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Emotional characteristics and personality traits: a study in mice of wild origin

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Emotional characteristics and personality traits:
a study in mice of wild origin

Abstract: The mechanisms driving animal personality (i.e., consistent individual differences in behavior across time and contexts) are still poorly understood. Recently, it has been proposed that personality traits may emerge from individual differences in emotional reactions. This thesis aimed to investigate how exploration tendency, one of the most frequently studied personality traits, is related to consistent individual differences in emotions in different age classes, using two rodent species of wild origin. In each chapter, we focused on one component of an emotional reaction (respectively, behavior, cognition and physiology), to assess either valence (i.e., positive or negative) or arousal (i.e., high or low) of the emotional experience. First, we showed that isolation call rate could be used to phenotype emotional profiles of young house mice, as pups' call rate was consistent over days and across three stressful situations. However, call rates were not associated with exploration tendency during adulthood. Second, our results suggested that a higher exploration tendency might be associated with a higher tendency to express negative affective states (i.e., a more negative judgement bias). Third, using infrared thermography, we found that fast explorative mound-building mice were characterized by a stronger sympathetic reactivity, as expressed by lower peripheral tail temperatures, than slow explorers shortly after a brief handling procedure. Overall, the findings of this research project contribute to the understanding of the emotional basis of personality traits and highlight the importance of considering individuality, through personality traits, when assessing emotions.

Keywords: *exploration tendency; affective state; isolation calls; cognitive judgement bias; infrared thermography; house mouse; mound-building mouse*

Caractéristiques émotionnelles et traits de personnalité : une étude chez des souris d’origine sauvage

Résumé : Les mécanismes qui sous-tendent la personnalité animale (c.-à-d., les différences individuelles de comportement stables à travers le temps et les contextes) sont encore mal compris. Il a été suggéré que la personnalité pourrait émerger à partir de différences individuelles dans les réactions émotionnelles. Cette thèse a pour objectif d’étudier comment la tendance à l’exploration, l’un des traits de personnalité les plus étudiés, est liée aux différences individuelles d’émotions, à différentes classes d’âge chez deux rongeurs d’origine sauvage. Chaque chapitre aborde un composant d’une réaction émotionnelle (comportement, cognition et physiologie), afin d’évaluer la valence ou l’intensité de l’expérience émotionnelle. Tout d’abord, nous avons montré que le taux d’appels d’isolement pouvait être utilisé pour caractériser les profils émotionnels de jeunes souris domestiques, celui-ci étant stable durant trois jours et dans trois situations stressantes. Cependant, ce taux n’était pas associé avec la tendance à l’exploration durant l’âge adulte. Deuxièmement, nos résultats ont suggéré qu’une tendance plus forte à l’exploration pourrait être liée à une plus grande tendance à exprimer des états affectifs négatifs (c.-à-d., un biais de jugement plus négatif). Troisièmement, nous avons constaté que les souris glaneuses plus exploratrices étaient caractérisées par une réactivité plus forte du système sympathique, exprimée par des températures périphériques de la queue plus basses, peu de temps après une procédure de manipulation brève. Dans l'ensemble, les résultats de ce projet de recherche contribuent à la compréhension de la base émotionnelle des traits de personnalité et soulignent l'importance de prendre en compte l'individualité lors de l'évaluation des émotions.

Mots-clés : *tendance à l’exploration ; état affective ; appels d’isolement ; biais de jugement cognitif ; thermographie infrarouge ; souris domestique ; souris glaneuse*

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1. General introduction

1.1. Animal personality: concepts and interest

1.1.1. Terminology and background

Animal personality has been a huge topic of interest in the past years. It has been reported in numerous clades that non-human animals (hereafter animals) within a population of a given species behave differently from each other and that such individual variation can be consistent over a long period of time (Carere & Eens, 2005; Carere & Maestripieri, 2015; Gosling, 2001; Sih et al., 2004; Vonk et al., 2017). Laboratory animals under strictly controlled environmental conditions, including individuals from inbred lines, also exhibit individual variation in personality (e.g., in laboratory mice *Mus musculus*: Freund et al., 2013; Lewejohann et al., 2011; Rödel et al., 2012). The field of animal personality benefits from studies of various disciplines as behavioral ecology (Dall et al., 2004; Sih et al., 2015), evolution (Reader et al., 2007; Réale et al., 2010); neuroendocrinology (Koolhaas et al., 1999), developmental biology (Stamps & Groothuis, 2010a,b), neuroanatomy (Corr & McNaughton, 2012; Montag & Panksepp, 2017; Wiese et al., 2018) and genetics (Dingemanse & Araya-Ajoy, 2015; Montag & Reuter, 2014; Phocas et al., 2006), contributing to its conceptual and experimental richness.

In the following, I will first give some main definitions underlying the concept of animal personality, present the personality traits typically assessed and briefly explain why personality traits are maintained within a population.

1.1.1.1. Definitions

Why and how consistent between-individual differences in behavior emerge and are maintained is now better understood due to the vast amount of research in the field and the synthesis work of several reviews. Among important challenges still faced by the researchers, and that some reviews aimed to overcome (Carter et al., 2013; David & Dall, 2016; Réale et al., 2007), are the variety of terms used to describe such differences and the traits measured, alongside with the diversity of experimental procedures and the associated behavioral variables to be quantified.

'**Animal personality**' and '**temperament**' are often used as analogous to describe between-individual differences in behavior that are consistent across time and contexts (Archard & Braithwaite, 2010; Bell, 2007; Gosling, 2001; Réale et al., 2007). Hence, individuals exhibit a particular 'personality type', for example, being repeatedly more aggressive in different contexts such as an agonistic encounter with a conspecific and a confrontation with a predator. Such differences between individuals, that is, their ranking within a population, are assumed to be maintained over time. However, this definition does not assume that the behavior cannot change over the lifespan of the individuals, particularly across crucial developmental steps, such as weaning or sexual maturation (Fawcett & Frankenhuis, 2015; Herde & Eccard, 2013). '**Behavioral syndromes**' refer to a set of correlated behaviors across multiple situations at the population level, while a 'behavioral type' characterizes the individual within the syndrome (Bell, 2007; Sih et al., 2004). That is, personality traits are often associated with each other; typically, the more aggressive individuals are also bolder, more explorative and more active. Finally, the concept of stress '**coping styles**' evaluates the consistent individual differences in behavioral and neuroendocrinological reactions to challenging situations (Koolhaas et al., 1999, 2010). The more recent view of coping style places the classical proactive-reactive continuum alongside three dimensions: more proactive individuals can be characterized by a low emotional arousal, a high reward sensitivity and a low executive control (de Boer et al., 2017).

1.1.1.2. Which traits are part of animal personality?

Five personality traits are predominantly investigated (also called the ‘Big Five animal personality traits’: Réale et al., 2007): exploration, boldness, activity, sociability and aggressiveness. Other traits can also be considered as part of an individual’s personality such as anxiety (Ibáñez et al., 2007; Schrader & Müller, 2005) or the equivalents of the human *Homo sapiens* Big Five personality traits, such as Extraversion or Neuroticism evaluated through ratings by human observers (e.g., orange-winged Amazons *Amazona amazonica*: Cussen & Mench, 2014; cotton-top tamarins *Saguinus oedipus*: Masilkova et al., 2018). Personality traits are seen as a continuum; for instance, animals can be evaluated along the shyness-boldness or the exploration-avoidance axis. Réale et al. (2007) define the shyness-boldness axis as “an individual’s reaction to any risky situation, but not new situations”, while the exploration-avoidance axis refers to “an individual’s reaction to a new situation”, including new environment, food, or object. The terminology proposed by Réale et al. (2007) has been adopted by a majority of studies. However, these definitions have not been always followed by authors referring to their terminology (e.g., Mazza et al., 2018), contributing to the confusion and lack of consistency among studies about the definition of the traits, the choice of the tests and the behavioral variables assumed to assess them. In this thesis, I will use the term ‘exploration’ in the sense of Réale et al. (2007), that we assessed by combining behavioral variables quantified in open field and novel object tests.

1.1.1.3. Maintenance of personality traits

Animal personality traits are heritable (van Oers et al., 2011, 2015) and are linked to reproductive success, survival and longevity (Boon et al., 2007; Careau et al., 2010; Seyfarth et al., 2012; Smith & Blumstein 2008), thus natural selection can act on them (Dall et al. 2004; Sih et al. 2004). Fitness advantages have been reported to be associated with the expression of certain personality types within a population. Typically, more aggressive, bolder and/or active individuals gain more food intake and have an increased growth, which may improve their reproductive success (Biro & Stamps, 2008). Yet, other personality types can also be favored through balancing selection processes. For instance, rare personality types can be advantaged through processes of negative frequency-dependent selection, similarly to the Hawk–Dove game of competition for resources (Dall et al., 2004; Wolf et al., 2008). Furthermore, various personality types can be favored depending on the environmental conditions, in particular within a heterogeneous environment (Dingemanse & Réale, 2005). For instance, fast explorer male great tits *Parus major* had higher survival rates during winters with high food availability while slow explorer males were favored during winters with low food availability (Dingemanse et al., 2004). Personality types may also influence how individuals disperse and occupy their environments (Cote et al., 2014; Sih et al., 2012). The maintenance of opposite personality types within a population could be explained by differences between individuals in their investment in the trade-offs between growth and mortality (Biro et al., 2004; Stamps, 2007) or between different life-history strategies (Wolf et al., 2007; Wolf & Weissing, 2012). Finally, positive assortative personalities within breeding pairs has also been shown to be associated with fitness advantages, such as a faster onset of reproduction (Rangassamy et al., 2015) and higher offspring growth (Both et al., 2005), also participating to the maintenance of personality variation.

1.1.2. Ontogeny of personality

The development of personality traits results from complex interactions between genetic effects (Dochtermann et al., 2014; Drent et al., 2003; Stamps & Groothuis, 2010b) and the environment in which the individual develops. Throughout ontogeny, various environmental conditions and factors have the potential to influence the set-up of individual (personality) differences (Hudson et al., 2011; Stamps & Groothuis 2010a; Trillmich & Hudson, 2007). Personality traits have been shown to be less consistent over large periods of time (Bell et al., 2009; Biro & Stamps, 2015; Stamps & Groothuis 2010b), which also depends on the personality trait investigated (Herde & Eccard, 2013). In particular, behavioral plasticity is often considered to be high in young animals before decreasing over time, in relation to the strong behavioral, neural and physiological changes that young animals undergo in their early life (Groothuis & Trillmich, 2011; West-Eberhard, 2003). This makes their emerging personality more flexible and sensitive to environmental changes, even though consistent individual behavioral profiles have been found in young animal (e.g., Hudson et al., 2015; Myers & Young, 2018; Rödel et al., 2017; Špinka et al., 2018).

However, only a few studies investigated whether early behavioral profiles may be predictive of later personality traits, especially across important developmental steps. These studies reported, for instance, associations between early vocalization profiles and emotionality in adults (Brunelli & Hofer, 2007), or sociability and spatial memory (Yoshizaki et al., 2017), which would deserve more attention (see section 5.2., p.146).

1.1.3. Personality and individual differences in cognition

Personality traits have also been studied as potential contributors of individual differences in cognitive abilities (Boogert, et al. 2018; Carere & Locurto, 2011; Griffin et al., 2015). '**Cognition**' is defined as the acquisition, processing, storage and use of information from environment (Shettleworth, 2010). Cognitives abilities are psychological processes and thus cannot be directly quantified; they are inferred from the evaluation of their observable manifestation: the behavior. Hence, research in animal cognition mainly focuses on the mechanistic basis of behavior, to know which cognitive abilities lead to changes in behavior. Numerous cognitive processes are investigated, as perception, learning, behavioral inhibition, self-recognition, language, memory or decision-making.

More recently, the individual variation in such abilities has become a topic of interest by its own, in order to understand the differences in performance between individuals within a population, as it is traditionally done in human psychology (Carroll & Maxwell, 1979). Individuals with distinct 'cognitive styles' consistently differ in the way they acquire, process, store and act on the information, that is, a cognitive style does not depend on the ability per se (Gruszka et al., 2010).

Hence, several authors suggested that personality types may be closely related to differences in cognitive styles (Carere & Locurto, 2011; Sih & Del Giudice, 2012). If the causal relationships between these two concepts remain to be elucidated (Griffin et al., 2015), one main hypothesis has received important attention across species: fast behavioral type individuals (that is, more proactive, aggressive, active, explorative and bolder individuals) should exhibit a higher speed but a lower accuracy cognitive style compared to slow behavioral types (speed-accuracy trade-off: Sih & Del Giudice, 2012). The fast type individuals would learn faster but also tend to form more routines, hence exhibiting more inflexible behavioral responses. That is, they would be less sensitive to small details of their environment and would perform poorly when the task requires to use fine details of the environment or to change the rules they previously learned (e.g., in reversal learning tasks). This hypothesis first received experimental support in several species (e.g., guppies *Poecilia reticulata*: Burns & Rodd 2008; guinea pigs *Cavia aperea*: Guenther et al., 2014; chickadees *Poecile atricapillus* Guillette et al., 2011; bank voles *Myodes glareolus*: Mazza et al., 2018; common ravens *Corvus corax*: Stöwe & Kotrschal, 2007).

However, a meta-analysis conducted by Dougherty & Guillette (2018) revealed inconsistencies in results both across and within species. Indeed, the direction of the proposed association presented above was highly dependent on the study species, the population and the context in which the personality traits were measured (see also Table 1 in Mazza et al., 2018). For example, boldness assessed in response to a predator, but not in response to a novel object or food, was positively associated with a high learning speed (Dougherty & Guillette, 2018). As also highlighted by Griffin et al. (2015), associations between personality traits and cognitive styles should be assessed repeatedly for the population of interest, without assuming its direction or its consistency over time (Boogert et al., 2018).

1.1.4. Application to animal welfare

Driven by pressure from public society and research, promoting a good welfare in captive animals is a current major interest. Not only the prevalence of negative emotions should be minimized, but positive emotional states should also be encouraged (Boissy & Erhard, 2014). Thus, animal welfare science aims to identify items and situations that induce positive or negative emotions and how their modification may influence the individual's mood (that is, longer-term emotional states, Mendl et al., 2010), with the ultimate goal to assess the animals' subjective affective state (Broom, 2008; Fraser, 2009).

To this aim, taking into consideration the individual behavioral profiles of the animals, that is, their personality traits, helps to advance knowledge in animal welfare (Richter & Hintze, 2019). Indeed, similar rearing conditions may have differential effects depending on the individuals' personality (Bolhuis et al., 2004; Melotti et al., 2011). Specific personality types may also be more likely to rely on environmental conditions to maintain a positive mood. For instance, proactive domestic pigs *Sus scrofa* were always more optimistic independently of their housing conditions whereas the mood of reactive pigs depended on the enrichment of their environment (Asher et al., 2016). Moreover, some personality traits may be more related to welfare issues than others, such as fearfulness (associated with pessimism: Lecorps et al., 2018b) or proactivity (with development of stereotypy: Joshi & Pillay, 2016). Hence, as a same situation may be experienced as positive by some individuals but may constitute a source of stress (thus leading to a poorer welfare) to others, developing housing conditions for group-housed species that fit different personality types remains a challenge (Richter & Hintze, 2019).

Taken together, these findings highlight the importance of considering individuality when assessing or aiming to improve animal welfare, instead of comparing groups or generalizing welfare improvements to the whole species (Fraser, 2009). Particularly, as emotions constitute a central topic in animal welfare, the assessment of their consistency over time and potential changes over ontogeny still remain to explore in more details (de Vere & Kuczaj, 2016; Richter & Hintze, 2019), even though the consistency of fear- and anxiety-related behaviors are increasingly considered in recent studies (Boissy & Erhard, 2014).

1.2. Emotions: theories and evaluation

1.2.1. History, definition and main approaches

The study of emotions is a key topic in human psychology and gave rise to numerous theories about their emergence (e.g., peripheral theory: James, 1884; central theory: Cannon, 1927; appraisal theories: Scherer, 1984; Lazarus, 1991). In humans, the subjective and conscious emotional experience is central in these theories but, as subjective feeling cannot be directly assessed in animals, the study of emotions in non-humans is quite recent. Darwin (1872/1998) was among the firsts to consider the question, proposing a continuity between human and animal emotions and mental lives. However, the scientific study of animal emotions was seen out of the scope of scientific study during the majority of the 20th century when the vision of the behaviorists was predominant. The ethologist Nicolaas Tinbergen (1963), at the origin of the four questions forming the basis of the discipline (causation, ontogeny, function and evolution of behavior), also followed that vision. Yet, other researchers of that period included subjective states in the study of animal behavior (for a review see Fraser 2009); Burghardt (1995) even suggested to add a fifth question focused on emotions as the study of behavior also helps to understand the affective states of animals. Nevertheless, the existence of an emotional life in animals has often been denied and has been excluded from a functional explanation of behavior (Fraser, 2009). Nowadays, emotions, regardless of whether they are felt consciously, have been recognized as essential to understand behavior (Wilson et al., 2019), and the question is not anymore about whether animals can have emotions.

Defining emotion is of major importance as the research question will differ depending on the key elements highlighted in the definition (Paul & Mendl, 2018). Even if there is no clear consensus, **emotions** can be defined as intense and brief states in response to a salient stimulus or event (from the internal or external environment) and they involve behavioral, physiological, neuronal, cognitive and subjective changes allowing the individual to respond to the eliciting stimulus (Désiré et al., 2002; Ledoux, 2012; Paul et al., 2005; Rolls, 2005). They are adaptive as they enable animals to avoid harm and punishers or to seek resources and rewards (Cardinal et al., 2002; Panksepp, 1994; Rolls, 1999). Other terms are also often encountered in animal emotion studies and may lead to confusion in the literature (de Vere & Kuczaj, 2016): **affect** may be used as analogous of emotion (Bliss-Moreau, 2017; Ledoux, 2012), as similar to mood (Paul et al., 2005) or to describe only the subjective experience (Ede et al., 2019; Fraser, 2009; Panksepp, 2010), whereas **mood** usually refers to more 'free-floating', longer-term states resulting from the accumulation of short-term emotions (Boissy & Lee, 2014; Mendl et al., 2010). In this thesis, I will use emotion and affect as interchangeable terms and the term 'mood' following the previous definition. Emotions can be separated in two categories: primary emotions (as fear, anger or joy) and secondary emotions (as shame, guilt or jealousy, which might be shared with humans only by a few species such as non-human primates) (Ha & Campion, 2019). The rest of this section will focus only on the primary or 'basic' emotions, as they are assumed to be shared among at least all vertebrates (Panksepp, 2011a).

Two main opposite views currently co-exist to describe emotions: discrete emotions and dimensional approaches. The **discrete** (also modular or basic) **emotion approach** argues that it exists distinct emotions (e.g., fear, rage or play) supported by underlying specific neural systems and related to specific autonomic changes (Tracy & Randles, 2011). The **dimensional** (or core affect) **approach**, in its most common definition, describes emotions along two dimensions (or axes): the valence (either positive or negative according to the rewarding or punishing value of the stimulus) and the arousal (that is, the high or low intensity), also under the control of specific neural circuits. Mendl et al. (2010) proposed an integrative framework to reunite the two approaches (Fig. A); see also Panksepp (2007a) and Izard (2007) for similar opinions.

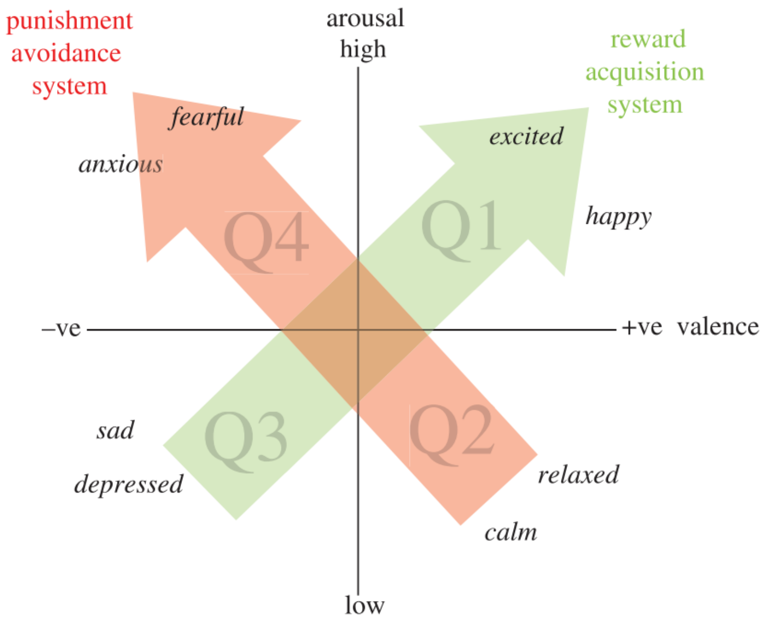


Figure A. Representation of the emotional experience according to the conceptual framework of Mendl et al. (2010) which integrates both the core affect approach, represented by two dimensions (arousal and valence), and the discrete emotion approach (basic emotions are noted in italics in their proposed locations along the two previous dimensions). In italics are also noted the subjective affective states possibly experienced by the individuals inside each quadrant (positive in quadrants Q1 and Q2 and negative in Q3 and Q4). The red arrow represents a possible bio-behavioral system to support punishment avoidance by changing the individual's affective state from a calm/relaxed (Q2) to a fearful/anxious (Q4) state. The green arrow represents a system that modifies a sad/depressed (Q3) into a happy/excited state, underlying the acquisition of rewards.

The major challenge in the study of emotions in non-human animals remains the assessment of its subjective, conscious part, as it cannot be assessed directly or, as in humans, via verbal reports. To overcome this issue, researchers in the field of emotion aim to assess the affective, internal state of the animals through the evaluation of observable and quantifiable variables, such as physiological and behavioral variables (Ledoux & Hofmann, 2018), without making inferences about a potential consciousness of the internal state (e.g., Ede et al., 2019; Mendl et al., 2010; but see 1.2.5., p.25, for a different opinion about how to assess the subjective experience). In that perspective, the whole emotional experience can be decomposed in different elements. I will follow here the classical classification (Dantzer, 1988; Désiré et al., 2002) with the addition of a more recent, cognitive component (Anderson & Adolphs, 2014; Clore and Ortony, 2000; Mendl et al., 2009; Paul et al., 2005). Thus, emotions can be divided in four components: behavioral, physiological, cognitive and subjective. Each of them will be further detailed in following sub-sections, focusing on mammals and particularly on laboratory rats *Rattus norvegicus* and mice, as the proxy measures of an emotional reaction are species-specific.

1.2.2. Behavioral component

The most widely assessed component of emotions is behavior as it can be directly observed. It may be of particular importance to evaluate the adaptive function of emotions (Frijda, 1986). In this context, behavior can be assessed at different scales, from general activity to fine facial expressions. Emotions are assumed to help individuals to avoid harmful situations and seek out positive ones. Hence, the most basic behaviors that can be assessed, to evaluate the elicited emotional valence, are approach and avoidance (or escape) from a stimulus. Usually, such approach/avoidance behaviors are complemented by the quantification of more specific behaviors, such as the duration of freezing (Galliot et al. 2012), exploratory activity (Ohl, 2003), consumption (or its absence in the study of anhedonia in depression models: Neumann et al., 2011).

Numerous tests have been specifically designed to assess emotional reactions of mammals, especially via the induction of negative emotions (mainly anxiety: Bouwknecht & Paylor, 2002; Harro, 2018, and fear: Galliot et al., 2012; Mobbs, 2018) during tests leading to unconditioned responses, such as the elevated plus maze (Carobrez & Bertoglio, 2005), open field (Perals et al., 2017), novel object (Ennaceur et al., 2009), startle test (Yuen et al., 2017) or light-dark box (Kulesskaya & Voikar, 2014) in rodents. Many of them have been widely applied to investigate new treatments or to assess the effect of drugs in neuropsychological human diseases (e.g., depression: Borsini et al., 2002; Czéh et al., 2016; schizophrenia: Sahin et al., 2016). For instance, exploration (also termed activity or exploratory activity) in the open field is one of the most discussed behaviors (Carter et al., 2013; Perals et al., 2017; Réale et al., 2007). Exploration, frequently considered to be “driven by an approach/avoidance conflict” (Augustsson and Meyerson, 2004), is often reduced after injection of anxiogenics and increased after the use of anxiolytics, leading researchers to interpret greater exploratory activity in the open field as being representative of low anxiety levels (but see Ennaceur et al. (2010) and Ennaceur (2014) for discussions on this phenomenon, as an increased activity may also represent a tendency to escape, induced by a high state of fear).

Learned responses can also be used and are central in animal welfare science to identify whether a specific stimulus is perceived as positive or negative. For instance, preference tests can be used to investigate which kind of surface (Telezhenko et al., 2007) or nest material (Blom et al., 1996) the animals value the most (that is, which one acts as a positive reinforcer). Yet, such tests may provide little information about the absolute perceived value as the results only provide a rank assessment (Ede et al., 2019).

Facial expressions can be used to assess emotional responses (Descovich et al., 2017), either negative (e.g., fear in laboratory mice: Defensor et al., 2012) or positive (e.g., in response to tickling in laboratory rats: Finlayson et al., 2016). For example, ears in a backward position were associated with a low emotional reactivity in house mouse (Lecorps & Féron, 2015). The ear position has also been used to distinguish the emotional valence in sheep *Ovis aries* (Reefmann et al., 2009a,b) and cows *Bos taurus* (Proctor & Carder, 2014). Also, ‘grimace scales’ have been designed to assess pain in rats (Sotocinal et al., 2011), mice (Langford et al., 2009) and horses *Equus caballus* (Dalla Costa et al., 2014).

