [5,*,†] and Patrick Pageat [5,†]

1 Animal Behaviour and Welfare Department, Research Institute in Semiochemistry and Applied Ethology (IRSEA), Quartier Salignan, 84400 Apt, France; m.marcet@group-irsea.com (M.M.-R.); f.menuge@group-irsea.com (F.M.)
2 Molecular Biology and Chemical Communication Department, Research Institute in Semiochemistry and Applied Ethology (IRSEA), Quartier Salignan, 84400 Apt, France; c.frosini@group-irsea.com
3 Animal Experimentation Service, Research Institute in Semiochemistry and Applied Ethology (IRSEA), Quartier Salignan, 84400 Apt, France; p.monneret@group-irsea.com
4 Data Management and Statistics Service, Research Institute in Semiochemistry and Applied Ethology (IRSEA), Quartier Salignan, 84400 Apt, France; e.descout@group-irsea.com
5 Research and Education Board, Research Institute in Semiochemistry and Applied Ethology (IRSEA), Quartier Salignan, 84400 Apt, France; p.pageat@group-irsea.com
* Correspondence: naima.kasbaoui@gmail.com (N.K.); a.cozzi@group-irsea.com (A.C.)
† These authors contributed equally to this work.

Simple Summary: Unwanted toileting is amongst the most undesirable behaviors in cats. The aim of this study was to test the effect of a chemical cue derived from cat anal glands on the elimination behavior of domestic cats tested individually. A total of 31 cats were tested for 23 h in an enriched test room where they had the choice between two litter trays, one sprayed with the composition tested and the other with the control. We measured the weight of elimination, the weight of urine, we recorded the type of elimination, and counted the urine spots and stool piles. We also looked at the duration of exploration of litter trays, and what trays the cats were choosing to toilet as a first and second choice. We demonstrated, across all parameters, that cats urinated and defecated significantly less in the litter trays where the chemical cue was sprayed. These results confirm and strengthen those of our previous study. Future studies could explore if a chemical cue derived from male cat anal glands might be used to manage unwanted toileting in cats.

Abstract: Unwanted toileting is amongst the most undesirable behaviors in domestic cats and can lead to conflicts between cats and the communities they are living in. This study aimed to confirm the effect of a semiochemical composition, reconstituted volatile fraction derived from cat anal glands, on the elimination behavior of domestic cats. A total of 31 cats were tested individually, for 23 h, in a blinded randomized choice test, with two litter trays, one sprayed with the treatment and the other with the control. Parameters included elimination weight, urine only weight, the record of the elimination type and counting of urine spots and stools, exploration duration of each litter tray, and first and second choice of litter tray to eliminate. Across all parameters, cats urinated and defecated significantly less in the litter tray where the semiochemical composition was sprayed than in the litter tray where the control was sprayed (for example: elimination weight $p < 0.0001$; urine only weight $p < 0.0001$; exploration duration $p < 0.0001$, and first elimination choice $p < 0.0001$). These results demonstrate that a semiochemical composition-derived from cat anal glands significantly decreases elimination at the location where it is sprayed. Future research is warranted to explore the possibility to manage unwanted toileting using this semiochemical composition.

Keywords: anal glands; cat welfare; domestic cat; elimination behavior; feces; semiochemistry; unwanted toileting; urine

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1. Introduction

Animals explore their physical and social environment and gather information to secure the necessary resources for their survival. This environment encompasses other animals, conspecifics, or other species. Gathering information about those animals, whether they are predators, conspecifics or preys, is an essential part of assessing the environment, to avoid threats and conflicts, find and select mating partners. Communication can be defined as the transmission of information from one animal to another [1] using different sensory modalities, such as visual, auditory, and chemical signals [2,3]. All animals emit volatile organic compounds (VOC), some of these compounds have evolved to become chemical signals emitted towards a receiver, either in the same species or other species [4]. Chemical communication allows signals to travel in the air or be deposited on a substrate, releasing the information they carry even when the animals are not present [5]. In a species that can sometimes adopt a solitary lifestyle [6] such as domestic cats, this is essential, as, in natural conditions (without human intervention) and except for cat colonies, conspecific encounters may not be very frequent [5].