Vocalizations have been one of the most studied parameters in numerous species as they allow a quick transmission of emotional states to others (Briefer, 2012; Brudzynski, 2018). They have been predominantly studied as representative of arousal (see Table 3 in Briefer, 2012), but animals also emit distinct calls in response to either positive or negative situations (Barker, 2018). Thus, vocalizations are often considered as indicators of the valence of an individual’s affective state. For instance, laboratory rats emit ultrasonic ‘chirping’ vocalizations when tickled, which have been considered as a form of ‘laughter’, as in apes (Davila-Ross et al., 2009) and in humans (Panksepp, 2007b; Panksepp & Burgdorf, 2010). Laboratory rats have been particularly studied for their vocalizations as they emit two types of vocalizations: ‘22-kHz calls’ in typically negative situations and ‘50-kHz calls’ in typically positive situations (but see Simola & Brudzynski (2018) for more details about the contexts of emission). Other species also express distinct calls depending on the valence as goats *Capra hircus* (Briefer et al., 2015), silver foxes *Vulpes vulpes* (Gogoleva, et al., 2010), domestic pigs (Ferreira da Silva Cordeiro et al., 2013) and cattle (Watts & Stookey, 2000). In laboratory mice, although no clear categorization of their vocalizations has been done, the emotional state of the individual also influences its calls (Granon et al., 2018). Vocalizations also provide the advantage that they can be studied across ontogeny (Peleh et al., 2019). Particularly dependent young, when separated from their caregivers and siblings, produce isolation calls (also named distress calls) whose number may depend on the intensity of the emotional distress experienced during isolation (Caruso et al., 2018; Winslow, 2009); that is, a higher number of such calls showing a stronger negative emotional state. Pups' isolation call rates will be quantified in **Chapter 1** to assess the individual consistency of such call rate over three consecutive days and across three stressful situations (Verjat et al., 2019).

1.2.3. Physiological component

Most of the physiological parameters assessed in emotion research overlap with those measured in stress research (Moberg & Mench, 2000), especially when investigating negative emotions such as anxiety or fear. Earlier definitions of stress often excluded the affective part related to the physiological stress reaction, although animals were usually placed in aversive situations that may have led to negative emotions (Moberg, 2000; Ramos and Mormède, 1998; but see von Holst (1998) for a different perspective). However, one of the last definition states that "the stress response is the sum of psychic arousal and changes in affect associated with physiological reaction where resources are mobilized, to sustain metabolic and behavioral adaptations, and allow the classical 'fight or flight' reaction" (Bombail, 2019).

As a consequences, the most studied parameters are the ones under the control of the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic-adrenal-medullary (SAM) system, after the confrontation with a stressor of interest (von Holst, 1998). For the HPA axis, ACTH and glucocorticoid levels (corticosterone mainly in birds, amphibians, reptiles, *Murinae*, and lagomorphs; cortisol in the majority of fish and in other mammals: Palme, 2019), that increase after the confrontation with a stressor, can be measured (Möstl & Palme, 2002; Vera et al., 2017; Wingfield, 2013). Traditionally, their analysis is done in blood, plasma or serum but, as the blood sampling itself may lead to an emotional reaction and be confounded with the response to the stressor of interest, non- or low-invasive techniques have gained in popularity and glucocorticoids can now be measured through the collection of saliva (Menargues Marcilla et al., 2008), urine (Palme et al., 1996), feces (Palme, 2019), milk (Yeh, 1984), hair (Keckeis et al., 2012), feathers (Bortolotti et al., 2008) or eggs (Rettenbacher et al., 2005).

Changes in the activity of the SAM axis can be assessed through heart rate, respiratory rate, blood pressure, skin conductance, levels of catecholamines or other circulating hormones (Caramaschi et al., 2013). Such markers of emotional reactions can be recorded in real time in large animals (except for hormone levels) without applying invasive telemetry techniques (e.g., by using a chest belt with an external heart rate monitor: Tamioso et al., 2018). However, the use of these techniques, such as heart rate or blood pressure telemetry, in small mammals as rodents typically requires surgery to implant a telemetry sensor (Krohn et al., 2003; Niemeyer, 2016). As an alternative, infrared thermography allows the recording of real-time changes in the body heat loss, a consequence of the cutaneous blood flow under the control of the sympathetic nervous system (Mufford et al., 2016; Stewart et al., 2005; Vianna & Carrive, 2012). This technique can be used in free-moving animals, at least within a smaller scale, small enough to be surveyed by an infrared thermal camera. By this technique, the body surface temperature can be correlated with the simultaneous behavior shown by the individual, allowing an assessment of its emotional reaction (Sato et al., 2018; Vianna &

Carrive, 2012). Infrared thermography has been proven useful to record stress and fear responses in the laboratory rat (Ågren et al., 2009; Vianna et al., 2005), the laboratory mouse (Gjendal et al., 2018) and the house mouse *Mus musculus domesticus* (Lecorps et al., 2016). In such species, the typical thermal response is a decrease in peripheral body parts (in particular in the tail) and an increase in body and eye temperature. We will use infrared thermography in **Chapter 3**, in the mound-building mouse *Mus spicilegus*, to assess their responses to handling in relation to their exploration tendency (Duparcq et al., 2019).

Nevertheless, measurements of the activity of the HPA axis and SAM system seem to mainly represent changes in the individual's arousal level, without informing about the valence of its affective state (Paul et al., 2005). A recent study showed specific changes in nasal temperature after the presentation of positive and negative stimuli in male common marmosets *Callithrix jacchus* using infrared thermography (Ermatinger et al., 2019). Although differences in arousal might also explain these results, further studies directly comparing positive and negative stimuli should be encouraged to potentially describe distinct physiological reactions depending on the valence of the situation (see Appendix 6.3., p.182). A few studies, mainly in farm animals, assessed the amount of visible eye white mediated via sympathetic control, but found contradictory results regarding the valence associated with an increase or a decrease in visible eye white (e.g., Sandem et al., 2002; reviewed in Ede et al., 2019). Hence, there is a possible limitation of the interpretation of physiological parameters as being unspecific proxies of the emotional intensity. However, positive emotions have received little attention so far (de Vere & Kuczaj, 2016), leading to less available information about physiological reactions to positive than to negative stimuli, but recent reviews should further stimulate such research (Ahloy-Dallaire et al., 2018; Balcombe, 2009; Berenbaum et al, 2016; Boissy et al., 2007). Moreover, biomarkers of emotions in humans, which could help to distinguish between positive and negative emotions, are currently investigated for their usefulness in animal research (e.g., immunoglobulin A: Staley et al., 2018).

1.2.4. Cognitive component

The influence between cognition and emotion is bidirectional, that is, distinct emotions may arise after the cognitive evaluation of the situation and of the internal state of the subject (appraisal theories) and the cognitive abilities of the individual may be modified by its emotional state (cognitive bias).

The term ‘appraisal theories’ refers to a theoretical approach started by Arnold (1960) and Lazarus (1966) and has been developed since then by several authors (e.g., Clore & Ortony, 2000; Ellsworth & Scherer, 2003; Roseman, 2013; Scherer, 2013). Appraisal theorists suggest that the appraisal (i.e., evaluation) of the environment, in interaction with the individual, plays the most important role in eliciting an emotion, as it will determine the type and intensity of the emotional experience (Moors et al., 2013). Scherer (2001, 2009) proposed that the evaluation (appraisal) of an event is based on four criteria of increasing complexity: (*i*) its relevance (suddenness, familiarity, predictability, goal relevance and pleasantness), (*ii*) its implications for the well-being and goals of the subject, (*iii*) the coping potential of the individual (its ability to cope with the consequences and to control the event), and (*iv*) the normative significance of the event and the response (compatibility with self-esteem, social norms and moral rules). Although these theories have been first designed for humans, animals have also been investigated for their appraisal abilities, mainly in farm animals with a welfare perspective (Boissy & Erhard, 2014; Désiré et al., 2002; Veissier et al., 2009). For instance, it has been shown that sheep (*a*) react to novelty and suddenness (Désiré et al., 2004, 2006) and express different responses depending on (*b*) the consistency of the event with their expectations (Greiveldinger et al., 2011), (*c*) their ability to control the situation (Greiveldinger et al., 2009) and (*d*) their social context (Greiveldinger et al., 2012). In rodents, only a few studies have been conducted but laboratory rats have been shown to develop less ulceration when given the possibility to control the presence of electric shocks (Weiss, 1972) or to predict their advent (Weiss, 1971). However, appraisal theorists do not make the assumption that the evaluation processes of the situation are systematically conscious, and such processes may be rather automatic for primary emotions (Paul et al., 2005).

In turn, the emotional state can also influence information processing through three types of cognitive bias: attention, memory and judgement bias (Mendl et al., 2009; Roelofs et al., 2016). Cognitive biases have been demonstrated in numerous species, since the first judgement bias study by Harding et al. (2004), and have been mainly applied in animal welfare science (Baciadonna & McElligott, 2015; Clegg, 2018; Crump et al., 2018) and psychopharmacology (Hales et al., 2014). An attention bias involves a modification of the attention toward negative or (potentially) threatening stimuli when the individual is in an anxious emotional state (rhesus monkeys *Macaca mulatta*: Lacreuse et al., 2013; sheep: Lee et al., 2016; Verbeek et al., 2019; cattle: Lee et al., 2018). Memory bias occurs when positive or negative events (that is, with an emotional load) are more readily remembered than neutral ones (laboratory rats: Burman & Mendl, 2018; rhesus monkeys: Lacreuse et al., 2013). The most frequently investigated one is the judgement bias: the emotional state (or the mood) is assumed to influence the interpretation of ambiguous information (see the supplementary material of Roelofs et al. (2016) for a review of the main judgement bias studies). In particular, judgement bias tests provide the major advantage to allow the evaluation of the valence of the individual's emotional state, as an individual with a positive mood would behave more optimistically (Boleij et al., 2012; Lalot et al., 2016). A positive judgement bias, for instance, can be due to an increased expectation of a reward or a decreased expectation of a punishment (Bateson & Nettle, 2015; Bateson 2016: Fig. B). A judgement bias test will be used in **Chapter 2** to assess the relationship between a personality trait (animals' exploration tendency) and individual differences in judgement bias.

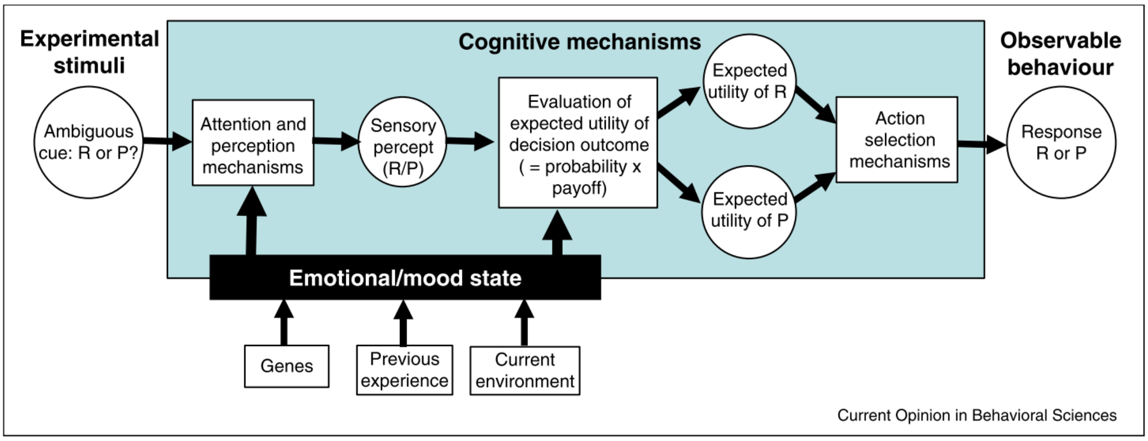
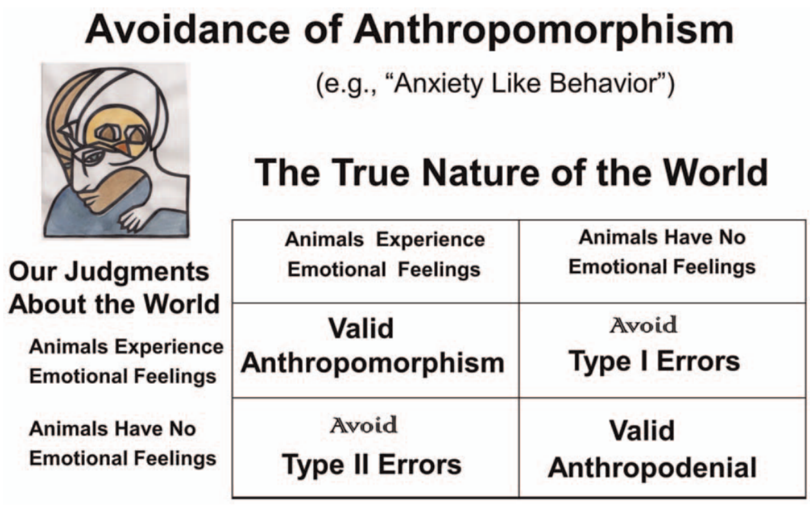


Figure B. Simplified diagram of some of the possible processes underlying the decision (evaluation and response) of an individual confronted to an ambiguous stimulus during a judgement bias task, from Bateson (2016; adapted from Mendl et al., 2009). P refers to cues predicting a punishment and R to cues predicting a reward. The affective state (or the mood), modulated by genes, experiences and environment, might influence the attention or perception of the cues as well as the attribution of the expected utility of the decision outcome. It is probable that the cognitive mechanisms occur in parallel, and not as a suite of processes.

1.2.5. Subjective component

As briefly introduced in the beginning of this section (see 1.2.1. History, definition and main approaches), the subjective, internal part of the emotional experience has been seen as out of the scope of scientific study for a long time, in part due to the influence of behaviorism. A majority of researchers have been, and still are, reluctant to attribute an emotional, internal life to animals. For instance, the emotional terms were often added with suffixes such as '-like', 'sham-' and/or putting the word between quotation marks (e.g., 'anxiety-like' instead of 'anxiety': Post et al., 2011; or 'sham-rage' for 'rage': Hess, 1954). Despite recent advances in cognitive science and neuroscience, attributing a subjective life to animals is still often seen as anthropomorphic (Fig. C) and out of a possible objective evaluation. As a result, most animal emotion researchers voluntarily avoid to refer to or to assume the existence of a subjective, conscious part in emotions (Dawkins, 2001; de Waal, 2011; Ledoux, 2012) and define them as "states that may or may not be experienced consciously" (Mendl et al., 2010).

However, Panksepp and co-workers argue that human and non-human animals share more than behavioral, physiological and cognitive components of emotion, but also their subjective part due to common underlying brain systems shared at least among mammal species (Panksepp, 2005; 2011). Contrary to the advocates of appraisal theories who consider that the evaluation of the situation is at the origin of the emotion, researchers in affective neuroscience argue that emotions emerge from distinct emotional brain circuits, without the need of a cognitive evaluation (Davidson & Sutton, 1995; Davis and Montag, 2019; but see Panksepp et al. (2017) for an exchange between researchers of both approaches).



Avoidance of Anthropomorphism

(e.g., "Anxiety Like Behavior")

The True Nature of the World

Our Judgments About the World	Animals Experience Emotional Feelings	Animals Have No Emotional Feelings
Animals Experience Emotional Feelings	Valid Anthropomorphism	Avoid Type I Errors
Animals Have No Emotional Feelings	Avoid Type II Errors	Valid Anthropodenial

Figure C. Diagram representing the relations between the 'true nature of the world' (i.e., the potential affective life of non-human animals) and how we judge the world on a scientific perspective, from Panksepp, (2011a). The author proposes that the scientific study of the subjective component in emotions, here the feelings, has been prevented due to a fear of anthropomorphism. During the 20th century, in order to avoid Type I errors, scientists denied that animals could have an affective nature ('anthropodenial'), thus leading to use terms as 'anxiety-like behavior' instead of anxiety. Panksepp argues that, based on the current data available, scientists should adopt a 'valid anthropomorphism' perspective, to avoid Type II errors.

By using deep brain electrical stimulations, pharmacological challenges and brain lesions in several mammal species, they described seven basic emotional circuits in the subcortical brain; four positives: SEEKING, LUST, CARE, PLAY and three negatives: FEAR, RAGE/ANGER and PANIC/SADNESS (Panksepp, 2011b). The stimulation of each circuit is assumed to give rise to the corresponding basic emotion, eliciting a specific behavioral and physiological pattern, and in particular inducing the subjective feeling associated to the raw emotion (Panksepp, 2011a,b). To support the existence of an affective life in animals, the author highlights several arguments: the subcortical structures underlying the seven emotions are homologous among the mammal species tested (Burgdorf et al., 2007; Kruk, 1991; Panksepp, 2016; Siegel, 2005), the ablation of the neocortex in the young does not prevent emotional reactions (Huston & Borbély, 1974; Panksepp et al., 1994; Valenstein, 1966), and, in particular, the electrical stimulation of the different emotional circuits alone can serve as a positive or negative reinforcer (Burgdorf et al., 2007; Delgado et al., 1954; Olds & Milner, 1954; Schlaepfer et al., 2013; Trowill et al., 1969). Finally, the stimulation of homologous brain regions in human can elicit an appropriate emotional experience that can be verbally reported (Panksepp, 1985, 1998). In conclusion, although it is not currently possible to know whether the type of evoked feelings differs between the systems or to assess their content, there is now strong support in favor of internal affective states in animals (Panksepp, 2005).

1.3. Personality and emotions

1.3.1. Biological basis of personality

The causal mechanisms driving individual differences in personality traits and shaping them through ontogeny are still poorly understood in animals, although they have been investigated in humans for a long time (Eysenck, 1967). The neuroanatomical correlates of personality have been predominantly investigated in humans (Kennis et al., 2013; Schmahl et al., 2006). They also start to be studied in more details in other animal species, mainly with the aim to better understand human personality and to develop new drugs to treat personality and mood disorders (Khan & Echevarria, 2017; Mehta & Gosling, 2008). For instance, bolder American minks *Neovison vison* presented more neurons in two regions of the amygdala, and such a higher number of neurons was also associated with a higher emotional arousal (Wiese et al., 2018). Recently, neuroimaging has been adapted to animals and used in the context of personality. Using magnetic resonance imaging (MRI) in chimpanzees *Pan troglodytes*, the percentage of gray matter of the subgenual cingulate cortex was found to be associated with a lower social dominance and a higher conscientiousness (Blatchey & Hopkins, 2010). Another large study in the same species highlighted the role played by asymmetries in the frontal cortex in explaining diverse personality traits (Latzman et al., 2015). However, these studies have been conducted on anesthetized individuals preventing a direct functional analysis. The successful and increasing use of functional MRI (fMRI) in awake dogs *Canis lupus familiaris* is promising (Andics & Miklósi, 2018; Czeibert et al., 2019; Haker et al., 2013) and may shed light on the neurobiological foundations of personality.

Such physiological correlates have been further investigated through the study of coping styles (Carere et al., 2010; Koolhaas et al., 1999, 2010), metabolic rates (Biro & Stamps, 2010; Holtmann et al., 2017) or molecular stress networks (Aubin-Horth et al., 2012). In particular, studies on coping styles highlighted two distinct behavioral types with related physiological and neurobiological correlates (de Boer et al., 2017; Coppens et al., 2010; Koolhaas et al., 2010), although on a continuous scale (Fig. D). Typically, proactive individuals are more aggressive, active and explorative, tend to form more routines and to have a lower emotional arousal, in association with a lower reactivity of the HPA axis but a higher SAM system reactivity. In contrast, reactive individuals exhibit opposite behavioral and physiological profiles. In small mammals such as rodents, the sampling method (e.g., blood collection after the exposition to a stressor) does not allow to assess simultaneously behavioral and physiological reactions. Such issue could be overcome by new non-invasive methods such as the use of infrared thermography to assess the reactivity of the sympathetic nervous system via changes in body surface temperature (Stewart et al., 2005; Vianna & Carrive, 2012; see 1.2.3. Physiological component, p.19).

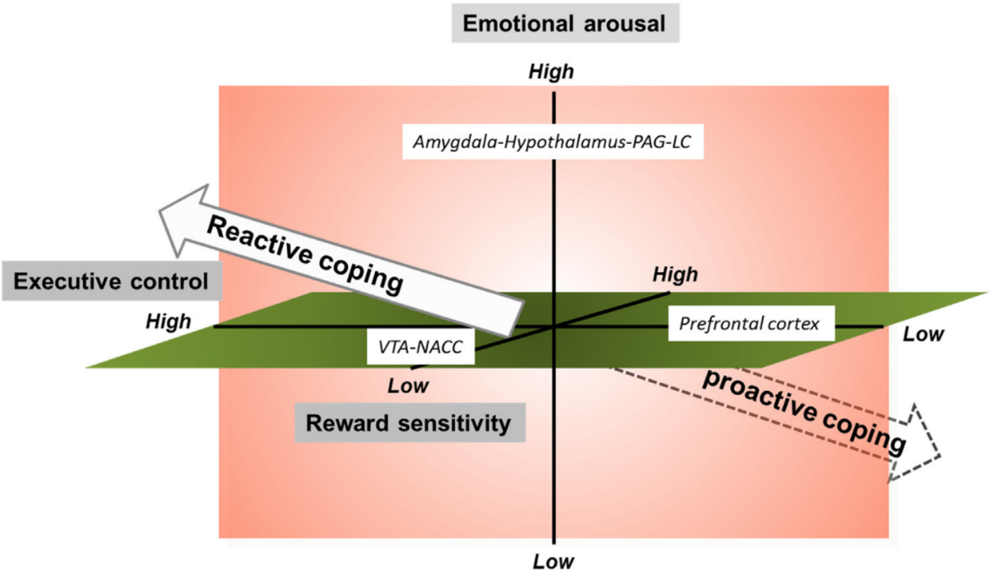


Figure D. Representation of the two coping styles according to the three-dimensional model developed by de Boer et al. (2017). On the X-axis, the degree of executive control is represented (under the control of the prefrontal cortex). The Y-axis reflects the intensity of emotional arousal, mediated by the amygdala-hypothalamic-periaquaductal gray-locus coeruleus brain circuit. The Z-axis figures the reward sensitivity, controlled by the activity of the ventral tegmental-nucleus accumbens pathway. Proactive individuals are graded low on the executive control and emotional arousal axes, but high on the reward sensitivity axis; that is, they behave with more impulsivity, lower emotional reactivity and are more sensitive to rewards than the reactive individuals.

Differences in emotionality as a causal mechanism of personality has been predominantly the focus of neurobiology and pharmacology fields, investigating treatment effects in animal models for potential applications to human mood or personality disorders, such as depression or chronic anxiety (Blanchard & Meyza, 2019; Czéh et al., 2016; Hernandez-Lallement et al., 2018). Although emotions were integrated into the early definition of animal personality ("consistent patterns of feeling, thinking, and behaving" in Gosling (2001) citing Pervin & John (1997)), they were later excluded by behavioral ecologists to only focus on the measurable part (i.e., behavior; e.g., Réale et al., 2007), but emotions remained under the scope of animal welfare scientists (e.g., Boissy & Erhard, 2014). Now, the most recent review in the field states that personality refers to “individual differences in behavior and emotion” (Wilson et al., 2019), but the two concepts are usually seen as only correlated. The hypothesis of an emotional basis of personality is increasingly considered by researchers in affective neuroscience, a discipline aiming to describe the neurobiological basis of seven primary emotions and their role in psychological well-being, affective brain disorders and consciousness of the affective life, both in human and non-human animals (Montag & Davis, 2018; Panksepp, 2005, 2006).

1.3.2. Emotions at the origin of personality: Affective neuroscience and personality

As previously introduced (see 1.2.5. Subjective component, p.25), Panksepp and his colleagues described seven basic emotions shared by all mammals, associated with specific physiological and behavioral patterns and subjective feelings (Davis & Montag, 2019; Panksepp, 2011b, 2015). Three have a negative valence: the RAGE/ANGER system (according to the author’s nomenclature) leads the individual to defend himself and his resources, the FEAR system helps to escape from threatening situations and the PANIC/SADNESS system leads the individual, in case of separation distress, to search for social support. The four other circuits have a positive valence: the most important, the SEEKING system promotes exploration and appetitive behaviors, in close relationship with the LUST (sexual urges), CARE (toward offsprings) and PLAY (driving pro-social activities in young animals) systems.

Affective neuroscience researchers argue that personality, considered as individual differences in behavior, emotion and cognition, emerges from individual differences in emotional reactions (Montag & Panksepp, 2017). Driven by genetic predispositions and environmental factors (Polderman et al., 2015), young individuals already differ in their subcortical brain functions (e.g., hypothalamus, amygdala, etc.) which elicit emotions in response to unconditioned stimuli. In particular, FEAR, RAGE, SEEKING and DISTRESS emotions should be present since the first days of life as they are critical for the survival of the young (Panksepp, 1998). For instance, two distinct lines of rats have been selected based on the amount of their 50-kHz vocalizations (emitted when "tickled" shortly after weaning: Burgdorf et al., 2005), produced during appetitive social behavior and rewarding situations (Knutson et al., 2002), and they showed distinct neural functioning (Burgdorf et al., 2009) and gene expression in the brain (Burgdorf et al., 2011).

During ontogeny, by the confrontation of the individual to various environmental factors, these primary-process emotions will lead to and control, in a bottom-up fashion, the development of behavioral habits and traits through learning processes (e.g., conditioning) via the upper limbic part of the brain (Panksepp, 2010). Furthermore, due to the maturation of the neocortex, in particular in its frontal part, emotional arousal will become increasingly regulated by higher-cognitive processes, at least in primates (Davidson et al., 2010; Panksepp, 1998). Hence, the personality is assumed to develop from/based on individual differences in emotional processes that will drive behavioral and cognitive differences, in interaction with the specific life experiences of the individual. As a result, the behavioral expression of personality is assumed to be the result of the combined reactivity of emotional primary-processes from the subcortical brain and of emotional regulation from the neocortex (Fig. E). In other words, personality is built from "the strengths and weaknesses found in the *basic* *affective systems*” in interaction with the environment and particularly the early experiences (Özkarar-Gradwohl, 2019).