Research has shown that several secretions such as urine, faeces and secretions from various glands can be vectors of chemical communication [7–10] and chemical compounds have been identified in urine [11,12], faeces [13,14], and anal glands [9]. In the domestic cat, the anal glands' structure has been described [15,16] and their chemical composition explored [9,16,17]. Scent communication using secretions from anal glands have been described in several species such as the honey badger (*Mellivora capensis*) [18], spotted hyena (*Crocuta crocuta*) [19,20] and red foxes (*Vulpes vulpes*) [7,21–24] Anal gland compounds may have a role in individual recognition [13], territorial marking or reproductive advertisement [11]. The research field of linking compounds to a specific role is expanding, such as exploring the interaction of chemical compounds and bacteria present in the glands [17] or the interaction between compounds and their binding proteins [25]. Cat elimination behaviour is a complex process [26,27] that fulfils the role of excreting waste from the body and providing information about species, sex, reproductive status of conspecifics and age for male cats [13,14]. This behaviour is different from marking behaviour where the cat may spray urine, scratch surfaces [28] and rub on objects and individuals [29–31] as a form of chemical communication. However, both in elimination behaviour and marking behaviour, compounds are present that seem to influence behaviour. Urinary extracts for example have been shown to influence the choice of location to eliminate in outdoor cats [32]. Regarding anal glands' secretions, they can be deposited on a substrate or are excreted on faeces. While some of the roles of chemical compounds isolated from cat anal glands have been explored [9], there is a lack of knowledge about the influence of very specific compounds can have on cat behaviour, especially on their choice of location to eliminate. Today, different repellents systems exist to manage cat inappropriate elimination, but elimination behaviour plays a crucial role for the cat and its communication. A new approach is to develop strategies to manage this behaviour, to promote human and cat cohabitation. A previous work performed in a cattery setting showed that a semiochemical composition, a reconstituted partial volatile fraction derived from the secretion of an entire mature male cat (13 years-old) anal glands, deterred cats from defecating at the location where it was sprayed [33]. The aim of our study was to use the same semiochemical composition on cats tested individually in a randomised controlled choice trial, to test its effect on cat elimination behaviour. Our hypothesis was that the semiochemical composition tested would have an influence on cat elimination behaviour and we predicted that the semiochemical composition would deter cats from defecating at the location where it is sprayed.

2. Materials and Methods

The study was approved by the IRSEA ethics committee (National French Ethics Committee C2EA125) under approval number CE_2019_07_CEIS_03.

2.1. Animals/Subjects

It was conducted on 31 cats (10 entire males, 10 entire females, 4 neutered males, and 7 neutered females, see Table 1), in an enriched test room where they were free to move. All cats were examined by a veterinarian before being included in the study and were in good health, free of chronic disease or lower urinary tract disorders.

Table 1. Cats participating in the experiment, cat sexes, and ages. Entire male and female cats were born within the same two weeks so the age presented is the average age for entire male and entire female cats.

Cat Name	Cat Sexe	Cat Age
Altesse Biscotte Brioche Choco Cannelle Gaia Mia Xarra Xena Venus	Entire female N = 10	2 years 10 months
Matisse Merlin Misty Next Simba Spicy Willy Woody Ying Yang	Entire male N = 10	2 years 10 months
Batcat Encre Garfield Guinness	Neutered male N = 4	13 years 10 months 13 years 9 months 8 years 8 months 8 years 8 months
Edelweiss Hermine Perle Rose Corona Elvira Guimauve	Neutered female N = 7	11 years 9 months 8 years 15 years 14 years 11 months 8 years 8 months 11 years 9 months 8 years 10 months

2.2. Material

The test room (12 m^2) contained shelves, bedding, hiding places, scratching posts, a tunnel, toys, and litter trays (Savic Aseo litter tray, L56 cm × L39 cm × H27.5 cm) (Figure 1). Dry food was provided in a puzzle feeder (Karlie flamingo NORTHMATE puzzle feeder) to match the specific needs of each cat (i.e., depending on the cat: Royal Canin Kibble VCN Adult Cat, VCN Senior Cat Stage 2 and Cat Satiety) and in a quantity sufficient for maintaining an adequate weight. Wet food (Hill's prescription diet i/d digestive care) was used as enrichment and put in an enrichment feeder (Kong classic and Kong Extreme, size S). Water was provided ad libitum in a water bowl. The room also contained a trap camera (Coolife trap camera model H881, 16 MP 1080P HD) with motion detection sensors, focused on the area where the litter trays were positioned.