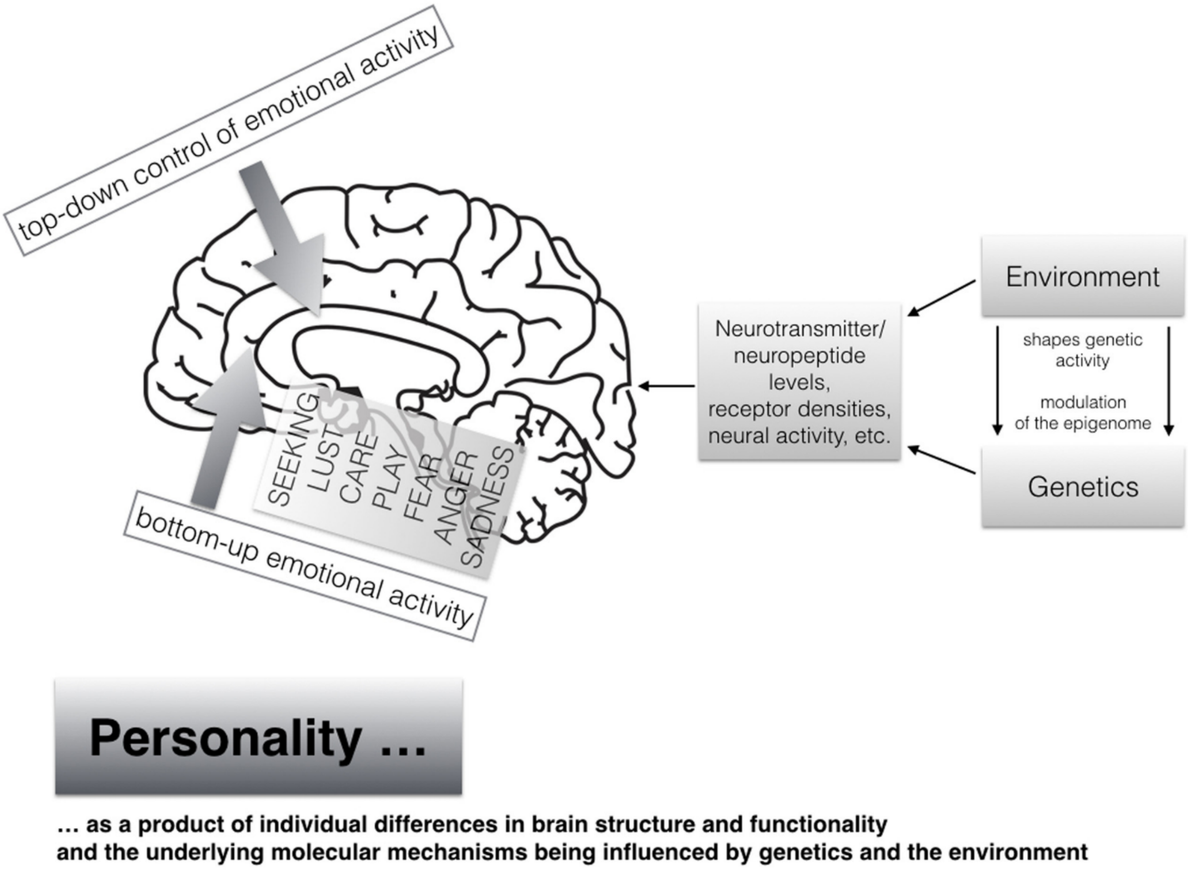


Figure E. Schema of the conceptual framework developed by Montag & Panksepp (2017) about the mechanisms underlying human personality. The environment influences the genetics of the individual and, together, they shape the structure and the functionality of the brain, thus leading to individual differences in brain activity. Personality emerges from these individual differences in activity of the emotional, subcortical systems and is also modulated by regulation processes from higher brain regions.

In 2003, the Affective Neuroscience Personality Scales (ANPS), a self-report questionnaire based on the neurobiological findings in affective neuroscience, was created to evaluate human individual differences in primary emotions in order to better understand the origin of personality traits and emotional disorders (Davis et al., 2003). Only six emotions were included, as questions related to LUST might have led to socially-oriented answers. The questionnaire has been validated by neurobiological studies (e.g., Reuter et al., 2009; van der Westhuizen & Solms, 2015). After a first study in Americans (Davis et al., 2003), the results have been applied to different cultures (e.g., Turkish: Özkarar-Gradwohl et al., 2014; Japanese: Narita et al., 2017; German and Serbian: Knežević et al., 2019; see Özkarar-Gradwohl, 2019 for a review). Strong correlations have been found between the primary emotions assessed through the ANPS and the personality traits evaluated via the Five Factor Model of Personality (McCrae & Costa, 2004), supporting the hypothesis that the primary emotions defined in affective neuroscience may represent the biological basis of personality (Davis & Panksepp, 2011; Fig. F).

As the ANPS evaluates the personal feeling via self-report, it is not possible to directly use it in other species. Yet, personality scales have been already applied to non-humans using the Five Factor Model of Personality to rate the animal's personality (e.g., in dogs; Gosling et al., 2003; chimpanzees: King & Figueredo, 1997) and future studies might adapt the ANPS to other species. Moreover, emotions are also considered as the evolutionary oldest parts of personality (Montag & Panksepp, 2017), as they are elicited by the subcortical and limbic parts, the phylogenetically oldest layers of the brain that are shared by all mammals (MacLean, 1990). Thus, if individual differences in emotions and personality have been found to be associated in humans, one might expect to find similar associations in other animal species as well.

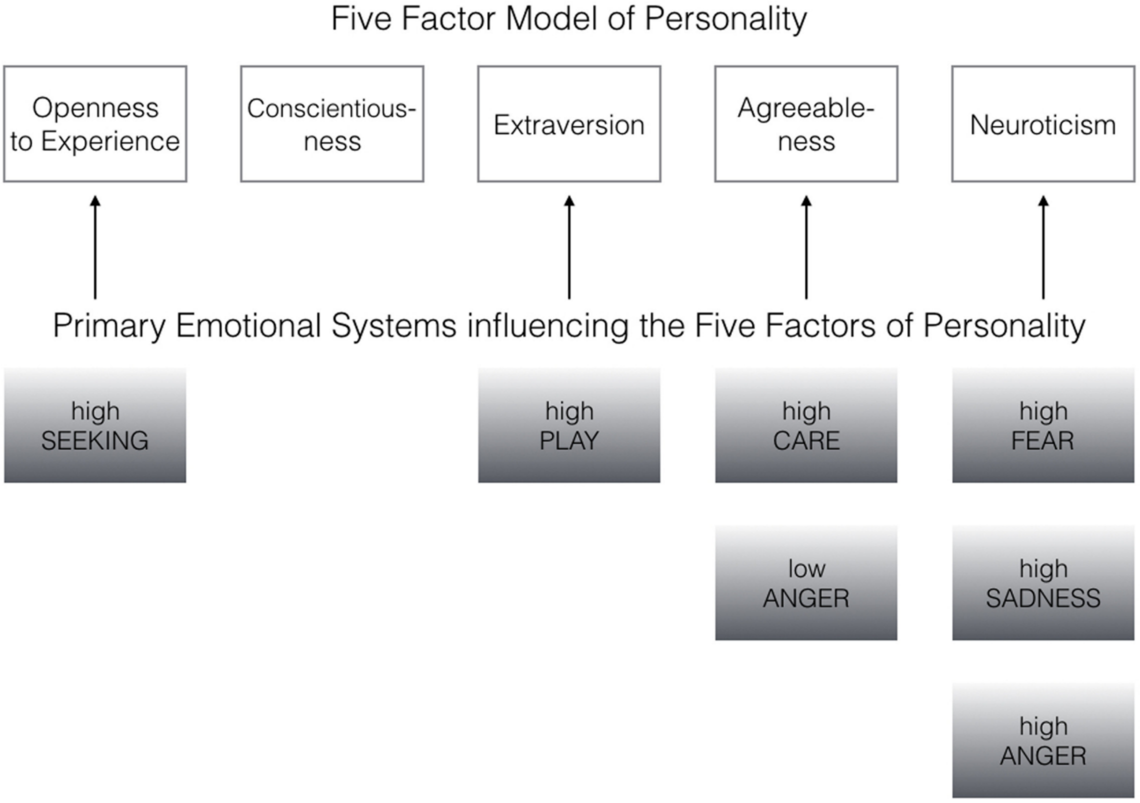


Figure F. Diagram of the most robust associations between primary emotional systems and the Big Five/Five Factor Model of Personality, from Montag & Panksepp (2017). Emotional systems influence the personality traits in a bottom-up fashion.

As a consequence, if emotions are underpinned by individual differences in subcortical brain functioning, they should be stable over reasonable time spans. Indeed, neuroanatomical modifications are costly in terms of time and energy (DeWitt et al., 1998), which also constitutes one of the hypothesis for the consistency over time of personality traits (Dall et al., 2004). Individual differences in affective styles, that is, considering emotions or at least the tendency of an individual to show certain emotional responses in a given situation as a trait, remain widely understudied. This particularly concerns positive emotions (de Vere & Kuczaj; Fraser, 2009), whilst consistencies of fear and anxiety over time and situations have recently received attention (Boissy & Erhard, 2014). We propose that individual consistency over time of emotional reactions should first be investigated, starting from a very young age. Furthermore, to increase our understanding of the emotional origin of animal personality, associations between individual differences in personality and in emotional reactions should be studied in various situations across the life span of the individuals.

1.4. Objectives of the thesis

I aimed to investigate in this project how consistent individual differences in emotions were related to personality traits in different age classes, using two different rodent species of wild origin, the house mouse *Mus musculus domesticus* and the mound-building mouse *Mus spicilegus*. We followed here a two-dimensional approach, that is, we aimed to evaluate the emotional valence and/or arousal rather than discrete emotions. Thus, in line with the argumentation that personality arises from individual differences in the reactivity of emotional systems, this project had two main goals. First, we investigated, using non-invasive methods, whether the individual differences in emotions were consistent over time and could be used to phenotype the individuals. Second, we evaluated the correlations between individual differences in emotion and in behavior (i.e., personality traits, with a focus on exploration tendency). Each chapter focuses on one of the components of an emotional reaction (respectively, behavior, cognition and physiology).

The **first chapter** focuses on the behavioral component by assessing the consistency of pup behavioral responses (pup calls) to isolation over three consecutive days and across different stressful situations (repeated isolation, presentations of odor cues stemming from their own nest and from the nest of an unfamiliar adult male), in house mouse pups. This first chapter has been already published in *Developmental Psychobiology*. The section 5.2. of the discussion (p.146) explores the association between pups' isolation call rates and adults' exploration tendency (under preparation).

Verjat, A., Rödel, H. G., & Féron, C. (2019). Isolation calls in house mouse pups: Individual consistency across time and situations. *Developmental Psychobiology*, *00*, 1–11. https://doi.org/10.1002/dev.21884

In the **second chapter** on the cognitive component of emotion, I investigated, in the house mouse, the association between individual judgment bias and exploration trait, with the aim to clarify whether the tendency to explore may be related to a higher tendency to express either positive or negative affective states. The manuscript has been submitted to *Animal Cognition*.

Verjat, A., Devienne, P., Rödel, H. G., & Féron, C. More exploratory house mice judge an ambiguous situation more negatively. Submitted to *Animal Cognition*.

Finally, the **third chapter** assesses the physiological component of emotion, in the mound-building mouse. We designed a test to evaluate short-term thermal responses (as a proxy of the sympathetic nervous reactivity) to a brief handling procedure, using infrared thermography. Then, we asked whether individual differences in thermal responses were correlated with individual differences in exploration tendency. This last chapter has been published in *Behavioural Brain Research*.

Duparcq, M., Jean, O., Verjat, A., Jaravel, L., Jacquet, D., Robles, F., Féron, C., & Rödel, H. G. (2019). Differences between fast and slow explorers in short-term tail temperature responses to handling in a rodent of wild origin. *Behavioural Brain Research*, *376*, 112194. https://doi.org/10.1016/j.bbr.2019.112194

1.5. General methods

1.5.1. Study animals

The main study animal of this project was the house mouse *Mus musulus domesticus* of wild origin. Wild mice were caught around Marcy L'Etoile (France) and bred for 10 generations at the laboratory RS2GP (VetAgroSup Lyon, France) before arriving at our laboratory in April 2013. Other wild mice were captured in several parts of France (Die and Beaujolais regions) and were added to our stock in 2013 and 2014 (last additions in September 2014). Our study animals were descendant of the 7-10th generations (see the Material and Methods of **Chapters 1 and 2** for more details about breeding and housing conditions). See Forestier (2018) for a detailed review of the life history and social organization of the house mouse. As we aimed to evaluate individual differences from an early age, we followed a longitudinal approach. Thus, mice were tested from postnatal day 9 to postnatal day 82 – 99 depending on the experimental group they were assigned to (Fig. G).

We used another mouse species of wild origin in **Chapter 3**, the mound-building mouse *Mus spicilegus*. For more details about the ecology of that species, please refer to a thesis previously conducted in our group (Rangassamy, 2016).

1.5.2. Planning of experiments

All house mice, males and females, were first tested as pups for their behavioral and thermal responses to isolation followed by three different treatments (Early emotional profiles: **Chapter 1**). At weaning, at postnatal day 21, siblings were separated by sex and groups of three or four males stemming from the same litter were formed. Between postnatal days 28 and 71, male subjects (*N* = 122) underwent repeated tests to phenotype them for their personality traits: sociability within the litter group (social interaction tests), exploration tendency (open field and novel object tests; following the definition of 'exploration' of Réale et al., 2007) and anxiety (elevated plus maze tests; but see the general discussion, section 5.3., p.150). Note that for the three latter tests, males were housed in sibling groups until the first session, they were isolated immediately after it and remained housed singly until the end of all the tests. Finally, males were divided in three experimental groups: Motivational Conflict Test ($N = 38$), Emotional Contagion Test ($N = 45$) and Judgement Bias Test ($N = 39$). Only the last one will be presented in my thesis (**Chapter 2**); the remaining data will be analyzed and published later on (Appendix 6.1. and 6.2.). The Judgement Bias Test group was trained to discriminate between two distinct locations (reinforced cues) before being repeatedly tested for their responses to an ambiguous cue. All experiments were filmed by an infrared thermal camera (T650sc, FLIR, Wilsonville, USA) to allow further analyses of both the thermal and behavioral responses of the individuals. Methods are detailed in the appropriate chapters or appendix.

Two groups of mound-building mice were tested. A first subset was used to validate the handling procedure (thermal-response test: $N = 5$), using individuals between 39 and 41 days old. Individuals of the second subset ($N = 54$) were tested for their exploration tendency (via repeated open field and novel object tests, on postnatal days 33 and 43) and their responses to the thermal-response test (repeatedly, on postnatal days 39 and 49). A more detailed description of the experiments conducted in the mound-building mouse is provided in **Chapter 3**.

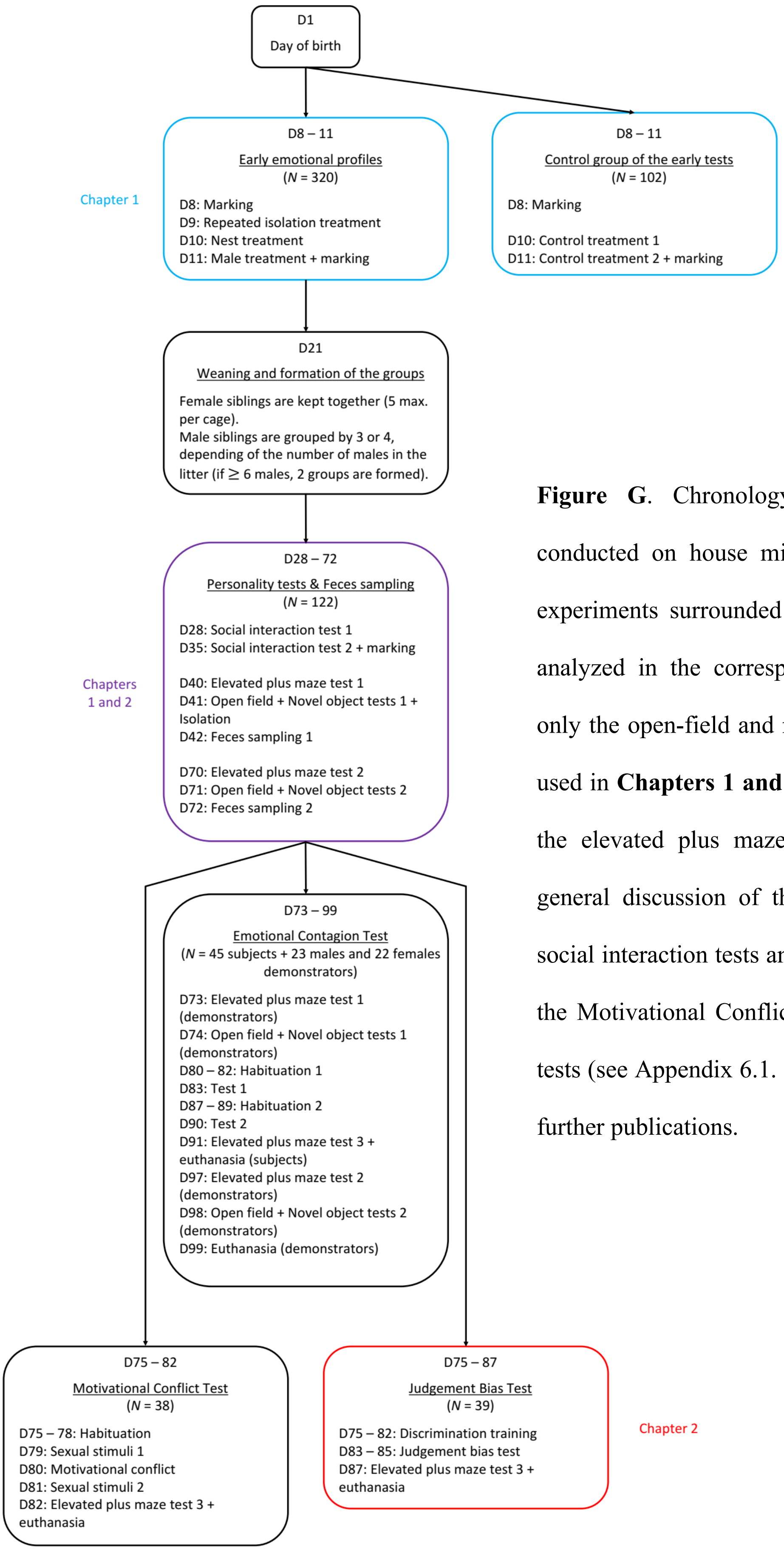


Figure G. Chronology of all the experiments conducted on house mice during the project. The experiments surrounded in color are described and analyzed in the corresponding chapters. Note that only the open-field and novel object tests have been used in **Chapters 1 and 2**; I explain why I excluded the elevated plus maze test from analysis in the general discussion of the thesis (5.3., p.150). The social interaction tests and feces sampling, as well as the Motivational Conflict and Emotional Contagion tests (see Appendix 6.1. and 6.2.) will be the topic of further publications.

2. Chapter 1: Isolation calls in house mouse pups: individual consistency across time and situations

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Author contribution statement: AV, HGR and CF conceived and outlined the study. AV planned and carried out the experiments, performed the statistical analysis and wrote the first version of the manuscript. CF conceived the R script to analyze ultrasonic vocalizations and thermal data. HGR and CF advised on the statistical analysis and revised the manuscript.

Supplementary results are given in the discussion (5.2. Complement of Chapter 1: Associations between individual differences in isolation call rate and exploration tendency; p.146).

ABSTRACT

Isolation calls are emitted by the offspring of many mammalian species when separated from caregivers and siblings. Some studies indicate that isolation call rates constitute a consistent individual trait; others show that the young adjust their vocalization rate to the current situation. We studied this in the house mouse (*Mus musculus domesticus*) by exploring individual consistencies in pup isolation call rates and their potential modulation in different social situations. We carried out experiments including three treatments (repeated measurements) during consecutive days, all starting with an initial isolation of the pup, followed by (1) a reunion with mother and littermates and a second isolation hereafter, (2) the confrontation of isolated pup with cues of its own nest or (3) with cues of an unfamiliar adult male. The first treatment induced a significant increase while the others induced significant decreases in pup isolation call rates. Pups showed consistent individual differences in initial call rates across the three days of testing (postnatal days 9-11), which were significantly associated with individual differences in call rates during the different treatments. We conclude that pup isolation calls represent a consistent, trait-like behavior in the house mouse, which can also express flexibility in response to social cues.

Keywords: *individual differences; Mus musculus; separation; social cues; ultrasonic vocalization*

1. INTRODUCTION

Vocalizations produced by dependent offspring when separated from their mother or other caregivers and from littermates are widespread among mammals (Ehret, 1980; Lingle, Wyman, Kotrba, Teichroeb, & Romanow, 2012). These isolation calls serve a communicative function as they trigger behavioral responses of the caregivers such as defensive behavior to protect the young or to search for and retrieve the offspring to the nest or burrow (Hahn & Lavooy, 2005; Lingle, Pellis, & Wilson, 2005; Noirot, 1972).

Recent studies in some altricial species have shown that offspring isolation call rates constitute a highly consistent individual trait, indicating its usefulness as behavioral measure to assess individual differences in young animals (e.g., *Homo sapiens*: Blum, Taubman, Tretina, & Heyward, 2002; *Felis silvestris catus* and *Mus spicilegus*: Hudson, Rangassamy, Saldaña, Bánszegi, & Rödel, 2015; *Sus scrofa*: Špinka, Syrová, Policht, & Linhart, 2018). Also studies in laboratory mouse pups (*Mus musculus*) have shown consistency over time in the number of ultrasonic calls emitted during isolation, even though there were strain differences in the general level of call rates (Barnes, Rieger, Dougherty, & Holy, 2017; Hahn et al., 1998; Hahn & Lavooy, 2005; Rieger & Dougherty, 2016).

Despite such individual consistency in isolation call rates, altricial young are also able to express contextual behavioral plasticity in isolation call production depending on the current situation (Rieger & Dougherty, 2016). For example, as it has been shown in studies in the laboratory rat (*Rattus norvegicus*) and the laboratory mouse, a decrease in ambient temperature increases call production of isolated pups shortly after birth, and smaller or younger pups with lower insulation have been reported to be more prone to such effects (Allin & Banks, 1971; Harshaw & Alberts, 2012; Sales & Skinner, 1979). Furthermore, pups can modulate isolation call emission in response to the presence of conspecific odour cues. For example the odour of mother and littermates typically leads to a diminution of pup isolation call rate in the laboratory mouse (D'Amato & Cabib, 1987; Marchlewska-Koj, Kapusta, & Olejniczak, 1999; Moles, Kieffer, & D'Amato, 2004). The effect of male odour cues is more controversial; some studies in the laboratory mouse did not find any effects on pup call rates while others reported an increase (Branchi, Santucci, Vitale, & Alleva, 1998; Santucci, Masterson, & Elwood, 1994). Furthermore, a second isolation after a short period reunited with mother or familiar adults makes the pups to vocalize at higher rates (Scattoni, Crawley, & Ricceri, 2009; Shair, 2007). However, to the best of our knowledge, the modulation of isolation calls by a sequence of different social situations has never been assessed by repeated measurements of same individuals, and using such an individual-based approach would allow to address both individual consistency and variability of isolation calls.

Our study, carried out in the house mouse (*Mus musculus domesticus*), had 3 main goals. First, we aimed to confirm previous results on laboratory mice by testing whether individual differences in pup isolation call production were consistent across consecutive days (Rieger & Dougherty, 2016). Second, we investigated in the same pups whether and how isolation call rates were modulated by three social situations and whether these responses were associated between the different situations. Third, we asked if individual differences in isolation call rates were predictive of how individual pups will respond to the three different treatments.

To answer the second question, we tested the pups in three different social situations, following an initial isolation. The first situation consisted of a repeated isolation separated by a short period back in the home cage with mother and littermates. For the second and third situations, we presented cues to the isolated pup, stemming from its own nest or from an unfamiliar adult male nest. Pups were expected to change their call rates in response to each situation, either with an increase or a decrease, thus exhibiting behavioral flexibility. Furthermore, we expected that pups showing a stronger response to one situation would also show stronger responses to the others. For the third question, we expected that individual differences in initial isolation call rates would be associated with differences in the amplitude of changes of call rates in response to each treatment, thus characterizing isolation calls as a consistent behavioral trait across situations.

2. MATERIAL AND METHODS

2.1. Study animals and housing conditions

The animals used for this study (house mice *Mus musculus domesticus*) were descendants (7th – 10th generation) of individuals caught from the wild around Lyon, France, and were bred in the animal facilities of the Laboratoire d'Ethologie Expérimentale et Comparée at the Université Paris 13. A total of 320 individuals (146 females and 174 males, mean litter sex ratio: 0.52 ± 0.20 SD; mean litter size: 7.9 ± 2.0 SD) were tested. For some of them (33 females and 37 males, mean litter sex ratio: 0.53 ± 0.22 SD, mean litter size: 7.8 ± 2.5 SD), the maximal peripheral temperature was analysed. Another group of 102 individuals was used as a control group; see below for details (52 females and 50 males, mean litter sex ratio: 0.48 ± 0.22 SD, mean litter size: 7.3 ± 2.0 SD). Animals were kept under a 14:10 light/dark cycle, with red light on at 09:00 am, a temperature of 20 ± 2.0 °C and a humidity of 50%.

From birth to postnatal day 21, pups of (unculled) litters were kept with their parents in polycarbonate cages (37.5 × 23.5 cm and 16 cm high, PLEXX, Elst, The Netherlands), with wood shavings, 15 cotton balls as nest material (COMED, Strasbourg, France) and two cardboard rolls as enrichment (7.5 × 3.8 cm of diameter, PLEXX, Elst, The Netherlands).

Food (rodent standard diet; Special Diet Services type M20, Witham, Essex, UK) and water were provided ad libitum.

At postnatal day 8, each pup was individually marked with a permanent nontoxic hair dye to allow individual recognition within the sibling group (Nyanzol-D, Greenville Colorants, Jersey City, NJ, USA). To this end, animals were held by the experimenter and a unique symbol was rapidly and softly drawn on their back using a fine paint brush.

Experiments (see details below) were conducted from postnatal days 9 to 11, as isolation calls in *Mus musculus* are emitted only during the first two postnatal weeks (Elwood & Keeling, 1982). Each pup was weighed at the end of the experiment (mean body mass at postnatal day 9: 4.66 g ± 0.81 SD; day 10: 5.05 g ± 0.86 SD; day 11: 5.47 g ± 0.93 SD). At postnatal day 21, sex was determined by external inspection of the anogenital region.