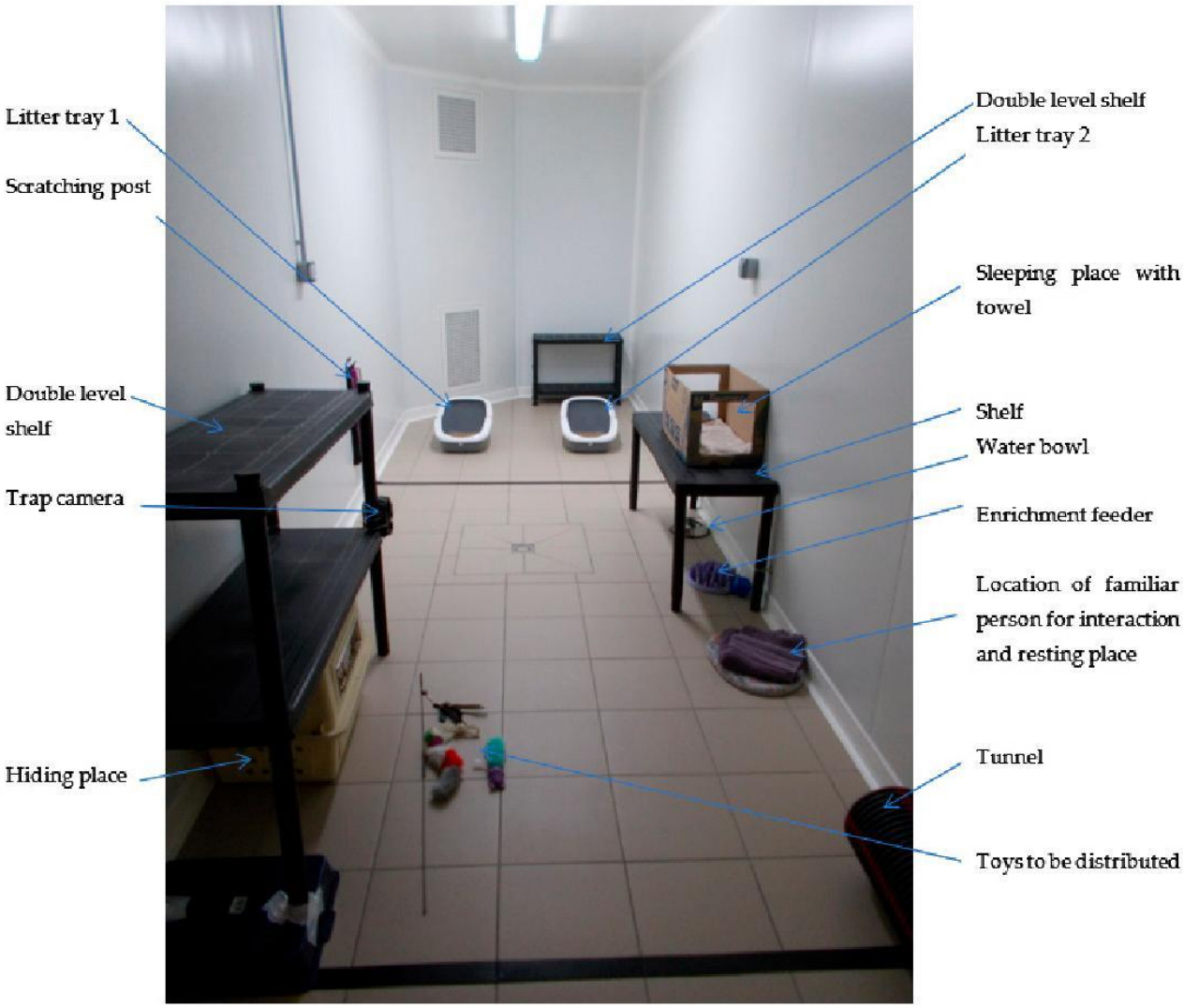

Figure 1. Enriched test room.