2.2. Experimental procedures

2.2.1. Description of the apparatus

The apparatus used for isolation experiments of pups was constituted of a square transparent plastic box (9.3 × 9.3 cm and 8.6 cm high), with top and bottom parts removed, placed on a heating plate keeping a constant temperature of 20.0 °C, to counterbalance slight changes of the room temperature (20.0 ± 2.0 °C). For each test and each individual, ultrasonic call rates were recorded, and we measured and analysed peripheral body temperatures for a subsample of individuals (see details below, 2.4). Peripheral body temperature was recorded with an infrared thermal camera (T650sc, FLIR, Wilsonville, USA), mounted 50 cm above the heating plate. Ultrasonic calls were recorded with a unidirectional ultrasonic detector (Ultramic 250K, Dodotronic, Castel Gandolfo, Italy) placed on the edge of the box by the aid of the software SoundChaser Pro (Acounect, France). Prior to the beginning of each test, the father was removed and placed in a separated clean cage until all pups of the litter were tested. The mother remained in the cage with the pups. The pups were handled by softly lifting them by the skin of their back and releasing them in the centre of the test apparatus once their paws touched the heating plate.

2.2.2. Isolation experiments

Isolation experiments were repeated once per day during postnatal days 9, 10 and 11. The first part of these experiments was always the same; each pup (one after the other) was taken out from its home cage and placed individually in the centre of the test apparatus. After 5 min (*repeated isolation treatment*) or 2 min (*nest treatment* and *male treatment*), the pup was confronted to one of the three following experimental treatments. This first part of these experiments was called thereafter “initial isolation” and only the first 2 min of each day were statistically analysed.

The experimental days were always conducted following the same order, as we aimed to investigate the consistency of individual differences in isolation call rates. Moreover, we chose this particular order to avoid a potentially aversive effect of the confrontation to cues of an unfamiliar adult male which could have affected the subsequent tests. All experiments were done by the same experimenter, who was not blinded to the experimental condition. However, the experimenter was blinded to the individual identity of the pups when analysing the recordings.

2.2.2.1. Repeated isolation treatment: Responses to a second isolation

On postnatal day 9, after the initial 5-min isolation (see 2.2.2.), the pup was brought back to its home cage (including mother and litter siblings, cf. Scattoni et al., 2009), for 5 min.

Then, the pup was isolated again for 5 min. At the end of this second isolation period, the pup was returned to its nest and the next litter sibling was tested.

2.2.2.2. Nest treatment: Responses to cues of pup's own nest

On postnatal day 10, a piece of cotton from the nest of the pup was presented to it after the initial 2-min isolation. To do so, the experimenter sampled a small piece of cotton (around 1 cm of diameter) from its home cage and carefully placed it close to the head of the isolated pup. This part of the test lasted for another 2 min before the pup was returned to its home cage.

2.2.2.3. Male treatment: Responses to cues of an unfamiliar adult male

On postnatal day 11, the addition of cues from an adult unfamiliar male's nest followed the initial 2-min isolation. A piece of cotton, sampled from the nest of an unfamiliar male was placed close to the head of the isolated pup. The test lasted for another 2 min before the pup was returned to its home cage. Each litter was tested with cues from a different unfamiliar adult male, which was not related to the pups for at least 2 generations.

2.2.3. *Control treatment*: Control condition to *nest treatment* and *male treatment*

The control group of pups underwent two days of control condition to test for potential effects of the addition of the cotton itself in *nest treatment* and *male treatment*. At postnatal days 10 and 11, each control individual was isolated during 2 min in the apparatus before a piece of clean cotton, constituting the control cue, was added close to the head of the pup. After 2 min, the pup was returned to its home cage. The isolation call rates were averaged between the two control days.

2.3. Analysis of isolation calls

For each experimental and control condition, we assessed the number of ultrasonic calls produced during each 10-s interval (call rate). For this, the audio files recorded were analysed with R version 3.4.4 (R Core Team, 2018) using the packages *tuneR* (Ligges, Krey, Mersmann, & Schnackenberg, 2018) and *seewave* (Sueur, Aubin, & Simonis, 2008). We only considered vocalizations as “isolation calls” which were longer than 1 ms and with a frequency higher than 40 kHz, as isolation calls in pups of *Mus musculus* have been described as ultrasonic vocalizations ranging between 40 and 90 kHz (Sales & Smith, 1978; Portfors, 2007).

2.4. Analysis of peripheral body temperature

The peripheral body temperatures of the pups during the different testing conditions were assessed by the analysis of a sample of 70 pups, for which we permanently recorded the experiments with an infrared thermal camera (see details above). For this, we captured the maximal peripheral temperature of the pup during each 30-s interval of testing. We first selected 1 out of 10 frames to obtain three images per second (videos were recorded with 30 frames/s). The images were then imported into R to select those for which the maximal thermal value corresponded to either the neck or the ear of the pup (see details on method of analysis in Zepeda et al., 2018). For each 30-s interval, we kept at least 10 images where the pup was in a similar position, with its four paws on the floor, and we calculated the median value of the maximal temperature of this set of images.

2.5. Statistical analysis

Statistical analysis were conducted using the software R version 3.4.4. We applied linear mixed effects model (LMM) using the package *lme4* (Bates, Mächler, Bolker, & Walker, 2015). All covariates variables were scaled for analysis. Homogeneity of variances was checked by visual inspection of the plot of the residuals versus fitted values, and normal distribution of the model residuals was verified by inspecting the normal probability plots (Faraway, 2006). For models with multiple covariates (see Table 1), we verified that there were no notable multicolinearities by calculating variance inflation coefficients (all < 1.1) (Zuur, Ieno, & Elphick, 2010). *P*-values were calculated by corrected *F*-tests using the Satterthwaite method (Bolker et al., 2009). Moreover, for all significant covariate effects, we calculated a marginal $pseudoR^2$ (package *MuMIn*; Barton, 2018), which can be interpreted as the proportion of variation explained by the fixed effect (Nakagawa, Johnson, & Schielzeth, 2017).

First, we tested for the changes in the number of isolation calls (dependent variable) during the initial isolation of each day (see Fig. 2), using LMM. The model included an interaction between the time (10-s intervals; covariate) and the day of test (postnatal days 9 to 11; factor with 3 levels), with litter identity and individual identity as random factors. We also tested for repeatabilities of the individual call rates as well as of the individual increases of call rates during the initial separation period with intra-class correlations (ICC), using a linear mixed effects model based on calculations of *P*-values with 1000 permutations using the package *rptR* (Stoffel, Nakagawa, & Schielzeth, 2017), with individual identity as a random factor. Increases in call rates were calculated by the individual regression slopes (using data in 10 s intervals) across the 2-min initial isolation period for each day and for each individual pup.

Second, we tested for potential changes in isolation call rate (dependent variable) in response to each experimental and control treatment to assess whether the isolated pups perceived the modification of the situation, using LMM. For this, we compared the mean number of calls emitted during the last 60 s before and the first 20 s (immediate response) or the second minute (1-min delayed response) after the treatment (factor with 2 levels: before or after the treatment; see Fig. 1). One model was run for each treatment and type of response; litter and individual identities were set as random factors. Then, we calculated these two differences (immediate and 1-min delayed responses), obtained for each experimental and control treatment, and used them as new dependent variables to compare the effect sizes of the responses to *nest* and *male treatments* and *control treatment* according to the condition (factor with 2 levels, as three models were run separately for each type of response), using litter identity as a random factor. All the following models were also run separately for immediate and 1-min delayed responses.

Third, we tested for correlations between the responses to the experimental treatments using LMM. Three models were done separately to compare them two by two (the independent variable was always the response to the treatment tested the day before the dependent variable) and the litter identity was set as a random factor.

Finally, we assessed the associations between different predictor variables and the (a) immediate and (b) delayed responses (that is, changes) in isolation call rates (see details above and in Fig. 1). Predictor variables were the averaged individual isolation call rates and increases in calls (individual regression slopes, see above) and the averaged maximal peripheral body temperature during first the 2-min initial isolation (all covariates), averaged body mass (covariate) and sex (fixed factor). We ran three different models (LMM), one per treatment, using litter identity as a random factor.

2.6. Ethics note

The study was conducted in accordance to the 'Guidelines for the Treatment of Animals in Behavioural Research and Teaching' (Animal Behaviour, 2012). All experimental procedures were approved by the French authority for animal care and use (APAFIS# 7585-201610121409165) and by the institutional ethics committee. The brief separation of the pups from their mother did not show any apparent effects on the pups' development as they all gained weight throughout the study in the same way as untreated pups. After the experiments ended on postnatal day 11, all animals were kept for further experimentation and for breeding.

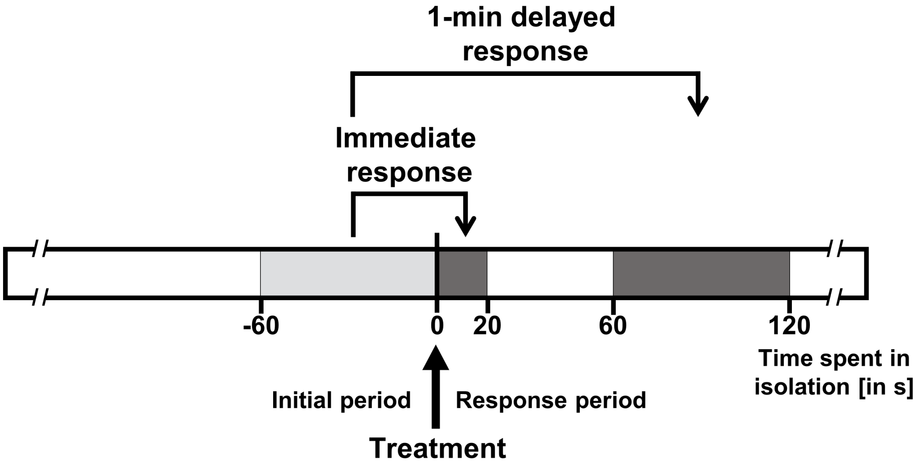

Figure 1. Schemata of the time scales used to assess immediate and 1-min delayed responses to each experimental and control treatment. Immediate responses were calculated as the difference between the mean number of isolation calls produced during the last 60 s (in light grey) of the first period and the first 20 s (in dark grey) of the second period of isolation. For 1-min delayed responses, the 2nd minute (in dark grey) was used instead of the first 20 s. See text for more details.

3. RESULTS

3.1. Changes in isolation call rates and consistency of individual differences across time during the 2-min initial isolation

During the 2 min of initial isolation on postnatal days 9 to 11, the pups showed a general, significant increase in the number of isolation calls across time, measured in 10-s intervals ($F_{1,11197} = 475.300$, $P < 0.001$; Fig. 2a-c). The interaction day × time was not significant ($F_{2,11195} = 1.85$, $P = 0.157$) indicating that this increase was not age-specific. However, the total number of isolation calls decreased significantly between the consecutive testing days ($F_{2,11197} = 182.910$, $P < 0.001$).

Moreover, the pups' initial increase in vocalization – as measured by individual regression slopes across the first 2 min of separation per day ($R_{\mathrm{ICC}} = 0.246$, $P < 0.001$), as well as the total number of calls during this period ($R_{\mathrm{ICC}} = 0.577$, $P < 0.001$), were significantly repeatable across the 3 days of testing. This indicates the existence of stable, individual differences in the pups' individual vocalization patterns in response to isolation.

3.2. Changes in isolation calls in response to different treatments

3.2.1. *Repeated isolation treatment*: Responses to a second isolation

During this first experiment carried out on postnatal day 9, the pups were individually isolated for a second time after a 5-min reunion with mother and siblings. We observed a significant increase in the number of isolation calls of the isolated pups when comparing the production during the last 60 s of the first isolation period and either the first 20 s (immediate response: $F_{1,319} = 23.168$, $P < 0.001$) or the second minute (1-min delayed response: $F_{1,319} = 4.791$, $P = 0.029$) of the second isolation period (Fig. 2a).

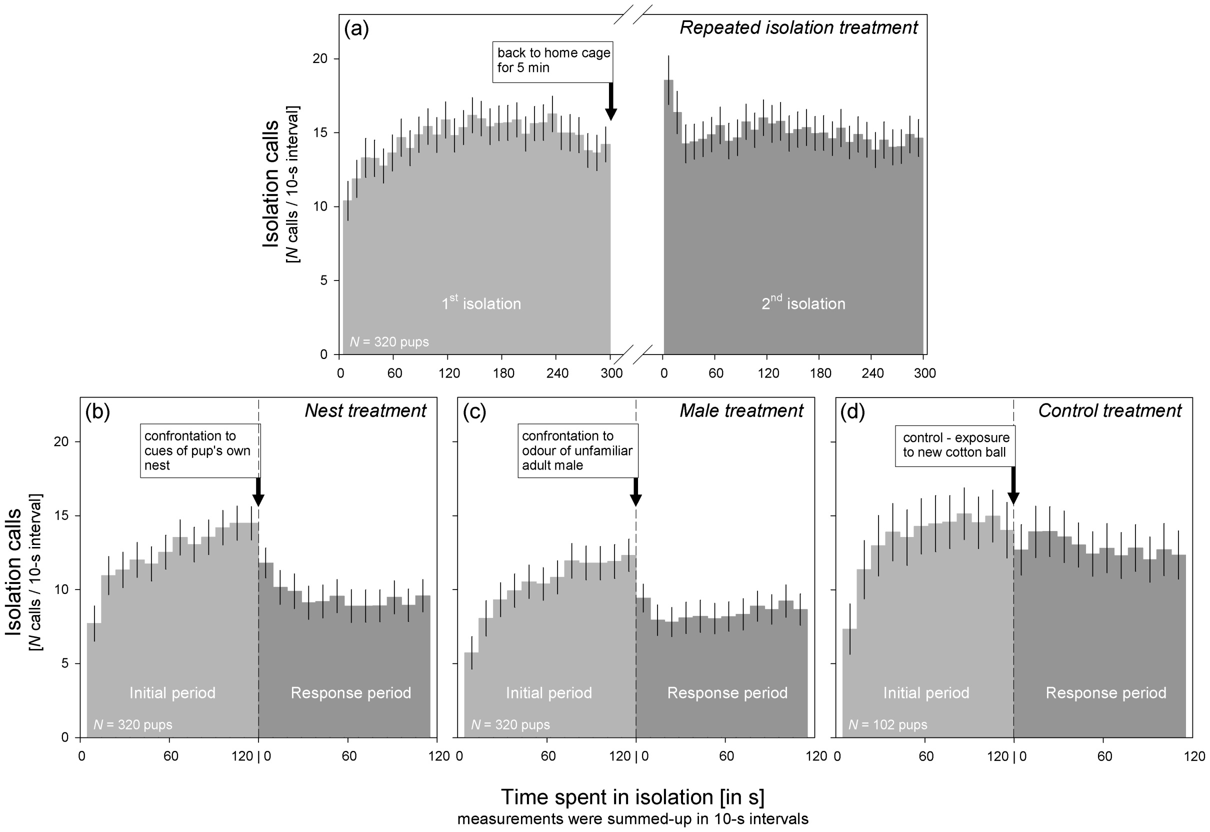

Figure 2. Average number of isolation calls (with 95% CI error bars) produced by house mouse pups during the initial isolation and after the treatment of three experimental and one control conditions. Pups were individually separated from the home cage for an initial isolation period of 2 min (or (a) 5 min) before being confronted to one treatment per day, at postnatal days (a) 9, (b) 10 and (c) 11. (d) The calls during control condition were averaged between postnatal days 10 and 11. See text for details on statistics.

3.2.2. *Nest treatment*: Responses to cues of pup's own nest

During the experiment on postnatal day 10, the isolated pups responded to the presentation of a small sample of their nest material by significant decreases in isolation call rates, both immediately ($F_{1,319} = 57.813$, $P < 0.001$) and 1-min delayed ($F_{1,319} = 148.84$, $P < 0.001$) after the treatment (Fig. 2b).

3.2.3. *Male treatment*: Responses to cues of an unfamiliar adult male

On postnatal day 11, the isolated pups were confronted to the nest material of an unfamiliar adult male. This procedure also led to significant immediate ($F_{1,319}$ = 71.961, $P <$ 0.001) and 1-min delayed ($F_{1,319}$ = 69.343, $P < 0.001$) decreases in isolation call rates (Fig. 2c).

3.2.4. *Control treatment*: Responses to control cues

During the control condition on postnatal days 10 and 11, the isolated pups also significantly decreased the number of isolation calls produced after the presentation of control cues, both immediately (day 10: $F_{1,101}$ = 7.566, P = 0.007; day 11: $F_{1,101}$ = 4.723, P = 0.032) and with a 1-min delay (day 10: $F_{1,101}$ = 9.661, P = 0.002; day 11: $F_{1,101}$ = 15.689, $P < 0.001$) after the presentation of the clean cotton (Fig. 2d).

3.2.5. Comparisons of effect sizes: *Nest* and *male treatments* and *control treatment*

The immediate decreases in isolation call rates in response to *nest treatment* and to *male treatment* did not differ significantly ($F_{1,319}$ = 0.137, P = 0.712; Fig. 3a). However, the decrease in call rate in response to *male treatment* was significantly stronger than the decrease in the control group ($F_{1,62}$ = 4.923, P = 0.030), and there was a similar tendency of a stronger decrease in call rate in response to *nest treatment* compared to the *control treatment* ($F_{1,65}$ = 3.268, P = 0.075).

At the longer-term, the decrease in isolation call rate in response to *nest treatment* was significantly stronger than in *male treatment* ($F_{1,319}$ = 11.903, $P < 0.001$; Fig. 3b) and the *control treatment* ($F_{1,63}$ = 6.021, P = 0.017). However, the decreases in response to *male* *treatment* and to the *control treatment* did not differ significantly from each other ($F_{1,59}$ = 1.397, P = 0.242).

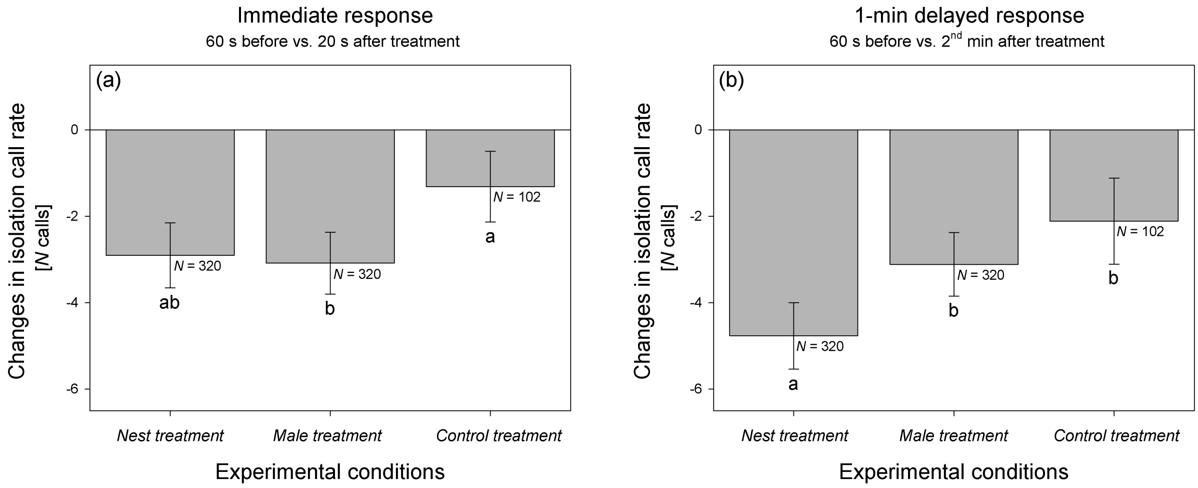

Figure 3. Comparison of the average changes in isolation call rates (with 95% CI error bars) in response to *nest treatment*, *male treatment* and *control treatment*, in house mouse pups. See Fig. 1 for details about the (a) immediate and (b) 1-min delayed responses. Isolated pups were confronted to cues from the pup's own nest (*nest treatment*; postnatal day 10), an unfamiliar adult male (*male treatment*; day 11) or a new cotton ball (*control treatment*; average of days 10 and 11). Statistics by LMM with litter identity as random factor, see text for details. Significant differences between treatments are indicated by different letters.

3.3. Associations between the changes in isolation call rates across the treatments

The immediate decrease in isolation call rates in response to cues of the pups' own nest was significantly and positively correlated with the decrease in response to cues of an unfamiliar adult male (*nest treatment* vs. *male treatment*: *pseudo*R^2 = 0.018; $F_{1,318}$ = 5.867, P = 0.016). All pairwise associations between the changes in response to the other treatments were not significant (*repeated isolation treatment* vs. *nest treatment*: $F_{1,314} = 0.336$, $P = 0.562$; *repeated isolation treatment* vs. *male treatment*: $F_{1,312} = 0.533$, $P = 0.466$).

The 1-min delayed decreases in response to *nest* and *male treatments* were also significantly and positively associated (*pseudoR*^{2} = 0.048; $F_{1,315} = 15.334$, $P < 0.001$). Moreover, the increase in call rates in response to the repeated isolation (*repeated isolation treatment*) was significantly and negatively correlated with the decrease in response to *male treatment* (*pseudoR*^{2} = 0.012; $F_{1,304} = 4.238$, $P = 0.040$). The changes in response to *repeated isolation treatment* and *nest treatment* were not significantly associated ($F_{1,300} = 1.591$, $P = 0.208$).

3.4. Associations between predictor variables and changes in response to treatments

3.4.1. Associations with initial isolation call rates and individual increases of initial call rates

The individual differences in initial isolation call rates were significantly and positively associated with the increase in response to the repeated isolation (*repeated isolation treatment*; Fig. 4a,b) and significantly and negatively associated with the decreases in response to the pup's own nest (*nest treatment*; Fig. 4c,d) and unfamiliar adult male cues (*male treatment*; Fig. 4e,f). That is, individual differences in initial isolation call rates showed significant effects both for immediate and 1-min delayed responses (Table 1).

Furthermore, the initial individual increases of call rates were also significantly and negatively associated with the immediate response to *repeated isolation treatment* (*pseudoR*^{2} = 0.036) and with the immediate (*pseudoR*^{2} = 0.021*)* and 1-min delayed responses (*pseudoR*^{2} = 0.036) to *male treatment* (Table 1).

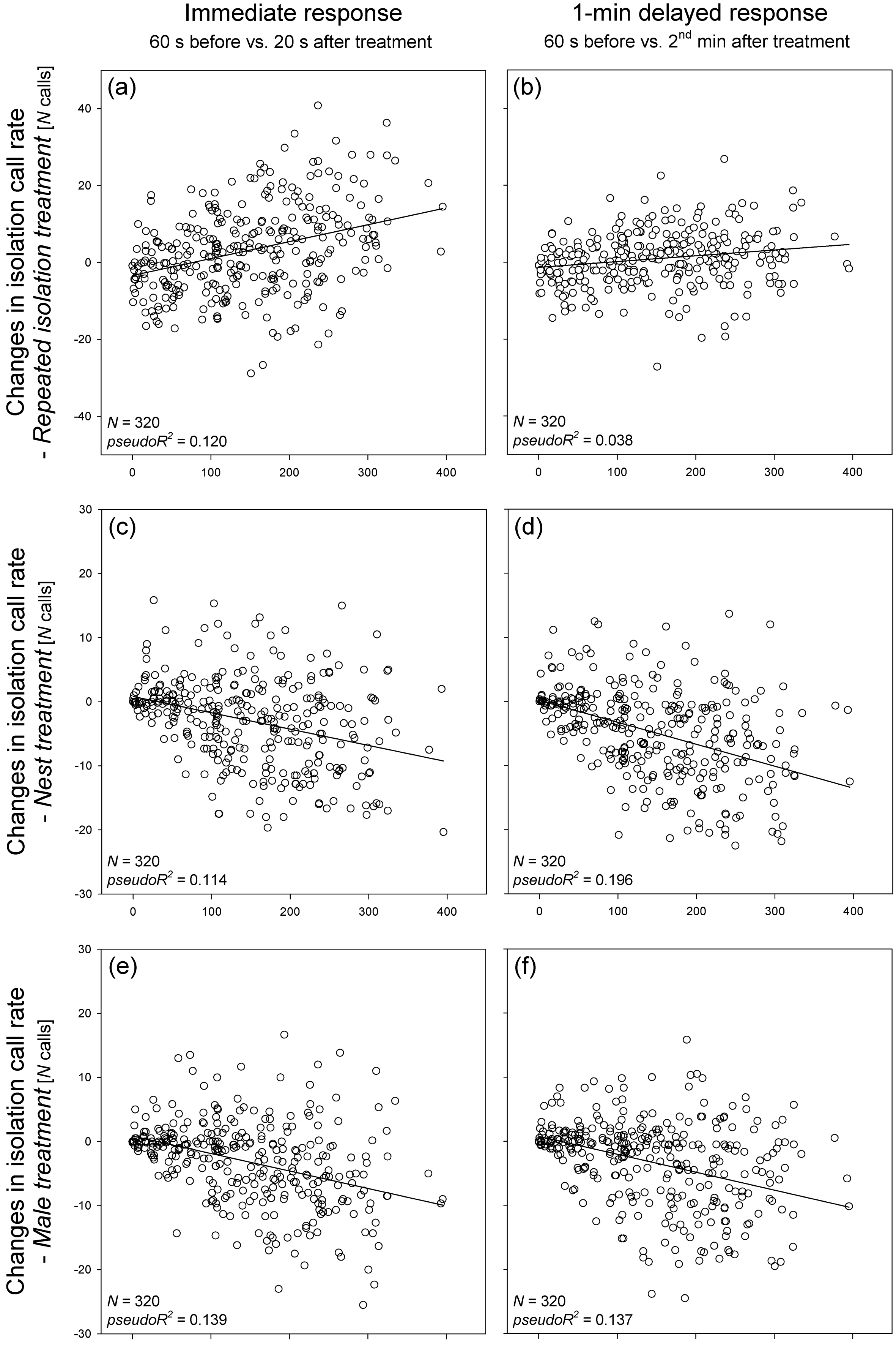
Immediate response
60 s before vs. 20 s after treatment
1-min delayed response
60 s before vs. 2nd min after treatment
Changes in isolation call rate - Repeated isolation treatment [N calls]
Changes in isolation call rate - Nest treatment [N calls]
Changes in isolation call rate - Male treatment [N calls]
(a)
N = 320
pseudoR² = 0.120
(b)
N = 320
pseudoR² = 0.038
(c)
N = 320
pseudoR² = 0.114
(d)
N = 320
pseudoR² = 0.196
(e)
N = 320
pseudoR² = 0.139
(f)
N = 320
pseudoR² = 0.137
40
20
0
-20
-40
30
10
-10
-30
100
200
300
400

Figure 4. Associations between the changes in isolation call rates in response to three treatments and the number of isolation calls produced during the 2-min initial isolation, in house mouse pups. Associations were tested for (a, c, e) immediate and (b, d, f) 1-min delayed responses (see Fig. 1). Pups were individually separated from the home cage for an initial isolation period of 2 min before the treatment was applied: (a, b) a second isolation after a short reunion with mother and siblings (*repeated isolation treatment*; postnatal day 9), the addition of cues from (c, d) the own nest of the pup (*nest treatment*; day 10) or (e, f) an unfamiliar adult male (*male treatment*; day 11). Each circle represents the values of one individual. All associations shown were statistically significant; analysis by LMM with litter identity as random factor, see text and Table 1 for details.

3.4.2. Associations with body temperature, body mass and sex

To untangle potential confounding effects that could explain the changes in isolation call rates in response to treatments, we also tested for associations with three individual characteristics (mean maximal peripheral temperature during the 2-min initial isolation, body mass and sex). As the temperature ($R_{\text{ICC}} = 0.315$, $P < 0.001$) and body mass ($R_{\text{ICC}} = 0.981$, $P < 0.001$) were significantly repeatable over days, they were averaged before testing for the associations.

The averaged body mass was significantly and negatively associated with the immediate ($pseudoR^2 = 0.026$) and 1-min delayed ($pseudoR^2 = 0.014$) responses to *repeated isolation treatment*, tended to be negatively associated with the longer-term response to *nest treatment*, and was not significantly associated with the decreases in response to *male treatment* (Table 1). The mean maximal peripheral temperature and sex were not associated with the responses to any of the treatments.

Table 1. Effects of different predictors on the changes in isolation call rates in response to three different treatments (see text for details) of house mouse pups ($N_{\text{total}} = 320$, except $N_{\text{temp}} = 70$ for analysis of max. peripheral temperature). Responses to treatments were calculated as difference in isolation call rate between 60 s before and (a) 20 s or (b) the 2nd minute after treatment: see details in Fig. 1. The call rate, the increase of calls (measured as individual regression slopes), and the averaged maximal peripheral temperature (measured in 30 s intervals) were assessed during the first 2 min of (initial) isolation. Analysis by linear mixed-effects models with litter identity as a random factor; significant effects are given in bold.

		Changes in call rate [*Repeated isolation treatment*]		Changes in call rate [*Nest treatment*]		Changes in call rate [*Male treatment*]	
	Predictor variables	*F*	*P*	*F*	*P*	*F*	*P*
(a)	Initial call rates	47.842	< **0.001**	37.356	< **0.001**	48.277	< **0.001**
	Initial increase in call rates	12.519	< **0.001**	1.281	0.259	6.725	**0.010**
	Max. peripheral temperature	0.276	0.601	0.012	0.911	1.102	0.298
	Sex	1.210	0.272	0.609	0.436	0.004	0.951
	Body mass	6.203	**0.016**	2.748	0.102	1.754	0.190
(b)	Initial call rates	12.951	< **0.001**	68.652	< **0.001**	46.436	< **0.001**
	Initial increase in call rates	0.021	0.884	0.015	0.902	12.443	< **0.001**
	Max. peripheral temperature	0.384	0.538	0.272	0.604	0.329	0.568
	Sex	0.022	0.881	0.420	0.517	0.966	0.326
	Body mass	4.876	**0.031**	3.368	0.070	0.312	0.578

4. DISCUSSION

House mouse pups showed highly stable individual differences in isolation call rates between postnatal days 9 and 11. These individual differences were also significantly associated with their individual responses to different social situations during the isolation. That is, pups characterized by high call rates responded stronger to the treatments either by an increase in numbers of calls when re-isolated or by a decrease when confronted to social odour cues.

As expected, pups showed a certain behavioral flexibility depending on the current situation. First, they vocalized at an increased rate when re-isolated after the reunion with mother and siblings. This finding is consistent with previous studies in laboratory mice (parental potentiation studies: Shair, 2007; Scattoni et al., 2009). Even though we cannot separate the effects of handling the pups and the reunion itself, our results underline the usefulness of this experimental paradigm leading to a general increase in pups' call rates.

Moreover, isolated pups modulated the number of calls when confronted to social odour cues. The here observed reduction of isolation call rate after the presentation of small parts from the pup's own nest suggests a calming effect of home cage or maternal odour cues, as it has been reported in the laboratory mouse (Caruso, Sabbioni, Scattoni, & Branchi, 2018; Moles et al., 2004) and laboratory rat (Shair, Masmela, & Hofer, 1999). As evident by our comparison with the control treatment, these cues had both immediate and longer-term effects. A similar pattern of response was observed when isolated pups were confronted to cues of an unfamiliar and unrelated adult male, but the responses to these cues occurred only immediately after their presentation. At first sight, this similar response, at least on the short term, to home cage and maternal cues and to the cues of a stranger male might be surprising. However, such similarities in responses to these different kinds of cues were also reported in a study on laboratory rats, in which pups were calmed down by maternal as well as by stranger male cues, but only when pups were raised in the presence of a male (Brunelli, Masmela, Shair, & Hofer, 1998). We propose that, in our study, the confrontation of odour cues of both kinds had a similar effect than the "contact quieting" which can be provided by the presence of conspecifics (Hofer, Shair, & Brunelli, 2002; Shair, 2007), but also by the presentation of wood shavings from the home cage (laboratory rats: Brunelli et al., 1998; laboratory mice: Wöhr, 2015).

Even though pups demonstrated flexibility in call rates, our study also provides evidence for individual consistency. As it has been shown already in several mammal species, individual differences in the pup isolation calls remained stable across time, supporting the usefulness of this behavioral measure to phenotype the young (Hudson et al., 2015; Špinka et al., 2018). Furthermore, our study confirms that the repeatability of this parameter is quite high (R_{ICC} = 0.577), at least during the relatively short study period of 3 consecutive days. A previous study in laboratory mice reported lower repeatabilities of 0.17-0.20 although over longer time spans (postnatal days 5-9 and days 3-14; Rieger & Dougherty, 2016), thus confirming the general pattern that repeatabilities tend to decrease with increased between-sampling intervals (Stamps & Groothuis, 2010b).

Furthermore, positive associations between responses to the two social odour cues were found at both time scales; pups generally responded with different amplitudes to each treatment but expressed consistency at the individual level. More importantly and in accordance with our main hypothesis, the consistent individual differences in initial call rates were associated with their responses to the three treatments. Thus, our study suggests the existence of different behavioral types (proactive and reactive, cf. Koolhaas, de Boer, Buwalda, & van Reenen, 2007) – although on a gradual scale, as pups with consistently higher isolation call rates also responded stronger to the different treatments (cf. laboratory rat pups in Brunelli & Hofer, 2007).

We found indications that other individual characteristics than individual profiles in call rate influenced the responses to the different treatments. This was evident as lighter pups – at least on postnatal day 9 – showed a stronger increase in isolation call rates compared to heavier ones when repeatedly isolated from mother and siblings. Maintaining a constant body temperature is critical for the survival of pups (Blumberg, 2001), and thus, as the temperature of lighter pups drops faster, they might increase the number of calls to be faster retrieved by the mother (Hahn & Lavooy, 2005; Stanier, 1975). However, the body mass only showed a weak effect on the increase in isolation calls (immediate response: $pseudoR^2 = 0.026$; 1-min delayed response: $pseudoR^2 = 0.014$) and we did not find a significant association between isolation call rates and peripheral body temperature to support this hypothesis. This latter absence of a significant association is consistent with previous studies in laboratory rat pups (Brunelli, Vinocur, Soo-Hoo, & Hofer, 1997; Hofer & Shair, 1978; Shair, Masmela, Brunelli, & Hofer, 1997), and it is also in accordance with previous results on the thermogenic performance of house mouse pups (Zepeda et al., 2018). This latter study showed that pups develop their ability to keep a stable body temperature during periods of isolation at around postnatal days 8-10.

Our results contribute to previous reports integrating isolation calls into the study of the development of individual differences in behavioral traits (Hudson et al., 2015; Yoshizaki, Koike, Kimura, & Osumi, 2017). For example, pups' isolation calls have been shown to be linked with sociability, spatial memory and emotionality in adults (laboratory rats: Brunelli & Hofer, 2007; laboratory mice: Winslow et al., 2000; Yoshizaki et al., 2017).

In conclusion, our study provides strong evidence that pups of *Mus musculus* are able to express consistent individual differences in isolation call rates, both across time and situations. Although isolation calls can be considered as a behavioral trait characterising the individuals, it also shows variability and thus represents a suitable behavioral measure for assessing how pups perceive changes in their environment and how they discriminate conspecifics or environmental conditions. Further studies are needed to explore whether such stable individual differences in vocal responses to isolation or to other social situations could be considered as an early predictor of stable individual differences in behavioral and emotional reactions in adulthood.

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3. Chapter 2: More exploratory house mice judge an ambiguous situation more negatively

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ABSTRACT

Exploration tendency, one of the most investigated animal personality traits, may be driven by either positive (when seeking interesting information) or negative (to reduce the uncertainty of the environment) affective/emotional profiles. To disentangle the valence of the affective state associated with exploration trait, we applied a judgement bias test to evaluate the animals' responses in an ambiguous situation, allowing an assessment of their affective state or mood. Experiments were carried out in male house mice (*Mus musculus*) of wild origin. Individual differences in exploration tendency were assessed by repeated open field and novel object tests. To evaluate the animals' judgement bias, we trained the subjects during 8 days in a 3-arm maze to discriminate between two extreme locations (outer arms: either positively reinforced with sugary water or less-positively reinforced with plain water), in terms of a shorter latency to approach the positively reinforced arm. After this learning criterion was reached, we repeatedly tested their responses to an ambiguous location (intermediate arm). The latencies to approach and consume the ambiguous reward were highly repeatable over the 3 days of testing, hence individuals expressed a stable judgement bias. Most importantly, more exploratory animals showed a more negative judgement bias, which supports the hypothesis that a higher exploration tendency was associated with a negative affective state. Further studies should investigate whether exploration in different situations might be due to distinct affective states.

Keywords: *animal personality; exploration; cognitive judgement bias; Mus musculus; affective state; emotion; curiosity*

1. INTRODUCTION

Animal personality can be defined as consistent individual differences in behavior across time and/or context (Gosling & John, 1999; Wolf & Weissing, 2012). Personality traits, such as aggressiveness, boldness or sociability, can be considered to be based on individual differences in emotional reactivity (Boissy & Erhard, 2014; Montag & Panksepp, 2017). Exploration tendency is one of the most frequently studied personality trait (Careau, Bininda-Emonds, Thomas, Réale, & Humphries, 2009; Carter, Feeney, Marshall, Cowlishaw, & Heinsohn, 2013; Duparcq et al., 2019; Réale, Dingemanse, Reader, & McDougall, 2007; Rödel et al., 2015) but its interpretation in terms of underlying emotions remains debated. According to a model formulated by Wolf and co-workers (Wolf, van Doorn, Leimar, & Weissing, 2007), exploration should be positively associated with boldness and aggressiveness, thus allowing more exploratory individuals to cope with unpredictable environments (Careau, et al., 2009). And in fact, such associations among these different personality traits are frequently found in various animal species (behavioral syndrome: Réale, et al., 2007; Sih, Bell, & Johnson, 2004). From a psycho-biological point of view, exploration towards new information is generally associated with curiosity, which may be defined as a motivation to ‘know’, to ‘see’ or to ‘experience’, leading to information-seeking behavior (Berlyne, 1960; Litman & Jimerson, 2004). It has been suggested that on the one hand, an animal’s curiosity/exploration tendency could be related to positive affective states involved in the act of seeking out for information of potential interest. But on the other hand, curiosity/ exploration could be also related to a negative affective state leading the animals to search for information resolving the perception of environmental uncertainty (Litman, 2007). We suggest that an increased, consistent exploration tendency might be associated with a specific affective profile. Thus, we propose that investigating relationships between judgment bias and exploration trait should help to clarify whether the tendency to explore may be related to a higher tendency to express either positive or negative affective states.

Since recently, the judgement bias test (or “cognitive bias test”) has been increasingly used to assess affective (or emotional) states and moods using different animal of different taxa, mainly mammals and birds (Clegg, 2018; Hales, Stuart, Anderson, & Robinson, 2014; Hintze et al., 2018; Roelofs, Boleij, Nordquist, & van der Staay, 2016). In such tests, each individual is first trained to discriminate between two highly distinct cues (e.g., two locations, tones, textures), one being associated with a positive outcome (food reward, access to the home cage, etc.) and the other with a negative, or less positive outcome (no or delayed food reward, air puff, etc.). In a second step, the subject is confronted to a novel, intermediate cue, for example a spatial cue located in the middle of the two previously learned ones, or an average tone or texture. The response to this ambiguous stimulus is assumed to depend on the valence of the affective state of the subject; that is, an animal in a more negative affective state is expected to respond to the intermediate cue more similarly to the negatively or less positively reinforced cue, i.e., with a higher latency to approach the intermediate cue (Mendl, Burman, Parker, & Paul, 2009; Roelofs et al., 2016). Such a response pattern is typically interpreted as a more “pessimistic” response. On the other hand, when the individual shows similar responses for intermediate and positive cues (e.g., approaching faster both cues), it will be interpreted as an “optimistic” response, i.e., the expression of a positive affective state. A negative judgement bias can be due to a decreased expectation of a reward or an increased expectation of a punishment (Bateson & Nettle, 2015). Judgement bias tests have been used to evaluate the changes in the valence of affective states after an experimental manipulation, mainly in relation to studies in the fields of psychopharmacology (Neville et al., 2019) or animal welfare (enrichment: Bethell, Holmes, MacLarnon, & Semple, 2012; pain: Neave, Daros, Costa, von Veyserlingk, & Weary, 2013; stereotypic behavior: Novak et al., 2016). Some recent studies also showed that individual differences in judgement bias were consistent over time, at least over short time spans (calves *Bos taurus*: Lecorps, Weary, & von Keyserlingk, 2018; bottlenose dolphins *Tursiops truncatus*: Clegg, Rödel, & Delfour, 2017).

As yet, some studies investigated the relationship between personality traits and individual differences in judgement bias, as the latter is assumed to reflect the valence of the subject's affective state. They highlighted positive associations between optimism (as a proxy of a positive affective state of the animal) and different personality traits such as sociability (dogs *Canis familiaris*: Barnard, Wells, Milligan, Arnott, & Hepper, 2018; bottlenose dolphins: Clegg et al., 2017) or proactivity (domestic pigs *Sus scrofa*: Asher, Friel, Griffin, & Collins, 2016). More pessimistic individuals were also more fearful (calves *Bos taurus*: Lecorps, Weary et al., 2018). Also in rodents, individual judgement bias has been shown to be related to individual differences in affective states. For example, more optimistic laboratory rats *Rattus norvegicus* were less vulnerable to stress-induced anhedonia (Rygula, Papciak, & Popik, 2013), were more motivated to obtain a reward (Rygula, Golebiowska, Kregiel, Kubik, & Popik, 2015) and were less anxious in open field and elevated plus maze tests (Parker, 2008). Yet, associations between personality traits, especially exploration, and judgement bias remain sparsely studied in rodents. In particular, judgement bias is often used to assess an animal's ability to react to and cope with stressful situations (e.g., unpredictable housing: Parker, 2008; pain: Lecorps, Ludwig, von Keyserlingk, & Weary, 2019) or to study how the judgement bias can be influenced by different living conditions (e.g., environmental enrichment in laboratory rats: Brydges, Leach, Nicol, Wright, & Bateson, 2011; Richter et al., 2012). To the best of our knowledge, judgement bias tests have never been used as a way to disentangle the emotional valence that may drive an animal's exploration tendency.

Based on the assumption that exploration tendency (assessed through open field and novel object tests) is related to the tendency to show a certain affective state (Alcaro & Panksepp, 2011; Montag & Panksepp, 2017), this condition should affect the judgement bias of the individuals. A positive correlation between exploration tendency and a positive judgement bias (hypothesis *i*) would support that high exploration is associated with a positive affective state (i.e., an increased interest for novelty in the environment) (Berlyne, 1967; Litman & Jimerson, 2004). In contrast, a negative correlation (hypothesis *ii*) would support that high exploration is associated with a negative affective state, e.g., due to the tendency to reduce perceived environmental uncertainty for reassurance (Hebb, 1955; Litman & Jimerson, 2004).

2. MATERIAL AND METHODS

2.1. Study animals and housing conditions

A total of 122 male house mice *Mus musculus domesticus* (mean litter size: 8.1, 95% CI [7.7, 8.4]) were tested for consistent individual differences in exploration behavior by repeated standard tests (see details in 2.2.1.). Animals were descendants of wild house mice caught around Lyon (France) and bred in the animal facilities of the Laboratoire d'Ethologie Expérimentale et Comparée (Université Paris 13, France) for 9-10 generations. Among them, a subsample of 39 individuals (mean litter size: 7.9, 95% CI [7.3, 8.6]) was also used for the judgement bias test. Study animals were kept under constant conditions with a 14:10 light/dark cycle (light off at 09:00 am), a room temperature of 20 ± 2.0°C, and with a humidity of approximatively 50%. The other males were used in other experiments, as phenotyping of exploration tendency was part of a larger project (not shown here).

At postnatal day 21, animals were weaned, and groups of males stemming from the same litter (3 groups of 2, 16 groups of 3 and 17 groups of 4 individuals) were formed. Females and surplus males were used for breeding and other experiments (not shown here). Groups were kept in polycarbonate cages (32.5 × 16.5 cm and 14.2 cm high, PLEXX, Elst, The Netherlands), with a bedding of wood shavings, 4 cotton balls (COMED, Strasbourg, France) per individual which the animals used as nest material, and two cardboard rolls as enrichment (7.5 × 3.8 cm of diameter). Food (rodent standard diet; Special Diet Services type M20, Witham, Essex, UK) and water were provided ad libitum.

At postnatal days 8, 11 and 35, each individual was marked with a permanent nontoxic hair dye to allow individual recognition within the group (Nyanzol-D, Greenville Colorants, Jersey City, NJ, USA). Animals were held by the experimenter and a unique symbol was rapidly and softly drawn on their back with a fine paint brush.

2.2. Experimental procedures

Experiments were conducted under red light condition, corresponding to the activity period of the animals, in an experimental room maintained under the same light regime, temperature and humidity than the housing room (see 2.1.). All experimental apparatuses (see description in 2.2.1. and 2.2.2.) were cleaned between testing of different individual with water and non-perfumed soap (Colgate-Palmolive, New York, USA).

2.2.1 Standard behavioral tests

Prior to the judgement bias test, subjects were phenotyped for their exploration tendency (N = 122 subjects) by repeated (2 test sessions) open field and novel object tests (Carter, et al., 2013; Réale, et al., 2007). Individuals were kept in sibling groups during the first test session (postnatal day 41, T_1). Immediately after, they were isolated and placed into a new clean cage (same dimensions and content than the group cage, see 2.1.) until the second test session (postnatal day 71, T_2) and remained isolated until the end of the study. Behaviors were video recorded using a camera (T650sc, FLIR, Wilsonville, USA) mounted over the test apparatuses, and video footage was stored for later analysis.

2.2.1.1. Open field test

The open field was a constituted of a circular arena (diameter of 60 cm; area of 2827 cm^2), surrounded by walls (69 cm high) made of white opaque polyethylene. A central circular area was defined (20 cm of diameter), representing one ninth of the total area.

Subjects were placed close to the wall of the arena and the test started for 5 min once the animal was released. The video camera was mounted 140 cm above the center of the arena. We quantified the total distance covered in the open field (Lecorps, Rödel, & Féron, 2016; Mazza, Eccard, Zaccaroni, Jacob & Dammhan, 2018; Rangassamy, Dalmas, Féron, Gouat, & Rödel, 2015; Yuen, Schoepf, Schradin, & Pillay, 2017), using Ethovision XT10 (Noldus Information Technology, Wageningen, The Netherlands).

2.2.1.2. Novel object test

The novel object test immediately followed the open field test: after the 5 min of the latter test, the individual was caught with a plastic box and placed again close to the wall of the arena. The object was positioned in the center of the arena, the individual was released and the test lasted for 5 min.

The object used on postnatal day 41 (T_1) was a small oval metal box (length: 9.5 cm; height: 2.7 cm) and on postnatal day 71 (T_2) the object was a round and opaque soft PVC toy (diameter of 8.5 cm and 4.5-5.0 cm high). The height of the objects allowed the animals to jump on them; due to their size and weight, objects could not be moved by the animals. We recorded the latency to approach and sniff the object for the first time and the percentage of time spent exploring of the object (% time exploring the object), measured as the sum of time spent sniffing, touching (with mouth or forepaws) and staying on the top of the object (Duparcq et al., 2019; Mazza et al., 2018; Rangassamy et al., 2015, 2016). All behavioral measures were analyzed using the software BORIS 6.2.2 (Friard & Gamba, 2016).

2.2.2. Judgement bias task

2.2.2.1. Description of the apparatus

The apparatus (Fig. 1) was made of opaque white PVC (3 mm thick). The walls separating the arms from the central platform were removable, allowing the experimenter to place the appropriate walls (with or without a swinging door, that is, a door that can swing open in both directions) in front of the reference arms before each trial. The walls of the apparatus were 50 cm high to avoid the animals to jump out of it. A video camera (FDR AX-100 4K, Sony, UK) was mounted 150 cm above the center of the apparatus.

2.2.2.2. Part 1: Training to the spatial discrimination task

The experiments started with a training phase during which individuals needed to learn the association between one location and one type of reward. The individuals (*N* = 39) were trained for 8 days, from postnatal day 75 to 82 and underwent one session of 4 trials per day. During the first day, only positively reinforced trials were conducted, as it has been shown to increase the speed of learning (Roelofs et al., 2016), although these data were not analyzed. During the next 7 days, two of the trials were positively reinforced and two were less-positively reinforced (see 2.3.2.1.). The trials were pseudo-randomized, as the individuals were confronted with a different order each day. During each trial, only one arm (either the left or the right reference arm; Fig. 1) was made accessible.

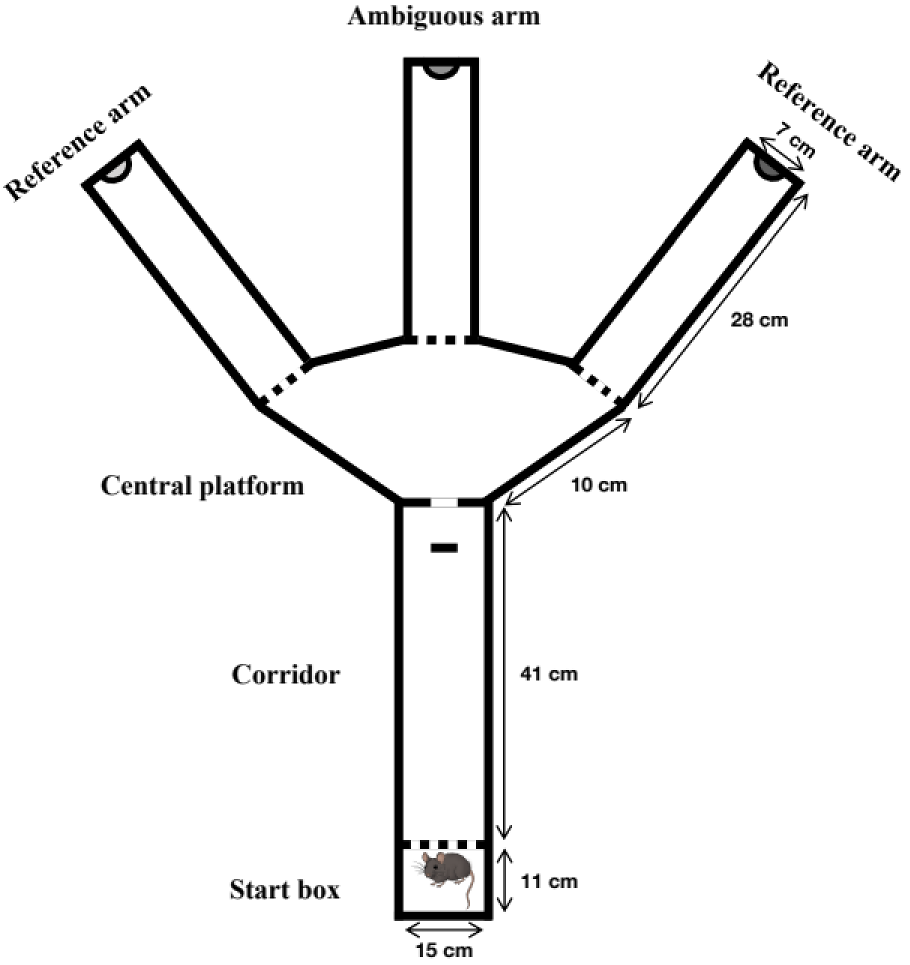

Figure 1. Schema of the judgement bias apparatus. It is composed of a start box, a corridor and a central platform leading to 3 same-sized arms placed at equal distance from the exit (4 × 4 cm) of the corridor. Dotted lines represent removable walls. Tested individuals could access the open arm through an opening (5 × 5 cm) with a squared swinging door. To avoid that the individuals could see which arm was opened before entering the central platform, a wall was placed 5 cm before the exit of the corridor with a rectangular opening (4 × 4 cm) at each side. See text for details on the experimental protocol.