2.3. Procedure

Before testing, the cats underwent a six-week progressive habituation program to ensure that they were comfortable staying alone in the test room, as they usually live in an enriched cattery environment with conspecifics. The habituation procedure consisted of first spending some time in the test room by groups of three to four cats living in the same cattery, a familiar human being present in the room. Then each cat spent some time only with a familiar human present during 20 min to provide positive interaction and opportunities to play. The familiar human then provided a small number of treats and left the room, for 20 min. The time the cat was left alone was gradually increased, from 20 min to five hours. The number of sessions depended on how each individual cat responded to being alone and was a minimum of six sessions and a maximum of ten sessions. Cats were monitored during the habituation process for signs of stress such as excessive agitation, pacing, continuous meowing, pawing/scratching at the door, refusing to eat or play and overgrooming. 32 cats entered the habituation process, and one cat was excluded from the study. All remaining cats (31) were comfortable being left alone in the test room at the end of the habituation process and didn't show any of the aforementioned signs of stress any

more. Their behaviour can be described as follows: they explored the room, played with toys, ate treats, or rested on the resting places.

The cats were tested individually and stayed 23 h in the enriched test room, alone, except during short sessions where they positively interacted (positive contact and play) with a familiar human. Four interaction's sessions (the first one of 30 min, then the following ones of 20 min each) were performed during the test.

The chosen experimental design was a Randomized Controlled Trial, where the cats had the choice between two litter trays (named 1 and 2) present in the test room. In each litter tray, one treatment was sprayed on the litter substrate. The treatment used was a reconstituted partial volatile fraction (named CEMS for Cat Elimination Modulation Semiochemical) of the secretion of a male cat's anal gland, as described in Kasbaoui et al., 2022 [33]. Briefly, the treatment is composed of several volatile compounds present in the anal glands secretions obtained from an entire male cat, following their identification by GC-MS analyses. The actual treatment product is made from these same several pure chemical compounds, industrially purchased (SIGMA, Saint-Quentin-Fallavier, France), at the final concentration of 2% in a mix of ethanol and water (60:40).

The other was the control (i.e., a mixture of ethanol and water, (60:40)).

The procedure was blinded and randomized, the treatment being applied randomly to litter tray 1 or litter tray 2 for the whole duration of the test of each individual cat. Litter trays were filled with 1.5 kg of non-agglomerant litter substrate (brand "Prop'chat NF) and treatments were sprayed (five sprays) on the litter substrate and mixed with it, according to the randomization list. Litter trays were weighed each day at the start of the experiment (Expondo digital weighing scale, model SBS-PT-40/1, with a precision of 1 g). The litter trays were then installed in the enriched test room and secured at their predetermined location with double-sided tape. The test started at 10 a.m. in the morning. The cat was brought to the test room approximately five minutes after the litter trays were put in place, to allow the ethanol to evaporate. The familiar human stayed with the cat for the interaction session during 30 min, then left the cat alone in the room, having given the Kong toy filled with wet food behind. The familiar human came back at 12:30 p.m., 2 p.m. and 4:30 p.m. for interaction sessions. After the last interaction session, the litter trays were removed from the test room in order to record data, perform the cleaning procedure, renew the litter substrates and re-apply treatments. The procedure was as follows: the litter trays were removed from the test room and weighed. The type of elimination present was recorded (urine only, stools only, or urine plus stools) and the counting of urine spots and stool piles was performed and recorded. Stools were then removed if present and the litter trays were weighed again (giving the parameter of the "urine only" weight). After all of the data were recorded, the litter substrate was discarded in a special bin. For cleaning, each litter tray was sprayed on the inside and the outside with a detergent disinfectant product that destroys liposoluble compounds (DNA02 LeVrai Professionnel, https://www.bernard.fr/, accessed 29 October 2021) and thoroughly wiped until completely clean. Then, it was sprayed a second time with water to rinse the detergent and avoid any contact between the detergent and the cats. After the litter tray was dried, 1.5 kg of fresh litter substrate was weighed and placed in the litter tray. Finally, according to the randomization list, the designated treatment was sprayed on the litter substrate and mixed with it. The litter trays were weighed to record the weight of the unused litter tray with the treatment applied and were put back in place. The whole procedure lasted less than 10 min. Then the cat was left alone for the night, and the test ended at 9 a.m. the next morning, when the cat was brought back to its cattery. Data were recorded again, and the test room was cleaned. The cleaning procedure was to remove all that was washable, then steam clean the floor and walls, spray a detergent that breaks down proteins and liposoluble compounds (VIGOR surpuissant, https://www.bernard.fr/, accessed 29 October 2021), then rinse the detergent with water, mop the floor, and wait 10 min for the room to dry completely before preparing the room for the next test.