2.2.2.3. Reinforcement of the reference arms

For one half of the individuals (randomly chosen), the right reference arm (Fig. 1) was positively reinforced (hereafter: 'positive arm') and the left reference arm was less-positively reinforced (hereafter: 'less-positive arm'), while the other half of the individuals was trained with the opposite location pattern. The positive reward consisted of a drop of sugar water (10% of sucrose 99%) and the less-positive reward of a drop of plain water. To allow video analysis of the consumption of the rewards, a blue food colorant (Vahiné, France) was added to the water for both positive and less-positive rewards. For each trial, the reward was deposited on the inner curved part of an open Petri dish (diameter: 3.5 cm) and fixed on the back wall of the arm, 1 cm above the floor, i.e., at the level of the animals' head to allow them to consume in a quadrupedal position.

2.2.2.4. Experimental procedure

At the beginning of each daily test session, individuals were placed singly into the start box (Fig. 1) and then the first trial started. During the first 3 min when the animal remained in the start box, the appropriate reward was prepared as explained above (see 2.2.2.3.) and the removable walls were placed to open or close the reference arms accordingly (Fig. 1). Then, the start box was opened and the individual was able to explore the apparatus for a maximum of 5 min. If during this time lapse, the individual entered the reference arm, it was given 1 min to consume the reward before the trial was ended. At the end of this minute, or after 5 min in case the animal did not enter in the arm, the individual was gently guided by the hand of the experimenter to the start box and the second trial started as soon as the door of the start box was closed. At the end of the four trials, the individual was returned to its home cage and the apparatus was cleaned.

2.2.2.5. Definition of the learning criterion

For each trial, the latency to approach and consume the reward after the animal entered in the central platform was recorded. To assess if the individuals learned to discriminate between the two reference arms, we plotted the averaged latencies to approach and consume the positive and the less-positive rewards (in s) for each training day and visually compared the two curves. The learning criterion was defined as follows: individuals had to approach and consume the positive reward with a shorter latency than the less-positive reward for at least two consecutive days. Furthermore, the threshold for the difference between these two latencies (positive and less-positive) was 5 s (mean difference among the individuals at the day they reached the learning criterion: 28.16 s, 95% CI [21.64, 34.68]). Individuals were considered as having learned the day this learning criterion (shorter latency to approach and consume the positive reward of at least 5 s during two consecutive days) was reached and they had to maintain the criterion until the end of the training period. Four individuals reached the learning criterion during the last training day (that is, they approached and consumed the positive reward faster during the last two training days) and were also considered as having learned. In total, 25 out of 39 individuals (64%) reached the learning criterion and continued the test (see 2.3.3.). The individual speed of learning, defined as the day at which the individual reached the learning criterion, was not significantly associated with individual differences in judgement bias ($P = 0.192$). Also, excluding the four 'late learners' from all statistical analyses did not lead to different results than the ones presented below (see result section).

2.2.2.6. Part 2: Judgement bias test

After the training, only the animals which reached the learning criterion were tested for their responses to an ambiguous location. During these trials, the centrally-located arm (hereafter: 'the ambiguous arm') was open while the two reference arms were closed. The test period lasted for 3 days, from postnatal day 83 to 85, with one session of 3 trials per day. The sessions were identical each day: a less-positive, a positive then an ambiguous trial. This allowed us to control for motivational effects that could influence the latency to reach the ambiguous reward depending on the valence of the trial preceding it. The ambiguous arm was also rewarded with plain (non-sugary) water to be able to measure the latency until consumption. The test sessions were performed following the same experimental procedure than the training sessions (see 2.3.2.2.).

During the three days of test, the individuals approached and consumed the positive reward after on average 6.25 s, 95% CI [4.09, 8.41], the less-positive reward after 61.84 s, 95% CI [55.99, 67.68] and the ambiguous reward with an in-between latency of 45.84 s, 95% CI [37.91, 53.77]. The latencies differed significantly from each other ($P < 0.001$).

2.2.2.7. Calculation of judgement bias index

For each individual successfully trained, we calculated a judgement bias index using the following formula, where L represents the latency to approach and start consuming the reward of the positively rewarded, the less-positively rewarded, or the ambiguous arm. The latency (with a resolution of 1 s) was measured from the time the animal introduced its head by the opening of the central platform until it started to consume the reward. Before the calculation of the index, the latencies of the positive, less-positive and ambiguous arms were averaged between the three testing days.

$$\text{Judgement bias index} = 1 - ((L_{\text{ambiguous}} - L_{\text{positive}}) / (L_{\text{less-positive}} - L_{\text{positive}}))$$

We obtained a judgement bias index ranging from 0 (for a latency to consume the ambiguous reward similar to the latency to consume the less-positive one) to 1 (for a latency to consume the ambiguous reward similar to the latency to consume the positive one).

2.3. Statistical analysis

All statistical analyses were done with the software R, version 3.4.4 (R Core Team, 2019). For all covariate effects, we calculated the marginal $pseudoR^2$ using the package *MuMIn* (Barton, 2018), which can be interpreted as the proportion of variation explained by the fixed effect (Nakagawa, Johnson, & Schielzeth, 2017). Prior to analysis, the latency to approach and sniff the novel object was log[x+1] transformed to adjust the data to a normal distribution. All covariates were scaled for analysis.

First, to summarize the behavioral variables in a single score, we applied a principal component analysis (PCA; R package *FactoMineR*: Lê, Josse, & Husson, 2008), using the behaviors quantified in the open field (total distance covered in the open field) and the novel object tests (latency to sniff the object, % time exploring the object), separately for each test session (T_1, postnatal day 41; T_2, postnatal day 71; N = 122 individuals). We only used the first component of the PCA for later analysis as it had an eigenvalue > 1; that is, the first component accounted for more variance than any of the original variables of the standardized data (Kaiser, 1991). This component was interpreted as ‘exploration’ score for later analysis.

The repeatability of the exploration scores (N = 122), as well as of the judgement bias index (N = 25, that is, the number of individuals that reached the learning criterion, see 2.2.2.5.), were calculated with intra-class correlations (R_{ICC}), using a linear mixed-effects model based on calculations of *P*-values with 10,000 Monte Carlo permutations (package *rptR*: Stoffel, Nakagawa, & Schielzeth, 2017), with individual identity as a random factor.

To test for associations between the exploration scores and the judgement bias index, we first performed a new PCA, using the averaged behaviors quantified across the two test sessions (*N* = 122). Then, we applied the *predict* function (package *FactoMineR*) to perform a new PCA on the subsample of individuals which successfully passed the judgment bias test (*N* = 25). This function allowed us to perform the PCA on the same space created for the PCA based on the 122 individuals, hence giving similar first components. We extracted the individual coordinates from the first component of the new PCA to obtain the exploration scores of the subjects which passed the judgement bias test (*N* = 25). Then, we first log[x+0.1] transformed the explanatory variable (judgement bias index averaged between the three days of testing) to increase the homogeneity of models residuals (Faraway, 2006). Second, we tested for the association between exploration scores (averaged between the two test sessions; independent variable, covariate) and individual judgement bias index (transformed; dependent variable, covariate) by running a linear mixed-effects model (LMM) with litter identity as a random factor, using the R package *nlme* (Pinheiro, Bates, DebRoy, Sarkar, & R Core Team, 2019). *P*-value calculations were based on 10,000 Monte-Carlo permutations.

2.4. Ethics note

All experimental procedures were approved by the French authority for animal care and use (APAFIS#7585-201610121409165) and by the institutional ethics committee (SBEA LEEC UP13). The individuals tested for their personality traits but not for their judgement bias were used for other experiments and for breeding. Animals which underwent the judgement bias test were euthanized at the end of the study, at postnatal day 87.

3. RESULTS

3.1. Consistent individual differences across time in exploration score

The first component of the PCA explained 49.7% (T_1) and 47.0% (T_2) of the variation of the data. During both times, higher scores indicated a greater distance covered in the open field (loadings during T_1: 0.423; loadings during T_2: 0.787), a shorter latency to sniff the object for the first time (T_1: -0.807; T_2: -0.858) and a higher % time exploring the object (T_1: 0.813; T_2: 0.231). The exploration score ($R_{ICC} = 0.511$, $P < 0.001$, Fig. 2) was significantly repeatable across the two test sessions ($N = 122$). Thus, individuals showed consistent individual differences in exploration behavior.

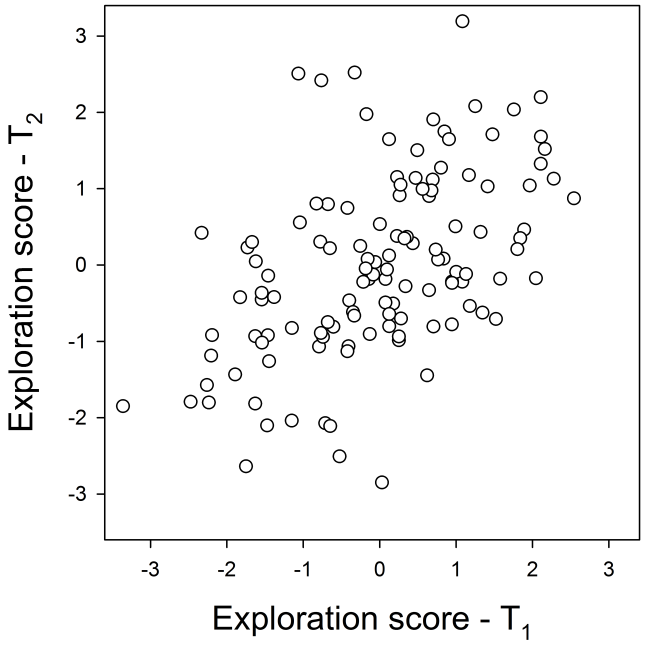

Figure 2. Consistent individual differences in exploration scores across the two test sessions T_1 and T_2 ($N = 122$ adult males). Individual scores correspond to the first component of a PCA, using the averaged behaviors quantified in repeated open field and novel object tests (postnatal days 41 and 71). Higher scores indicate a higher exploration tendency (greater distance covered in the open field, shorter latency to sniff and higher % time exploring the object). The association between the two test sessions was statistically significant and tested by intra-class correlation, see text for details.

3.2. Consistent individual differences across time in judgement bias index

The judgement bias index was significantly repeatable at the individual level across the three days of testing ($R_{\mathrm{ICC}} = 0.711$, $P < 0.001$, Fig. 3). Thus, individuals showed stable individual differences in the relative latencies to approach the ambiguous cue.

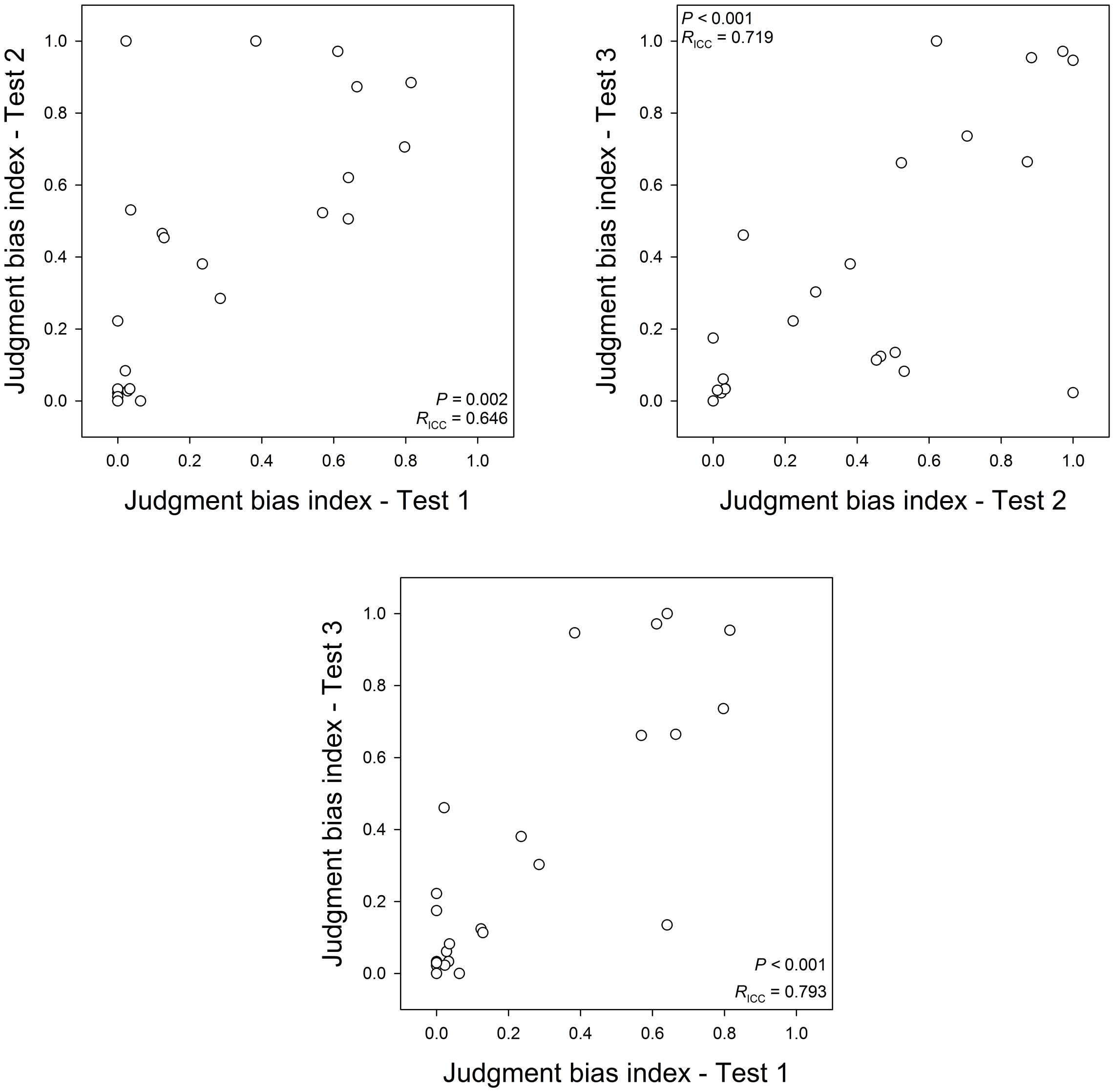

Figure 3. Consistent individual differences of the judgement bias index across three days of testing (*N* = 25 adult males). The calculation of the judgement bias index is detailed in the text. Judgement bias ranges from 0 (negative bias) to 1 (positive bias). All associations shown were statistically significant and tested by intra-class correlation, see text for details.

3.3. Associations between individual differences in judgement bias index and exploration scores

Individual differences in judgement bias index were significantly and negatively associated with individual exploration scores (*pseudoR*^{2} = 0.189, P = 0.035, Fig. 4). That is, individuals which were more explorative during the open field and novel object test, showed a longer latency to approach the ambiguous cue during the test situation (that is, they responded with a latency more similar to the one they showed when approaching the less-positive cue).

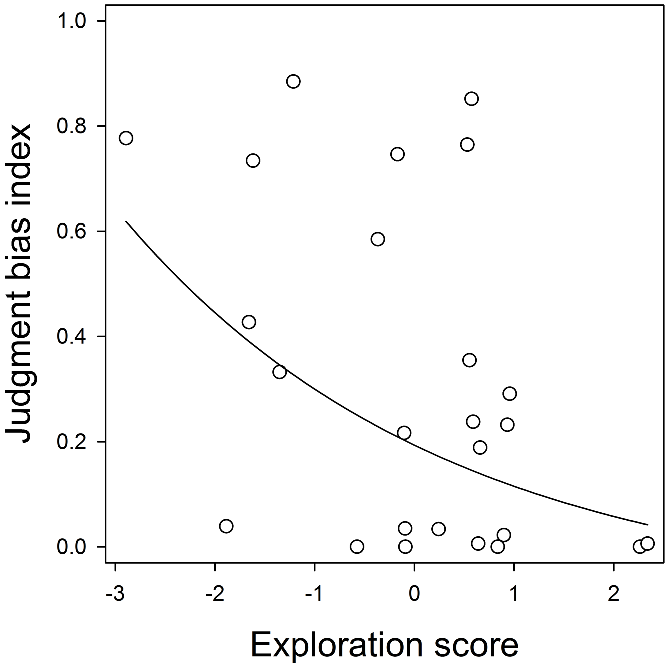

Figure 4. Association between judgement bias index and exploration score (N = 25 adult males). Judgement bias ranges from 0 (negative bias) to 1 (positive bias) and were averaged between the three days of testing. Higher exploration scores (averaged between the two test sessions, at postnatal days 41 and 71) indicate a higher exploration tendency (greater distance covered in the open field, shorter latency to sniff and higher % time exploring the object). The association was statistically significant (details in text); analysis by LMM.

4. DISCUSSION

As expected, individual judgement bias was significantly associated with the animals' exploration tendency. In accordance to our second hypothesis, more explorative subjects showed a more negative judgement of the ambiguous test situation.

Our results on the existence of consistent individual differences in exploration confirm the findings of previous studies in laboratory mice *Mus musculus* (Brust, Schindler, & Lewejohann, 2015; Lewejohann, Zipser, & Sachser, 2011; Rödel et al., 2012) and other rodent species of wild origin (mound-building mouse *Mus spicilegus*: Duparcq et al., 2019; Rangassamy et al., 2015; common vole *Microtus arvalis*: Herde & Eccard, 2013; bank vole *Myodes glareolus*: Mazza et al., 2018; Eurasian harvest mouse *Micromys minutus*: Schuster, Carl, & Foerster, 2017). To the best of our knowledge, our study is the first to demonstrate consistent individual differences in exploration in house mice of wild origin.

We validated our judgment bias protocol based on positive and less-positive rewards, as our individuals responded to the presentation of the ambiguous cue with an intermediate latency between the positive and less-positive cues. It is important to develop judgement bias tests which do not require the use of punishments, such as mild electric shocks (Enkel et al., 2010) or air puffs (Brajon, Laforest, Schmitt, & Devillers, 2015), as they can directly modify the affective state of the subjects and lead them to avoid the ambiguous cue, hence exhibiting more pessimistic responses (Mendl et al., 2009). Others have already developed such tests using for example the presentation of 1 versus 2 food pellets in the laboratory rat (Parker, Paul, Burman, Browne, & Mendl, 2014) or of small versus large rewards in the domestic pig (Roelofs, Nordquist, Josef, & van der Staay, 2017). An appropriate selection of the reinforcers becomes more crucial when the baseline judgement bias is assessed, as it has been done in our study. Furthermore, in accordance with studies in calves *Bos taurus* (Lecorps, Weary et al., 2018; Lecorps, Kappel, Weary, & von Keyserlingk, 2018), domestic pigs (Asher et al., 2016) and bottlenose dolphins (Clegg et al., 2017), we demonstrated that wild-origin house mice displayed consistent individual differences in judgement bias over three days of testing.

Returning to the main goal of our study, we found that individuals expressing a more negative judgement bias were also the more explorative ones in open field and novel object tests. Exploration of novel situations (environment and objects) is often used as a proxy to assess anxiety and emotional reactivity (Harro, 2018; Ohl, 2003). Especially the behaviors we quantified (overall activity in the open field, % time exploring the object and latency to approach the objects) are likely to refer to the SEEKING system, a positive emotional system underlying explorative and approach behavior proposed by Panksepp and co-workers (Alcaro & Panksepp, 2011; Montag & Panksepp, 2017; Panksepp, 2005). According to this approach, individuals with a more sensitive SEEKING system are more motivated to search for rewards and to explore new stimuli, which could greatly impact the responses when confronted to an ambiguous stimulus. In this context, we might expect that more explorative individuals would have positive expectations about the outcome of the ambiguous cue (i.e., positive judgement bias) and thus would faster approach and consume the ambiguous reward. Contradicting this prediction, our results show a correlation to the opposite direction, suggesting that more explorative individuals might have more negative expectations in an ambiguous situation.

We conclude that our results are consistent with others suggesting that information seeking behaviors may be mediated through curiosity reduction (curiosity-drive theory: Berlyne, 1954, 1960), i.e., individuals are motivated to explore their environment in order to reduce uncomfortable states due to environmental uncertainty, or motivated by a lack of available information (curiosity as a feeling-of-deprivation: Litman & Jimerson, 2004). These models, which involve a degree of negative affectivity during exploration would imply that more explorative individuals have more difficulties to sustain the novelty of the environment in the open-field and the novel object tests, pushing them to increase their explorative activities. These more explorative individuals might be characterized by a more pronounced tendency to express negative affective states (i.e., more negative affective profiles), leading them to engage in the exploration of novel and uncertain situations for reassurance. We suggest that findings obtained by judgement bias test indicate such negative affective profiles. Indeed, during the judgement bias test, individuals may have invested more time in exploratory activity, instead of consuming the reward, to reduce the negative affective state induced by the uncertainty of the novel, ambiguous cue, finally leading to a negative bias. However, the association we reported here might remain highly dependent on the context we measured the exploration tendency. For futures studies, we propose that associating a judgement bias test with the quantification of exploration in other experimental paradigms, such as ‘free exposure’ open field and novel object tests (Fonio, Benjamini, & Golani, 2009; Griebel, Belzung, Misslin, & Vogel, 1993) would bring further insights into the association between individual differences in exploration tendency and affective profiles.

Finally, such explorative and proactive individuals (in the sense of Koolhaas et al. (1999); as fast exploration is a key component of proactivity), despite a higher novelty-seeking, are also characterized by a lower executive control (de Boer, Buwalda, & Koolhaas, 2017). The ambiguity in judgement bias tests might create a conflict between the tendency to explore and the behavioral inflexibility (i.e., low executive control) of proactive individuals. Proactive individuals are also less sensitive to changes in their environment and being more likely to form routines (Coppens, de Boer, & Koolhaas, 2010; Sih & Del Giudice, 2012). On the contrary, slow explorers take more time to gather more detailed information, which allow them to be more flexible when the environment changes. Such associations between individual differences in exploration types and cognitive abilities have already been highlighted by several studies (Guenther, Brust, Dersen, & Trillmichh, 2014; Guillette, Reddon, Hoeschele, & Sturdy, 2010; Mazza et al., 2018; Mazza, Jacob, Dammhahn, Zaccaroni, & Eccard, 2019; Verbeek, Drent, & Wiepkema, 1994). For instance, proactive pigs were less successful in a reversal learning task, due to difficulties to inhibit the behavioral patterns they previously learned (Bolhuis, Schouten, Leeuw, Schrama, & Wiegant, 2004). In our study, consistently with a study in carpenter ants *Camponotus aethiops* (d'Ettorre et al., 2017), more explorative individuals showed more pessimistic responses, that is, they showed longer latencies to reach the ambiguous reward. Hence, according to this hypothesis, the here observed negative judgement bias in more exploratory animals might not be an expression of a negative affective state but might rather be explained by a greater inflexibility in adjusting their behavior when confronted to a new situation due to routines formed during the training period (Coppens et al., 2010; Sih & Del Giudice, 2012). This explanation would challenge the prevalent interpretation of a negative judgement bias as the expression of a negative affective state or mood.

In conclusion, our study is consistent with others showing associations between individual differences in judgement bias and a personality trait (here, exploration tendency). In particular, judgement bias and personality tests could complement each other to help determining the affective states underlying the classical personality traits, although the observed associations may remain highly dependent on the context of evaluation of the personality traits. Further studies should also investigate the potential confounding effects of behavioral inflexibility of proactive (and possibly more exploratory) individuals when aiming to assess their affective state through judgement bias tests.

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4. Chapter 3: Differences between fast and slow explorers in short-term tail temperature responses to handling in a rodent of wild origin

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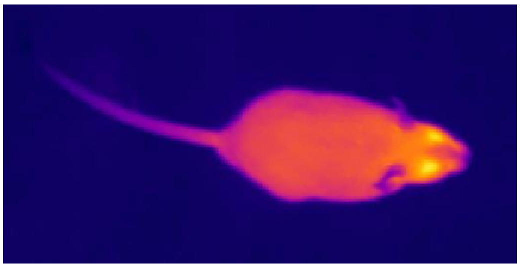

ABSTRACT

Animals of different behavioral types typically show associated differences in their physiological stress response, including differential reactivity of the sympathetic nervous system. Infrared thermography offers the possibility to explore this link in a non-invasive way via the quantification of fine-scale changes in peripheral body temperature due to changes in cutaneous blood flow. We used this technique to investigate the association between exploration tendency, a behavioral trait frequently used to phenotype mammals and birds, and short-term thermal responses to challenge in a small rodent of wild origin, the mound-building mouse (*Mus spicilegus*). We applied a brief handling procedure consisting in the transfer of subjects into a small arena. This procedure led to a significant increase in subjects' maximum peripheral body temperature (mainly reflecting the temperature of the eyes) and to significant decreases in maximum temperatures at different positions on the tail. Maximum peripheral body and tail temperatures showed significant individual-level consistencies in response to repeated applications of the handling procedure, suggesting stable individual differences in the animals' sympathetic activity. We then compared the thermal responses to handling between 'fast' and 'slow' explorers, who were phenotyped through repeated open field and novel object tests. Fast explorers showed significantly lower tail temperatures than slow explorers shortly after handling, suggesting a stronger sympathetic reactivity in the former. Comparisons within sibling groups kept in different cages showed that the differences between explorer types were particularly pronounced during the first minute after handling, and increased in magnitude along the first millimeters distal to the tail base.

Keywords: *exploration tendency; infrared thermography; Mus spicilegus; personality; stress; sympathetic activity*

1. INTRODUCTION

A large and still growing body of evidence from a wide range of taxa suggests that animals show consistent individual differences in behavior over time and across contexts, frequently termed as animal personality, behavioral syndrome or coping style (Gosling & John, 1999; Koolhaas et al., 1999; Réale, Reader, Sol, McDougall, & Dingemanse, 2007; Sih, Bell, Johnson, & Ziemba, 2004; Stamps & Groothuis, 2010). These differences in behavioral phenotypes are typically associated with underlying differences in neurophysiology, such as in the animals' stress response (Carere, Caramaschi, & Fawcett, 2010; Ebner & Singewald, 2017; Koolhaas, de Boer, Coppens, & Buwalda, 2010; Korte, Koolhaas, Wingfield, & McEwen, 2005).