The parameters studied included total elimination weight (urine plus stools, which was calculated by subtracting the weight of the unused litter tray with the treatment from the weight of the used litter tray), urine weight (which was calculated by subtracting the weight of the unused litter tray from the weight of the used litter tray without stools), type of elimination (0 = no elimination, 1 = urine only, 2 = stools only, 3 = urine plus stools), and frequency of urine spots and stools (counting each urine spot and stool pile for each cat). The videos recorded were exported twice a day and stored. Video analysis allowed to study the exploration duration of each litter tray (i.e., time when the cat sniffed the litter tray, had his head inside the tray, or scratched the litter tray) and the first and second choice of litter tray to eliminate.

2.4. Statistical Analysis

Data analysis was performed using SAS 9.4 software Copyright (c) 2002–2012 by SAS Institute Inc., Cary, NC, USA. The significance threshold was fixed at 5%.

Continuous parameters (total elimination weight, urine weight, and exploration duration) were analysed according to the treatment applied (Y and Z), the sex of cats (entire male, entire female, neutered male, and neutered female), and the treatment sex interaction. The normality of residues from raw data was verified using the UNIVARIATE procedure. If normality was verified, the different effects listed on these variables were evaluated with a GENERAL LINEAR MIXED MODEL using the MIXED procedure with "block" in the random statement. If normality was not verified, Box-Cox data transformation was performed using the TRANSREG procedure to try to obtain normality. After a Box-Cox transformation, normality was obtained and transformed data were modelled using the MIXED procedure. If there were significant differences, multiple comparisons were analysed with the TUKEY test using the LSMEANS statement.

Binary variables (first and second choices of litter tray for elimination) were analysed according to the treatment applied, the sex of cats, and the treatment × sex interaction. These effects were evaluated with a GENERALIZED LINEAR MIXED MODEL (Block being considered as a random effect) using the GLIMMIX procedure, specifying the BINARY distribution in the MODEL statement. If there were significant differences, multiple comparisons were analysed with the TUKEY test using the LSMEANS statement.

Polytomous variables (type of elimination, frequency of urine spots and stools) were analysed according to the treatment applied, the sex of cats, and the treatment × sex interaction. These effects were evaluated with the help of the GENERALIZED LINEAR MIXED MODEL (Block being considered as a random effect) through the GLIMMIX procedure, specifying the MULTINOMIAL distribution.

3. Results

3.1. Elimination Weight

For the total elimination weight (Table 2), we found a significant effect of:

(i) **treatment** (**GLMM**; **DF = 1**; **F = 43.44**; $p < 0.0001$) where the elimination weight was significantly lower in the litter tray sprayed with CEMS than in the litter tray with the control;

(ii) **sex** (**GLMM**; **DF = 3**; **F = 7.48**; $p = 0.0009$) where the weight of elimination in females and the weight of elimination in males were significantly lower than in neutered males; and

(iii) **interaction between treatment and sex** (**GLMM**; **DF = 3**; **F = 4.60**; $p = 0.0100$).

Table 2. Means and standard error of the total elimination weight in kilograms and the urine only weight in kilograms, according to treatment and sex.

Variable	Effect		Mean	SE
Total elimination weight	Treatment	CEMS	0.02	0.00
		Control	0.07	0.01
	Sex	Entire male	0.04	0.01
		Entire female	0.03	0.01
		Neutered male	0.09	0.03
		Neutered female	0.06	0.01
Urine only weight	Treatment	CEMS	0.02	0.00
		Control	0.05	0.01
	Sex	Entire male	0.03	0.00
		Entire female	0.03	0.01
		Neutered male	0.07	0.02
		Neutered female	0.04	0.01

There was a significant difference between females and neutered males (**Tukey's test**; **DF = 27**; ***t* value = −4.28**; ***p* = 0.0011**) and males and neutered males (**Tukey's test**; **DF = 27**; ***t* value = −4.22**, ***p* = 0.0013**). Regarding the significant interaction effect, there was a significant difference between CEMS and control within the neutered males (**Tukey's test**; **DF = 27**; ***t* value = 5.37**; ***p* = 0.0003**) and between neutered males and males within control (**Tukey's test**; **DF = 27**; ***t* value = −5.58**; ***p*-value = 0.0002**).