The physiological stress response mainly involves two complementary systems, the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic adrenomedullary system (von Holst, 1998). Consistent findings in mammals and birds show a generally higher or more chronic activation of the HPA axis in individuals with more reactive (as opposed to more proactive individuals (Koolhaas et al., 1999)), more passive (De Miguel et al., 2011; Rödel, Monclús, & von Holst, 2006; von Holst, 1986), more anxious or fearful (Dhabhar et al., 2012; Jones, Satterlee, & Ryder, 1994; Rangassamy et al., 2016), less aggressive (Veenema, Meijer, De Kloet, Koolhaas, & Bohus, 2003) and less exploratory phenotypes (Carere, Groothuis, Möstl, Daan, & Koolhaas, 2003; Montiglio, Garant, Pelletier, & Réale, 2012; Stöwe, Rosivall, Drent, & Möstl, 2010). Furthermore, more proactive (Ågren, Lund, Thiblin, & Lundeberg, 2009; Hessing, Hagelsø, Schouten, Wiepkema, & van Beek, 1994; Koolhaas et al., 1999; von Holst, 1986), aggressive (Sgoifo, De Boer, Haller, & Koolhaas, 1996), and more active and exploratory types (Ferrari et al., 2013; Fucikova, Drent, Smits, & Van Oers, 2009; Montiglio et al., 2012) frequently show indications of a higher reactivity of the sympathetic nervous system, which activates immediate physiological changes related to the body's 'fight-or-flight' response when individuals perceive a threat or danger (von Holst, 1998).

Measurements of circulating hormone concentrations related to the activation of the sympathetic adrenomedullary system require invasive blood sampling procedures, and thus hardly allow repeated measurements over a short span of time. Therefore, low- or non-invasive methods such as recordings of heart rate and breathing rate are frequently applied in studies exploring changes in sympathetic activity in response to environmental perturbations (Ferrari et al., 2013; Fucikova et al., 2009; Hessing et al., 1994; Montiglio et al., 2012). During recent years, infrared thermography has opened new directions and possibilities towards the non-invasive quantification of sympathetic nervous system activity (Gjendal, Franco, Lund Ottesen, Bratbo Sørensen, & Olsson, 2018; Herborn et al., 2015; Rekant, Lyons, Pacheco, Arzt, & Rodriguez, 2016; Stewart, Webster, Schaefer, Cook, & Scott, 2005). This method consists in assessing the animals' body heat loss through the instantaneous measurement of peripheral temperature, reflecting cutaneous blood flow (Vianna & Carrive, 2012). Changes in this parameter, i.e., by vasoconstriction or vasodilatation, constitutes a consequence of the stimulation of the sympathetic nervous system (von Holst, 1998). For example, a study in laboratory rats *Rattus norvegicus* showed sympathetic cutaneous vasoconstriction in tail and paws in response to a fear-inducing treatment while back, head and eye temperatures increased (Vianna & Carrive, 2005). Similarly, house mice *Mus musculus domesticus* exposed to open field and elevated plus-maze tests showed a decrease in tail temperature and an increase in eye temperature (Lecorps, Rödel, & Féron, 2016). So far, infrared thermography has been hardly applied to assess sympathetic nervous system reactivity in different behavioral types (Ågren et al., 2009), and continuous recordings exploring the time course of such differences directly after challenge are still lacking.

Using infrared thermography, we investigated whether differences between behavioral types in short-term peripheral body temperatures responses – as a proxy of the animals' sympathetic nervous reactivity – were detectable after a brief handling challenge. For this, the animals were transferred from their cages into a novel environment (a small arena), a procedure which is part of the routines carried out during care of and experimentation with rodents kept under laboratory conditions. Experiments were conducted under standardized laboratory conditions by using a rodent of wild origin, the mound-building mouse *Mus spicilegus*. Animals of wild origin are advantageous models for the study of consistent individual differences in behavior since such animals often show a larger inter-individual variation in behavioral types compared to inbred laboratory strains (Koolhaas et al., 2010). In our study, we focused on differences in exploration tendency ('fast' and 'slow' explorers), a main behavioral ('personality') trait frequently used to phenotype mammals and birds (Carere & Maestripieri, 2013; Dingemanse, Both, Drent, & Tinbergen, 2004; Réale et al., 2007; Rödel et al., 2015), and a major trait component of the proactive-reactive continuum proposed by Koolhaas and coworkers (Koolhaas et al., 1999; Koolhaas, De Boer, Buwalda, & Van Reenen, 2007). Furthermore, previous studies in the mound-building mouse have already shown notable and consistent individual differences in behavioral responses during repeated standard tests such as in the open field and during novel object presentation, frequently used to phenotype differences in exploration tendency (Rangassamy, Dalmas, Féron, Gouat, & Rödel, 2015; Rangassamy et al., 2016).

In a first step, we (*i*) tested whether our procedure consisting in handling and transfer of subjects from their cages into a novel environment was sufficient to induce detectable stress responses through short-term changes in peripheral body temperatures. We quantified (*a*) the maximum peripheral body temperature, which can be interpreted to mainly reflect the temperature of the eyes (Lecorps, Rödel, & Féron, 2019), and (*b*) the surface temperature at different positions on the tail. In accordance with previous findings in other rodent species (Lecorps et al., 2016; Vianna & Carrive, 2012), we expected a significant increase in the maximum peripheral body temperature, but a significant decrease in tail surface temperature. Furthermore, we (*ii*) explored consistencies in the animals' thermal responses to repeated applications of our stress-inducing procedure, which would suggest the existence of stable inter-individual differences in sympathetic responses (Koolhaas et al., 2007; Koolhaas et al., 2010). We (*iii*) tested for differential responses in animals with different exploration types. As suggested by previous studies in mammals using other kinds of stressors and other measures of the animals' sympathetic responses (Koolhaas et al., 1999; Montiglio et al., 2012; Montiglio, Ferrari, & Réale, 2013), we predicted a higher maximum peripheral body temperature (mainly reflecting the temperature of the eyes), but a lower tail surface temperature in response to our handling and transfer procedure in fast compared to slow explorers, indicative of a higher sympathetic reactivity in the former behavioral type. In particular, we investigated the potentially differential response dynamics of fast and slow explorers during the following minutes after the application of the stressor, and we compared the differences between explorer types at increasing distances from the base of the tail.

2. MATERIAL AND METHODS

2.1. Study animals and housing conditions

Mound-building mice of our breeding stock at the animal facilities of the Laboratoire d’Ethologie Expérimentale et Comparée, Université Paris 13 were descendants of 80 animals caught from the wild at different sites in Hungary in 1999. To maintain a high genetic variation, additional individuals were captured at the same Hungarian collection sites every 2-4 years. This has been done recently in 2016, 2.5 year before the onset of the present study, when 10 wild-caught animals were added to our breeding stock.

For the production of experimental animals, breeding couples were housed with their offspring in transparent polycarbonate cages (40.5 × 26.5 cm and 15 cm high, Tecniplast, Buguggiate, Italy) containing wood shavings as bedding material. Around 15 cotton balls were always provided for nest building and cages were enriched with 2 cardboard rolls (3.7 cm diameter and 9.6 cm long). Water and food (rodent standard diet, Special Diets Services, type M20, Witham, UK) were supplied ad libitum. The breeding and experimental rooms had a 14:10 light/dark cycle with red light from 09:30 am until 07:30 pm, at a relative humidity at approximatively 50% and a steady ambient temperature of 20 ± 0.5 °C (see controversial suggestions on keeping animals in the laboratory under higher ambient temperature conditions, close to their thermoneutral zone (Fischer, Cannon, & Nedergaard, 2018; Speakman & Keijer, 2012)).

In total, 59 juvenile mound building mice were used for experimentation in this study. Until weaning on postnatal day 28, juveniles were housed with both parents. After weaning, litter sibling groups were transferred to separate cages (40.5 × 26.5 cm and 15 cm high), also enriched with several cotton balls and 1 cardboard roll. To allow individual recognition of litter siblings by the experimenter, animals were marked with different symbols on their back with black, non-toxic hair dye (ProDye, Weaver Leather, Ohio, USA) on postnatal day 9, and then again during the following weeks, in case the symbols started to fade. For this purpose, the dye was quickly applied with a brush while pups were held by their flanks. For drying, pups were put on a paper towel sheet under a heating lamp for 5 min before they were returned to the nest of their home cage.

A subset of the animals (*sample A*, $N = 5$ individuals from 5 different litters, 2 females and 3 males) was used for a validation experiment of the thermal-response test. The other subset (*sample B*, $N = 54$ individuals from 8 different litters, 22 females and 32 males) was first behaviorally phenotyped and then underwent the thermal-response test (details below).

2.2. Experimentation

Experiments were carried out in an experimental room adjacent to the breeding room under the same light, temperature and humidity conditions. All tests (open field test, novel object test, thermal-response test) were conducted under red light conditions, i.e., during the animals' activity period. Between the tests of different individuals, apparatuses were cleaned with soap water and were dried afterward.

2.2.1. Behavioral phenotyping

Repeated open-field and novel object tests were used to assess the animals' exploration tendency. For each individual, the novel object test (duration: 5 min) was carried out right after the open field test (duration: 5 min). Tests were recorded by video cameras (FDR AX-100 4K, Sony, UK) mounted 120 cm above the center of the test arena, and video footage was stored for subsequent behavioral analyses. Five min before the beginning of testing, home cages including experimental animals were transported from the breeding room to the experimental room. Litter siblings were then separately taken from the home cage in a random order for testing. Each individual was repeatedly tested, on postnatal day 33 (T_1) and day 43 (T_2). The experimenter always left the room after the animals were entered into the test apparatus.

2.2.1.1. Open field test

The apparatus consisted of a white opaque polyethylene circular arena of 60 cm diameter, including a central and a peripheral part, surrounded by a 65 cm high wall. The central part was a central circle not visible for the animals, with a diameter of 23 cm. Individuals were always entered into the test arena at the same marked location close to the wall of the apparatus. The test started when the individual was released. The total distance covered and the distance covered by individuals in the central part of the open field were quantified using the software EthoVision XT 10 version 10.1 (Noldus, Wageningen, The Netherlands).

2.2.1.2. Novel object test

Once the open field test was completed, the subject was captured by the aid of a transparent plastic tube open at one end (8 cm diameter and 17 cm high). Then, a novel object was added in the center of the arena and the subject was released from the tube at the same starting location, close to the wall of the arena, as previously described during the open field test.

On postnatal day 33 (T_1), the novel object was a circular glass pot (6.7 cm diameter and 4.6 cm high), whereas on postnatal day 43 (T_1) it was a cylindrical silicone cake tin (8.9 cm diameter and 4.6 cm high). Three different behaviors were quantified during the tests using the software BORIS version 6.2.2 (Friard & Gamba, 2016), the latency to approach and to sniff the novel object, the time spent exploring it (sniffing, touching and climbing), and the number of times that the animals climbed on the object (with all 4 paws on it).

2.2.2. Thermal-response test

The purpose of this test was to assess the animals' short-term thermal responses to handling including the transfer into the test apparatus by measuring their peripheral body temperatures via infrared thermography. The test apparatus, into which the animals were entered singly, consisted of a squared white PVC arena (23.5 × 23.5 cm) with 40 cm high walls. An infrared thermal camera capable of recording video sequences (T650sc, FLIR Systems, Wilsonville, OR, USA; resolution: 640 × 480 pixels, sensitivity of < 20 mK at 30 °C with an emissivity fixed at 0.95; 30 frames per s [30 Hz]) was mounted 136 cm over the apparatus. The test procedure for animals from *sample A* (N = 5) and *sample B* (N = 54) differed slightly (see below).

2.2.2.1. Procedures during the thermal-response test

In a first step, we aimed to carry out a validation of the thermal-response test using individuals of *sample A* (*N* = 5), who were between 39 and 41 days old at the time of testing. For this sample, the test was only carried out once per individual. We aimed to explore the changes in thermal response by comparing the (basal) thermal values quantified shortly before handling, when subjects were still in their standard cages, with the response values after handling when transferred into the test apparatus. The procedure began by the isolation of individuals in the evening of the day before testing. Being solitary is not an unusual situation in the mound-building mouse; solitary females and males have been frequently found under natural conditions (Gouat, Katona, & Poteaux, 2003; Simeonovska-Nikolova, 2012). For this, we removed subjects from their home cages (in which they were housed with their siblings) and placed them singly in a clean transparent polycarbonate cage (26.5 × 16 cm and 13 cm high, Charles River, Wilmington, USA). The cage was left open to allow recordings from above by the infrared thermal camera. To prevent the animals from jumping and climbing out of their cages, the cage walls were extended by additional 60 cm high white and opaque PVC walls. This isolation cage contained wood shavings, food pellets, and a filled water bottle. The day after, two hours before the beginning of the test, the experimenter silently entered the experimental room to switch the infrared thermal camera system into standby mode and then left the room. For testing, the experimenter returned into the room, did not approach the cage but switched on the camera via a cable connection to start the infrared thermal recording. The isolated individual was filmed for 3 min within the cage, then was captured using a plastic tube open at one end (8 cm diameter and 17 cm high) and moved to the right top corner of the test apparatus (see description above). During this procedure, the apparatus was placed in such a way that it was in focus of the thermal camera mounted above. Then, after this handling and transfer procedure, which lasted on average around 30 s per individual, thermal recordings of subjects were continued in the absence of the experimenter for another 5 min. Four different thermal parameters were recorded, see details below.

In a second step, responses in thermal parameters were measured in individual of *sample B* (*N* = 54 individuals). Testing was repeatedly done, on postnatal days 39 and 49. However, the animals were not separated from their siblings during the night before. That is, we only recorded thermal videos after the animals were placed singly into the test apparatus for 5 min. Subjects were taken singly and in random order from their home cages containing sibling groups and were placed into the test apparatus in the same way as described above.

2.2.2.2. Analysis of infrared thermal data

Measures of peripheral body temperatures (from whole body and tail, including the eyes) were obtained from thermographic recordings with the software ResearchIR version 4.40.4.17 (FLIR System, Wilsonville, OR, USA).

Using recordings with a resolution of 30 frames per s, the maximum peripheral body temperature for each individual and of each frame was saved to file, i.e., 900 measurements per 30-s interval. For each 30-s interval, we only chose the 10% highest maximum temperatures recorded to select frames in which the maximum temperature usually corresponded to the temperature of the eyes or possibly (depending on the position of the animal) to the inner parts of the ears (Lecorps et al., 2019). Based on this selection procedure, we calculated the median of these 90 measurements.

For the quantification of tail temperature parameters, thermal data were imported into the program R version 3.5.3 (R Core Team, 2019) using the packages *Therimage* (Tattersall, 2019) and *exiftoolr* (O'Brian, 2019) and were processed using a script (written by one of the co-authors, CF) to sample one out of ten successive frames from the thermal videos. Manually, two points were defined on each side of the tail at the base in order to define the extremities of a first segment of pixels crossing the tail. A third point was then manually chosen in order to orientate and trace 10 successive parallel segments, each 1 pixel distant from the other toward the distal part of the tail. Maximum temperatures of each segment of pixels were then used to capture the tail temperature along 10 pixels (1st to 10th pixel distal to the base of the tail), corresponding to a length of around 7 mm. Note that the complete tail length of a mound building mouse of this age class (around 45 days) is approximately 6 cm; see (Lecorps et al., 2016) for a thermal image of a mouse including its tail using the same infrared thermal camera system (*Mus musculus*, similar in size to *M. spicilegus*). Five frames were analyzed using this method at each 5-s interval of the thermal video footage for individuals of *sample A* (to reach a high, but time-consuming accuracy), and at each 30-s interval for individuals of *sample B*. Median thermal values were calculated for each 5-s interval (*sample A*) or for each 30-s interval (*sample B*) for each tail position (1st, 5th and 10th pixel distal to tail base).

2.3. Ethics note

Animals were kept and treated according to accepted international standards (Vitale et al., 2018) and to the ethics and animal care guidelines of France, where the experiments were carried out. Experimental procedures were approved by the local authority for laboratory animal care and use (Comité d'Éthique en Expérimentation Animale Charles Darwin; #17922-2018112916198301) and by the institutional ethics committee (SBEA UP13). Individuals were bred for the purpose of a long-term study of which the presented results are part of; thus, animals were kept for further follow-up experiments.

2.4. Statistical analyses

Statistical analyses were done using the software R version 3.5.3 (R Core Team, 2019). First, we analyzed data from *sample A* (N = 5 individuals). For comparisons across time of thermal parameters measured during the thermal-response test, we used linear mixed-effects models LMM using the R package *nlme* (Pinheiro, Bates, DebRoy, Sarkar, & R Core Team, 2019). As the sample size was rather small, we used a permutation test (10,000 Monte-Carlo permutations, R package *pgirmess* (Giraudoux, 2018)) to calculate the P-values. The individual identity of subjects was used as a random factor to account for repeated measurements within the test.

We used a principal component analyses PCA to capture the information of the animals' (*sample B*, N = 54) behavioral responses in the open field (total distance covered, and the distance covered in the center of the arena) and in the novel object test (latency to approach/sniff, time spent exploring the novel object, and number of climbing events on the novel object) in a single score. This was done separately for the 2 days of testing T_1 (postnatal day 34) and T_2 (postnatal day 44). During T_1 and T_2, only the first component (first axis of PCA) which we interpreted as an 'exploration score' for later analysis, had an eigenvalue > 1 indicating that this component accounted for more variance than any of the original variables of the standardized data.

The exploration scores obtained by PCA were tested for repeatability across time (T_1, T_2) using intra-class correlation (Lessells & Boag, 1987). We used LMM-based calculations of intra-class repeatability (R_{ICC}) with the R package *rptR* (Stoffel, Nakagawa, & Schielzeth, 2017). *P*-value calculation was based on 10,000 Monte-Carlo permutations, with individual identity as a random factor. Furthermore, we considered cage identity as an additional (potentially confounding) random factor as litter sibling groups were housed in the same cage, thus sharing the same environment and maternal origin.

Using LMM-based calculations of intra-class repeatability, we also checked for individual-level (N = 54) and within-cage-level (N = 8) repeatabilities of the different peripheral temperature measures taken from animals of *sample B* during the 2 days of testing (postnatal days 39 and 49). Individual identity and cage identity were used as random factors, and the 30-s time interval after handling (10 levels, see Figs. 2, 3) and sex as fixed factors. This was done to account for the variance explained by these two fixed effects by removing it from the repeatability estimate (enhanced agreement repeatability (Stoffel et al., 2017)). Temperature data were standardized (scaled) within each day of testing to account for the animals' potentially different peripheral temperature levels on the different days of testing, e.g. due to age-dependent differences in fur cover.

We aimed to assign individuals with clear fast and slow exploration tendency for later analysis. Thus we discarded the central quartile (25%) of the distribution of exploration score, i.e., animals (13 out of 54 individuals of *sample B*) which were ambiguous with respect to exploration trait. By this procedure, 12.5% of the individuals above and 12.5% below the median exploration score were excluded from further considerations (Rangassamy et al., 2016). The remaining animals with exploration scores above this threshold interval were referred to as ‘fast explorers’ (*N* = 20), and individuals below this threshold interval as ‘slow explorers’ (*N* = 20). This procedure was done overall, i.e., across all individuals of the sample. Furthermore, due to the strong and significant differences in thermal response parameters among cages (i.e., across litter sibling groups; see Fig. A in the Suppl. Material), we also assigned an alternative, within-cage ranking of explorer types following the above-mentioned procedure. That is, for each cage, we assigned fast and slow explorers relative to their litter siblings and discarded ambiguous individuals whose exploration scores were within the 12.5 percentiles above or below the median exploration score of each cage (*N* = 8 cages; mean litter size of sibling groups within cages: 7.4, range: 4–9 juveniles/cage).

Based on data from *sample B*, we tested for differences between fast and slow explorers (fixed factor with 2 levels) for the different thermal parameter, using absolute peripheral temperatures (body and tail) as well as relative temperatures calculated as the deviation from the cage mean (details in Table 2). We always included the 2-way interaction between explorer type and time step (fixed factor with 10 levels) into the models to test for a potentially differential dynamics in temperature values over time in fast and in slow explorers. The models included two random intercept factors: individual identity and cage identity. In case of the analysis of absolute temperature values, we provided the *P*-values of the random factor ‘cage identity’ (by a likelihood ratio test, R package *lmerTest* (Kuznetsova, Brockhoff, & Christensen, 2017)) to assess whether among-cage differences significantly contributed to explaining the overall variance in the different thermal parameters. Analyses were carried out by multifactorial LMM (R package *lme4* (Bates, Maechler, Bolker, & Walker, 2015)). For the calculation of *P*-values, we applied corrected *F*-tests with Kenward-Roger approximation (Bolker et al., 2009).

In cases where parametric statistics were used, we verified that model residuals were well adjusted to a normal distribution by normal probability plots, and we checked for the homogeneity of variances by plotting the fitted values versus the residuals (Faraway, 2006).

3. RESULTS

3.1. Thermal-response test

We compared the values of different thermal parameters 3 min before and 3 min after subjects (*sample A*, $N = 5$) were transferred from the isolation cage into the apparatus (light gray bars in Fig. 1), taking into account a delay directly after the end of the transfer which was not considered for analysis (dark gray bars in Fig. 1). After a delay of 2 min, the maximum peripheral body temperature (mainly reflecting the temperature of the eyes) increased significantly by on average 0.65 °C (LMM with 10,000 Monte-Carlo permutations: $P < 0.001$; Fig. 1a). Already after a delay of 1 min, the maximum surface temperature close to the tail base (distance of 1 pixel: $P < 0.001$; Fig. 1b) and at more distal positions on the tail surface (5 pixels: $P < 0.001$, Fig. 1c; 10 pixels: $P < 0.001$, Fig. 1d) decreased significantly by on average 0.64 °C, 0.42 °C and 0.25 °C, respectively.

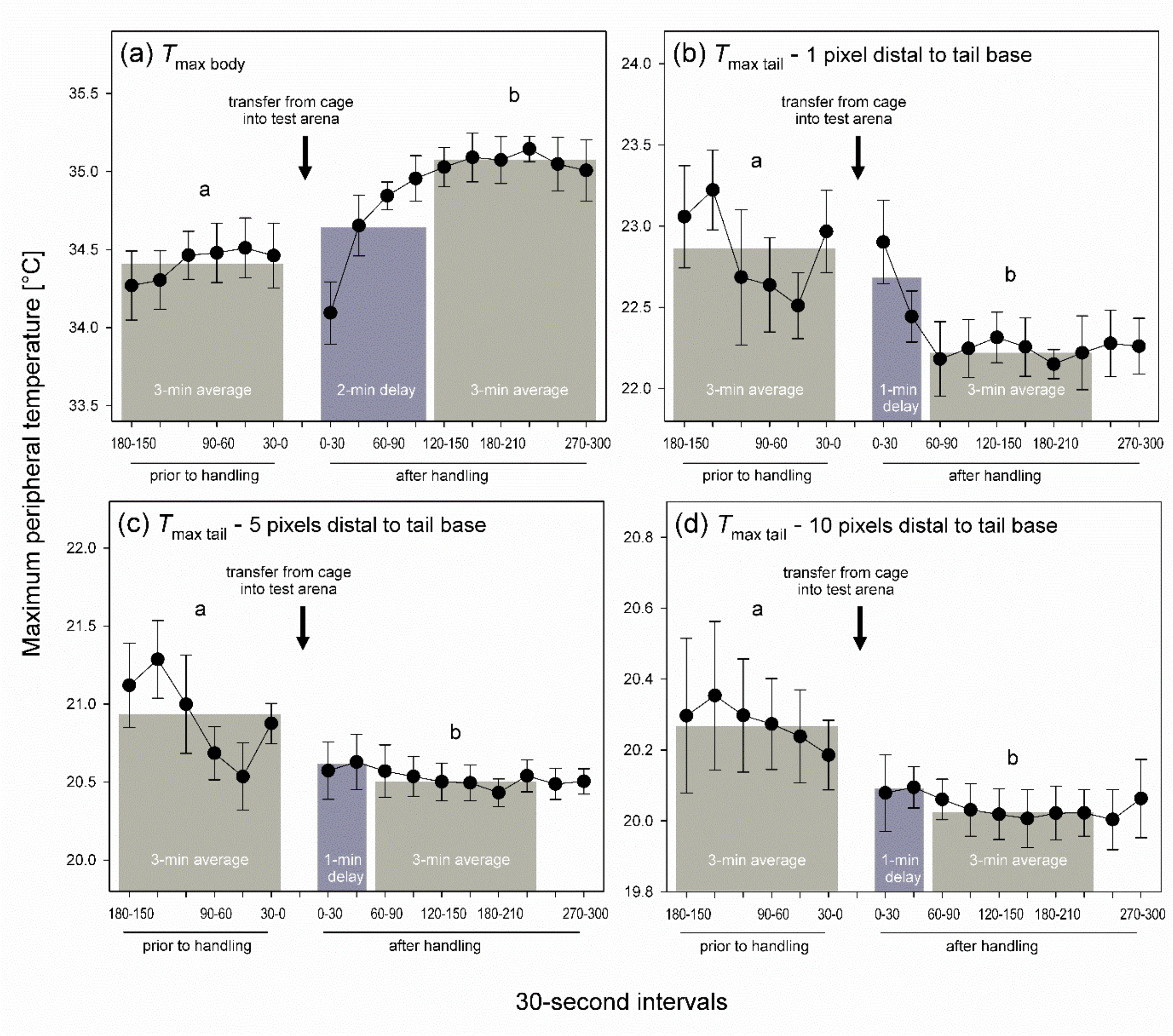

Figure 1. Time courses of 4 thermal parameters quantified during the validation of the thermal-response test by infrared thermography. The transfer from the home cage into the test arena (handling) lasted around 30 s. Each point represents the mean value ± SE of $N = 5$ individuals (*sample A*). Gray bars represent the average temperature values over the indicated time spans. Mind the differences in scaling in (a-d), and note that the maximum peripheral body temperature ($T_{\text{max body}}$) can be interpreted to mainly reflect the temperature of the eyes. Statistical comparisons were carried out between values of the 2 light gray bars by LMM with 10,000 Monte-Carlo permutations; significant differences are indicated by different letters.