For the weight of urine only (Table 2), we observed a significant effect of:

(i) **treatment** (**GLMM**; **DF = 1**; **F = 41.23**; ***p*-value < 0.0001**) where the weight of urine only was significantly lower in the litter tray sprayed with CEMS than in the litter tray with the control;

(ii) **sex** (**GLMM**; **DF = 3**; **F = 6.81**; ***p*-value = 0.0006**) where the weight of urine only in females and the weight of urine only in males was significantly lower than in neutered males;

(iii) **interaction between treatment and sex** (**GLMM**; **DF = 3**; **F = 4.46**; ***p*-value = 0.0072**). There was a significant difference between females and neutered males (**Tukey's test**; **DF = 54**; ***t* value = −3.78**; ***p* = 0.0022**) and male and neutered male (**Tukey's test**; **DF = 54**; ***t* value = −4.06**; ***p* = 0.0009**).

3.2. Type and Number of Eliminations

We observed a significant effect of treatment for the type of elimination (**GLMM**; **DF = 1**; **F = 15.61**; ***p*-value = 0.0005**), the number of urine spots and the number of stool piles per litter tray (urine spots: **GLMM**; **DF = 1**; **F = 14.42**; ***p*-value = 0.0008**; stool piles: **GLMM**; **DF = 1**; **F = 6.66**; ***p*-value = 0.0152**). Cats significantly chose to eliminate less (score of elimination) in the litter tray sprayed with CEMS than in the litter tray sprayed with the control. They also urinated and defecated significantly less in the litter tray sprayed with CEMS than in the litter tray sprayed with the control (Table 3).

Table 3. Score of type of elimination, frequencies of urine spots and stool piles according to treatment.

Variable	Effect		Score [1]			
			0	**1**	**2**	**3**
Type of elimination [1]	Treatment	CEMS	17	9	1	4
		Control	3	12	3	13
Variable	**Effect**		**Frequency [2]**				
			0	**1**	**2**	**3**	**4**
Urine spots [2]	Treatment	CEMS	18	9	4	0	0
		Control	6	9	11	4	1
Variable	**Effect**		**Frequency [2]**		
			0	**1**	**2**
Stool piles [2]	Treatment	CEMS	26	3	2
		Control	15	15	1

[1] For the type of elimination score 0 = no elimination; 1 = urine only; 2 = stools only; 3 = urine plus stools. [2] For the urine spots and stool piles, frequency represents the total daily number of urine spots and stool piles in the litter tray.

3.3. Exploration of the Litter Trays and Choice of Litter Tray to Eliminate

Cats explored the litter tray with CEMS significantly less than the litter tray with the control (mean ± SE: CEMS 58.16 ± 8.04 s versus control 132.56 ± 13.60 s; **GLMM**; **DF = 1**, **F = 27.86**; ***p*-value < 0.0001**) and chose the litter tray with the control to eliminate significantly more than the litter sprayed with CEMS, for the first choice (**GLMM**; **DF = 1**; **F = 100.33**; ***p*-value < 0.0001**) and second choice (**GLMM**; **DF = 1**; **F = 5.24**; ***p*-value = 0.0293**) of elimination.

For the first choice, a significant difference in the sex effect is observed (**GLMM**; **DF = 3**; **F = 23.49**; ***p*-value = <0.0001**). No significant difference in the sex effect is found for the second choice (**GLMM**; **DF = 3**, **F = 0.02**; ***p*-value = 0.9949**).

These results showed that cats avoided the litter tray sprayed with the CEMS treatment (Table 4).

Table 4. Scores of first litter tray chosen to eliminate and on the second litter tray chosen to eliminate.

Variable	Effect		Score	
			0	**1**
First choice of elimination	Treatment	CEMS	26	5
		Control	7	24
Variable	**Effect**		**Score**	
			0	**1**
Second choice of elimination	Treatment	CEMS	23	8
		Control	14	17

Score 0 = litter tray not chosen; 1 = litter tray chose; Given the binomial nature of these data (choices represented by 1 and 0), differences in numbers could be partially explained by differences in choices, not only by differences in numbers of cats.

During the study, two cats out of 31 urinated outside the litter tray (6% of the population tested).

Taking into account all results, cats significantly chose to eliminate (urinate or defecate) in the litter tray sprayed with the control rather than the litter tray sprayed with CEMS.

4. Discussion

The aim of this study was to assess the effect of a semiochemical composition, reconstituted volatile fraction derived from male cat anal glands (of which some effect had been shown in a previous study [33]), on the elimination behaviour of cats tested individually, in a randomised controlled choice trial. We showed that cats urinated and defecated significantly less in a litter tray sprayed with the treatment CEMS than in a litter tray sprayed with the control. They also explored the litter tray treated with CEMS significantly less than the one with the control, and their first and second choice for elimination was significantly the litter tray sprayed with the control treatment. There was a significant effect of sex (females versus neutered males and males versus neutered males) for the elimination weights (urine plus stools and urine only) where neutered male produced a higher weight of total elimination and a higher weight of urine only then both females and males.

4.1. Effect of Treatment

Our study showed that cats urinated and defecated significantly less in the litter trays sprayed with the CEMS treatment, across all of the elimination behaviour parameters: elimination weight, type of elimination, urines spots, and stools piles. These results confirm the ones of our previous study regarding defecation and give more data about urination (urine weight and urine frequency).

The CEMS treatment also had a significant effect on the first and second choice of litter tray for elimination, with the control litter trays being significantly chosen for the first elimination event and for the second elimination event. Some cats even always chose the control litter tray. So, the CEMS treatment seems to have an aversive effect and significantly deter cats from choosing the litter tray where it is sprayed. This is confirmed by the parameter "exploration duration of the litter trays", with the litter tray sprayed with the CEMS treatment being explored significantly less than the litter tray with the control treatment. The exploration duration parameters and the choice of elimination parameters were co-dependent, as the exploration of the litter tray included the scratching of the litter substrate.

Urine, feces, and sebaceous glandular secretions contains chemical compounds that are vectors of chemical communication [7,8]. Anal gland secretions can be excreted in cat urine [7] and also on cat feces [9]. When allowed outside and living in a colony, cats bury their feces in their core home range and tend to bury them less in the peripheral areas of their home range [8]. Entire male cats also sometimes do not cover their feces as well [8] and cat feces contain the chemical basis of species, sex, and individual recognition [13]. The semiochemical composition tested in this study is specific to an entire male cat. Therefore, it is possible that the avoidance behaviour was triggered by this semiochemical composition, considered as a territorial scent marking.

4.2. Effect of Sex

There was a significant effect of sex in three out of eight parameters. The first effects were on the elimination weight (i.e., total elimination weight (urine plus stools) and urine only weight). For both parameters, the statistical analysis showed that there was a difference between female and neutered males, and males and neutered males. From a previous study of cats in our cattery [33], we showed that neutered male cats were also significantly heavier than female cats. Neutered male cats are also older than male and female cats. As water intake and urine production can be influenced by cat weight [34], it is possible that the elimination weight of neutered male cats, both depending on their average weight and their condition, could be higher than that of male and female cats that were much younger (approximately three years old) and for the females, significantly lighter. This hypothesis is also supported by the fact that there was no effect of sex on the number of urine spots. Therefore, neutered males did not urinate more frequently, but produced more urine during their urination.

The avoidance effect depended on the cat, and in our study, it was present in the four sex categories tested (entire males and females, neutered males, and females), despite significant differences between them. During the study, no cats showed signs of stress making necessary to stop the test. A few cats were more sensitive than others and refused to use either litter tray and sprayed and urinated outside the litter tray. It is possible that the perception of this semiochemical composition was perceived as stressful for the most sensitive cats [35] in our enclosed study setting. However, in the test conditions, the cats could be away from the litter trays, but could not leave the room. In real life conditions, cats could freely leave the location where the CEMS treatment would be applied, rendering less likely the possibility to be stressed by continuous exposure to a signal triggering avoidance. Moreover, chemical signals are a part of the cats' daily lives [5] so the signal would be, in real life, one signal present in a wealth of other chemical signals, and part of the cats' natural environment.

5. Conclusions

We demonstrated that a semiochemical composition, reconstituted volatile fraction derived from cat anal glands, deterred the subjects from defecating and urinating in the litter tray where it was sprayed. This semiochemical composition does not prevent the elimination of the cat but could promote redirection of elimination. Future research is warranted to explore the possibility of using this semiochemical composition to help manage unwanted toileting in real life conditions and if this composition, while triggering avoidance, may also modulate other behaviours of cats and/or have stressful effects on cats.

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