3.2. Behavioral phenotyping

Before the thermal-response test carried out with animals of *sample B* (N = 54), subjects were behaviorally phenotyped by repeated open field and novel object tests. By a PCA, we extracted scores (the first axis of the PCAs for T_1 and T_2, respectively) based on the animals' behavioral responses in these tests.

During both times of testing, higher individual score values of these first PCA axes were associated with behaviors indicating high exploratory activity, such as a greater distance covered in the open field arena (loadings T_1: 0.402; loadings T_2: 0.511) and in the center of the open field (T_1: 0.430; T_2: 0.518), a shorter latency to approach and sniff the novel object (T_1: –0.329; T_2: –0.272), a longer time spent exploring it (T_1: 0.542; T_2: 0.414), and a higher number of climbing events on the novel object (T_1: 0.501; T_2: 0.475). The first axis of the PCA of T_1 explained 54.5% and the first axis of T_2 explained 49.2% of the total variance of the data.

Exploration scores were significantly repeatable across time at the individual level, i.e., animals which had higher scores during T_1 also tended to have higher scores during T_2 (intra-class correlation by LMM with 10,000 Monte-Carlo permutations: $R_{\mathrm{ICC}} = 0.349$, $N = 54$, $P = 0.003$). For further analysis, we assigned individuals with low and high exploration tendency, which was based on the averaged exploration scores obtained during T_1 and T_2. To this end, individuals with exploration scores of more than half a quartile below the median were referred to as 'slow' explorers and individuals with scores of more than half a quartile above the median were considered as 'fast' explorers. This procedure was applied (*a*) across all individuals of the sample, as well as (*b*) for all individuals within each cage ($N_{\mathrm{cages}} = 8$) to obtain a categorization of relatively fast and slow explorers with respect to litter siblings within the same cage. As a consequence, for both procedures (*a*, *b*) the sample sizes (*sample B*) used for later analyses were reduced from $N = 54$ to $N = 40$ individuals.

3.3. Consistent individual differences in thermal responses

All thermal parameters considered, the maximum peripheral body temperature, and the maximum surface tail temperatures measured at a distance of 1, 5 and 10 pixels to the tail base, were significantly repeatable across the two times of testing, on postnatal days 39 and 49 – although intra-class repeatabilities were moderate or low (Table 1). Cage-level repeatabilities were not statistically significant, but there were statistical tendencies for some temperature parameters (Table 1).

Table 1. Individual-level and cage-level repeatabilities of different measures of peripheral body and tail temperatures quantified by infrared thermography after subjects were handled and transferred into a novel environment. Note that the maximum peripheral body temperature ($T_{\text{max body}}$) can be interpreted to mainly reflect the temperature of the eyes. Experiments were carried out on postnatal days 39 and 49, with $N = 54$ individuals (*sample B*) kept in 8 cages (sibling groups). Analysis by LMM-based intra-class correlations with 10,000 Monte-Carlo permutations, including individual identity and cage identity as random factors. Significant effects are highlighted in bold.

	Individual-level repeatability			Cage-level repeatability		
	R_{ICC}	$N_{\text{individuals}}$	P	R_{ICC}	N_{cages}	P
$T_{\text{max body}}$	0.122	54	**< 0.001**	0.200	8	0.142
$T_{\text{max tail}}$ – 1st pixel	0.046	54	**< 0.001**	0.170	8	0.062
$T_{\text{max tail}}$ – 5th pixel	0.061	54	**< 0.001**	0.085	8	0.079
$T_{\text{max tail}}$ – 10th pixel	0.065	54	**< 0.001**	0.021	8	0.184

3.4. Differences in thermal responses in fast and slow explorers

3.4.1. Maximum peripheral body temperature

Overall differences: In accordance with the changes over time observed in *sample A* (*N* = 5; Fig. 1a), the measurements in *sample B* (*N* = 40) also showed significant increases in the maximum peripheral body temperature (mainly reflecting the temperature of the eyes) after handling and transfer into the apparatus (Fig. 2a; Table 1a). However, there were no significant differences between fast and slow explorers. Furthermore, the absence of a significant interaction between time and explorer type indicated that there were no significant time-specific differences in maximum body temperature (Table 2a).

There was a significant effect of between-cage variation in the maximum peripheral body temperatures (Table 2a). This was also evident with respect to some tail temperatures, see Table 2b, c (see also Fig. A in Suppl. Material). Thus, we decided to also investigate differences between fast and slow explorers within sibling groups kept in the same cage, respectively.

Within-cage differences: There was a significant interaction between the differences in exploration type within cage and the time of measuring (Table 2a). Post-hoc comparisons revealed a significantly lower relative maximum body temperature (deviation from the cage mean) in faster than in slower explorers only during the last time interval, 270-300 s after the end of the handling and transfer procedure (Fig. 2b).

There were no significant differences between males and females, neither with respect to absolute or relative (within-cage differences in) maximum body temperatures (Table 2a).

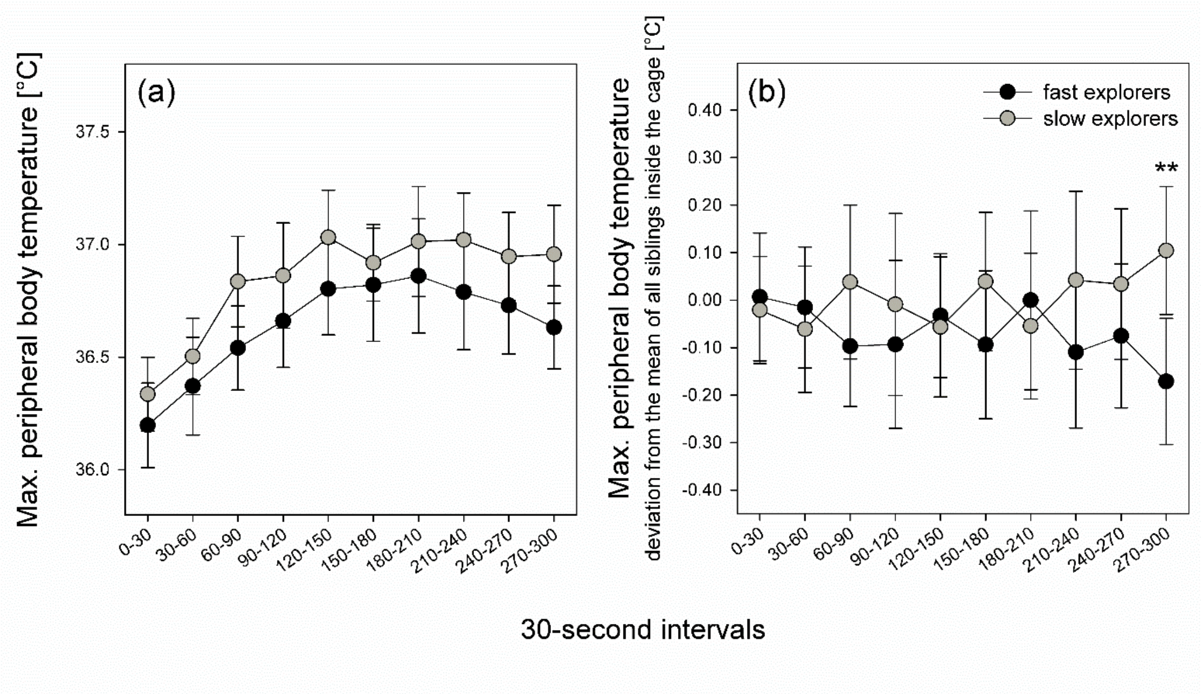

Figure 2. Time courses of the maximum peripheral body temperature quantified in the thermal-response test (sample B) during 5 min after handling; (a) absolute values and (b) relative values within cage calculated as the deviation from the cage mean. Note that the maximum peripheral body temperature can be interpreted to mainly reflect the temperatures of the eyes. Mean values ± $CI_{95\%}$ of fast explorers (black circles) and slow explorers (gray circles) are shown for each 30-s interval. (a) Differences between fast and slow explorers were not significant. (b) The interaction between time interval and behavioral explore type was significant, thus post-hoc comparisons were carried out for each interval; significant differences are given (**$P < 0.010$). See Table 2 for details on statistics.

3.4.2. Maximum surface temperature of the tail

Overall differences: Also here, in accordance with the pattern observed in *sample A* ($N = 5$; Fig. 1b-d), the measurements of tail temperatures in *sample B* ($N = 40$) showed significant decreases over time at the 3 different positions sampled (Fig. 3a-c; Table 2b-d).

Overall, at 1, 5 or 10 pixels distal to the tail base, the maximum tail surface temperatures were significantly lower in fast than in slow explorers, and the average differences in temperatures accounted 1.00 °C at the position closest to the tail base (pixel 1), 1.06 °C at the middle position (pixel 5), and 1.06 °C at the most distal position considered (pixel 10). There were no significant interactions between time interval and explorer type, indicating that the differences between fast and slow explorers were not time interval-specific (Table 2b-d).

Also here, there were indications for significant between-cage variation in temperature values, at least with respect to peripheral maximum tail temperatures measured at the positions of pixel 1 and 5 (Table 1b, c; see also Fig. A in Suppl. Material).

Within-cage differences: For all 3 positions, at the 1st, 5th and 10th pixel distal to the tail base, we found significant interactions between the differences in exploration type within cage and the time of measuring (Table 2b-d). Overall, the time course of the average differences in temperature deviations from the cage mean revealed more pronounced differences between fast and slow explorers mainly during the earlier time intervals after handling. This trend was increasingly evident with respect to measurements taken more distal from the tail base, confirmed by the pattern of statistically significant post-hoc comparisons between relatively fast and slow explorers at the 3 different tail positions (statistics in Fig. 3d-f).

Again, there were no significant differences between males and females, neither with respect to absolute or relative (within-cage) maximum tail temperatures (Table 2b-d).

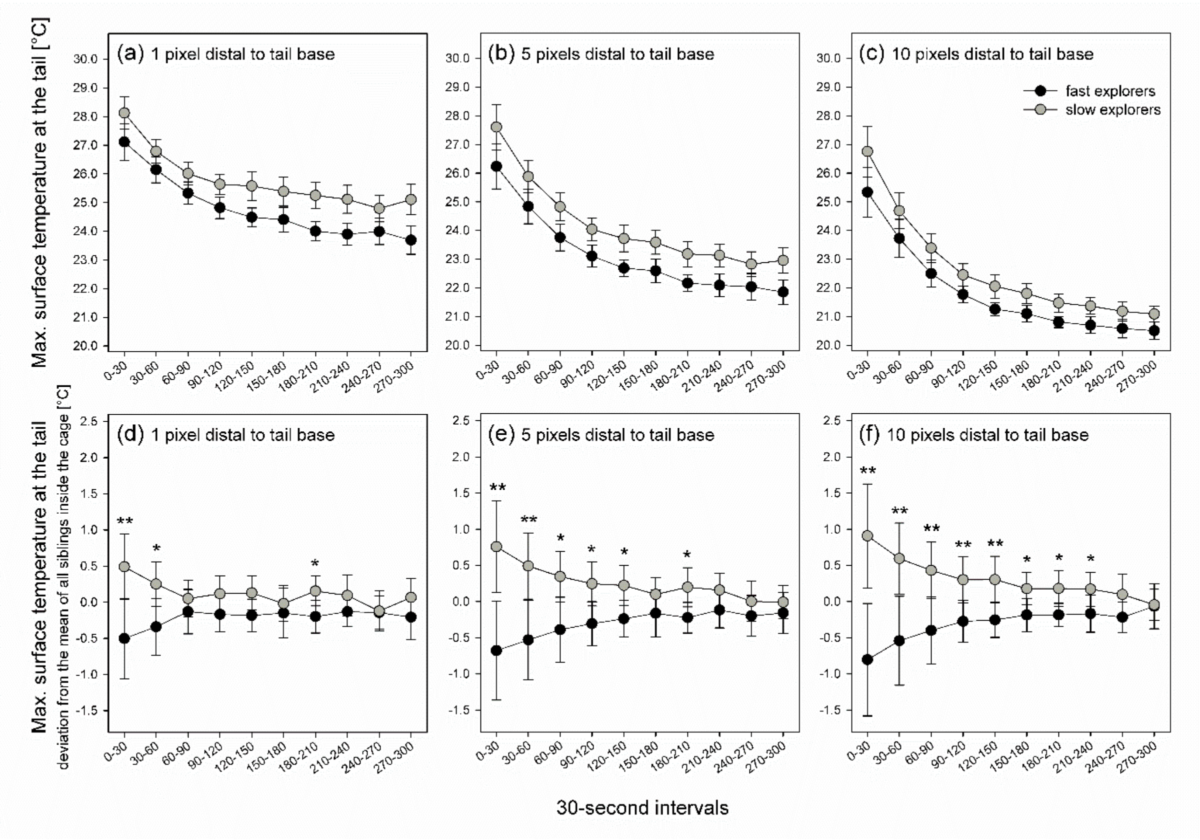

Figure 3. Time courses of the maximum surface tail temperatures quantified in the thermal-response test (*sample B*) during 5 min after handling; (a-c) absolute values and (d-f) relative values within cage, calculated as the deviation from the cage mean. Mean values ± $CI_{95\%}$ of fast explorers (black circles) and slow explorers (gray circles) are shown for each 30-s interval. (a-c) Thermal values of fast explorers were significantly lower than in slow explorers. (d-f) The interactions between time interval and behavioral explore type were significant, thus post-hoc comparisons were carried out for each interval; significant differences are given (*$P < 0.050$; **$P < 0.010$). See Table 2 for details on statistics.

Table 2. Effects of different explanatory variables on thermal parameters, (a) the maximum peripheral body temperature (mainly reflecting the temperature of the eyes) and (b-d) the maximum surface temperature of the tail measured 1, 5 or 10 pixels distal to the tail base (animals from *sample B*). Overall temperature differences as well as relative differences within cages, calculated as the deviation from the cage mean (*N* = 8 cages), were analyzed. The factor exploration tendency contained 2 levels, fast (*N* = 20) and slow (*N* = 20) explorers; the factor time step contained 10 levels as shown in Figs. 2, 3. Analysis by multifactorial LMMs including individual identity and cage identity as random factors. Significant effects are highlighted in bold.

		Overall differences in max. temperature			Relative differences in max. temperature (within cage)		
Dependent variable	Explanatory variables	*F*	*df*	*P*	*F*	*df*	*P*
(a) $T_{\text{max body}}$	Sex	0.090	1	0.329	1.502	1	0.229
	Exploration tendency *E*	0.646	1	0.427	0.685	1	0.415
	Time step *T*	26.223	9	**< 0.001**	0.101	9	0.999
	E × *T*	0.700	9	0.709	1.965	9	**0.043**
	[1]Cage identity	[2]16.125	1	**< 0.001**	--	--	--
(b) $T_{\text{max tail}}$ – 1st pixel	Sex	1.554	1	0.222	0.040	1	0.842
	Exploration tendency *E*	5.198	1	**0.029**	7.599	1	**0.010**
	Time step *T*	89.844	9	**< 0.001**	0.187	9	f0.995
	E × *T*	1.119	9	0.348	2.482	9	**0.009**
	[1]Cage identity	[2]22.158	1	**< 0.001**	--	--	--
(c) $T_{\text{max tail}}$ – 5th pixel	Sex	0.001	1	0.981	0.504	1	0.482
	Exploration tendency *E*	6.839	1	**0.013**	10.188	1	**0.003**
	Time step *T*	150.159	9	**< 0.001**	0.177	9	0.996
	E × *T*	0.363	9	0.952	4.284	9	**< 0.001**
	[1]Cage identity	[2]7.814	1	**0.005**	--	--	--
(d) $T_{\text{max tail}}$ – 10th pixel	Sex	0.097	1	0.758	1.511	1	0.227
	Exploration tendency *E*	12.529	1	**0.001**	10.401	1	**0.003**
	Time step *T*	203.558	9	**< 0.001**	0.131	9	0.999
	E × *T*	1.148	9	0.328	5.937	9	**< 0.001**
	[1]Cage identity	[2] < 0.001	1	> 0.999	--	--	--

[1] Cage identity was used as a random factor; the statistical significance of among-cage variance was calculated by likelihood-ratio test and thus [2] chi-square values are provided.

4. DISCUSSION

4.1. Thermal-response test

We successfully verified that our experimental design, consisting in a brief handling and transfer procedure of subjects from their cage into a small arena caused significant and quick changes in the maximum peripheral body and tail temperatures, assessed by infrared thermography (see Fig. 1). The observed decreases in tail surface temperatures corroborate previous findings in rodents using other kinds of brief challenges (Lecorps et al., 2016; Vianna & Carrive, 2005; Vianna & Carrive, 2012) and can be considered to result from the stimulation of the sympathetic nervous system causing vasoconstriction of tail arterio-venous anastomoses thus leading to a reduction of the cutaneous blood flow (Blessing, 2003; de Menezes, Ootsuka, & Blessing, 2009).

Thermal recordings of the visible body surface in adult mice indicate that the maximum temperature usually corresponds to the temperature of the eyes, mostly driven by the blood flow in the rich capillary beds surrounding the eyes (Valera et al., 2012). Furthermore, eye temperatures as measured in studies using infrared thermography have been shown to well reflect an animal’s body core temperature (Collins et al., 2018; Perez de Diego et al., 2013). Eye temperature typically shows short-term increases in stressful situations due to vasodilatory effect by sympathetic activation (Dai et al., 2015; Lecorps et al., 2016; Travain et al., 2015), thus explaining the increase in the maximum peripheral body temperature in response to handling observed in our study (but see (Gjendal et al., 2018)).

4.2. Association between exploration tendency and the animals' consistent thermal responses

We could successfully phenotype the animals based on their consistent individual differences in exploration tendency in repeated open field and novel object tests, confirming previous findings in the mound-building mouse (Rangassamy et al., 2015; Rangassamy et al., 2016), and in accordance with studies in other rodent species (Ferrari et al., 2013; Herde & Eccard, 2013; Mazza, Eccard, Zaccaroni, Jacob, & Dammhahn, 2018; Rödel & Meyer, 2011; Yuen, Pillay, Heinrichs, Schoepf, & Schradin, 2015). The results of our study also suggest the existence of consistent individual differences in sympathetic responder types as exemplified by the significantly repeatable peripheral maximum body and tail temperatures in response to the handling and transfer procedure carried out at different ages. The existence of consistencies in an individual's sympathetic (re)activity is part of the concept of coping style, referring to individual differences in both behavioral and physiological responses to environmental perturbations (Koolhaas et al., 1999; Koolhaas et al., 2010). This is for example supported by studies in calves *Bos taurus* (Van Reenen et al., 2005) and in wild Alpine marmots *Marmota marmota* (Ferrari et al., 2013) showing individual consistencies in breathing rates and/or in heart rates in response to stressful test situations. To date, there are only very few published studies suggesting consistent individual differences over time in sympathetic activity based on infrared thermographic measurements (Herborn et al., 2015), and to the best of our knowledge, ours is the first to show such individual consistencies in tail temperature responses to challenge. Repeatabilities in our study were low or moderate (see Table 1), which might, on the one hand, indicate a higher degree of intra-individual plasticity in sympathetic stress responses.

Returning to the main aim of the study, our findings provide clear support for a higher sympathetic reactivity in fast than in slow explorers by means of their consistently lower maximum tail surface temperatures measured repeatedly during 5 minutes following the handling and transfer procedure. Similar results have been obtained in studies using other markers of the animals' sympathetic reactivity. In alpine marmots, higher breathing rates and heart rates were found in animals who were more active in the open field test (Ferrari et al., 2013), and a study in Eastern chipmunks *Tamias striatus* found a comparatively higher increase in heart rates during a restraint test in animals phenotyped as fast explorers based on their open field behavior (Montiglio et al., 2012). Furthermore, great tit chicks *Parus major* from a selected line of fast explorers showed a stronger increase in breathing rates in response to isolation and handling stress than slow explorer individuals (Fucikova et al., 2009). However, we surprisingly did not find clear and significant differences between fast and slow explorers with respect to the maximum body surface temperature, which is usually interpreted to reflect the temperature of the eyes (Lecorps et al., 2019). We propose that studies using more direct measurements of eye temperatures might be done to further investigate potential differences between different personality types.

Although not part of the original focus of our study, we detected notable differences in tail temperatures among sibling groups housed in different cages (see Fig. A in the Suppl. Material). This was further supported by highly significant effects of cage identity in our statistical analyses when comparing fast and slow explorers (see Table 2), and by statistical tendencies pointing towards cage-level consistencies in peripheral body and tail temperatures (see Table 1). The potential underlying causes for such a comparatively lower within-cage but higher between-cage variance in peripheral body temperatures in response to our stress-inducing procedure could be manifold, ranging from cage-specific differences in huddling behavior thus affecting the overall peripheral temperature of all individuals per cage, to (epi)genetic effects driving similarities in stress responsiveness between litter siblings (Wilson, 2017; Young & Morrison, 1998). Thus, we decided to run additional analyses to explore possible associations between within-cage (and thus within-litter) differences in exploration tendency and within-cage differences in thermal responses to our handling and transfer procedure. By this approach, we intended to eliminate potential biases due to between-cage differences, which we suggest might be an easily overseen issue in thermographic experimentation with laboratory animals. Interestingly, even within sibling groups, relatively fast explorers showed significantly lower maximum temperatures on the tail surface than their littermates with slow exploration type. And most importantly, these within-cage analyses revealed more differential temporal response pattern between explorer types, with most pronounced differences during the first min after the handling and transfer procedure. Furthermore, the magnitude of these differences increased with increasing distance to the tail base, although we quantified surface temperatures only along the first 7 mm (i.e., 10 pixels) distal to the base. These findings might have applied implications for the design of thermographic studies in laboratory rodents.

4.3. Conclusions

The major strength of our approach using infrared thermography was the possibility to record the temporal dynamics of individuals' responses to a brief stress-inducing procedure without any further disturbance of the animals by the measurement itself. By such recordings, we could show consistently lower maximum tail surface temperatures in fast compared to slow explorers, indicating a higher sympathetic activity in the former. We emphasize the possibility to use thermal recordings of body surface temperatures in response to standard handling procedures as a quick and feasible method to phenotype the animals’ sympathetic (re)activity, and thus potentially their coping style (Ågren et al., 2009).

Furthermore, by our findings, we draw attention to differences among groups of animals (here: litter sibling groups) kept in different cages, as in our study such cage effects accounted for a significant part of the variance in peripheral body temperatures. Keeping laboratory rodents in groups is conform to animal welfare legislation, and for example in our case mixing different litters of juvenile wild-origin mound building mice was not feasible as this would have led to considerable perturbations within cages. Thus, we suggest the analysis of within-cage (or within-litter) differences, for example by calculating deviations from average values per cage, as a useful complementary approach to overcome potential biases driven by cage or litter effects (Lazic & Essioux, 2013; Rödel, Bautista, Roder, Gilbert, & Hudson, 2017).

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SUPPLEMENTARY MATERIAL

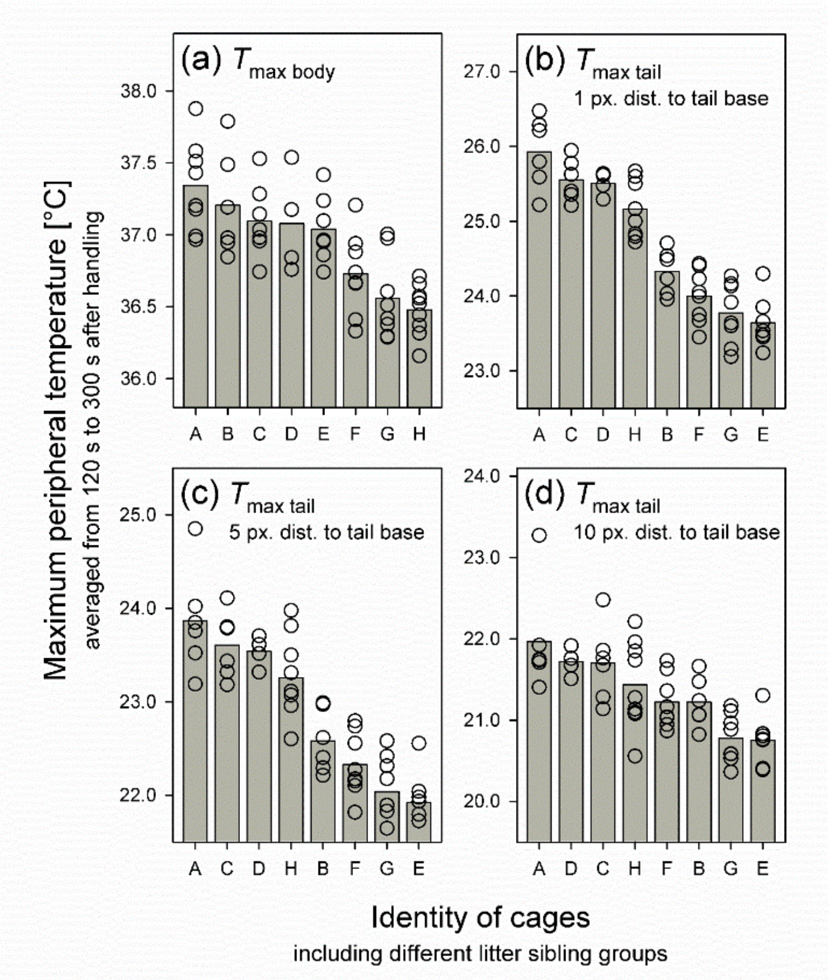

Figure A. Between-litter differences in (a) the maximum peripheral body temperature and (b-d) the maximum surface temperatures of the tail 1 pixel, 5 pixels and 10 pixel distal to the base, averaged over a period of 120 s to 300 s after handling and transfer of subjects to the apparatus (*sample B*, *N* = 54 individuals). Note that the maximum peripheral body temperature can be interpreted to mainly reflect the temperatures of the eyes. Open circles correspond to individual temperature values (averaged over two times of measurements during postnatal days 39 and 49) of litter siblings within each cage, and gray bars represent the average within cage. There were highly significant differences between the different litter sibling groups kept in different cages, with respect to all 4 thermal parameters (linear models with 10,000 Monte-Carlo permutations: all $P < 0.001$).

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5. General discussion

5.1. Overview of the results

The aim of this project was to contribute to the field of animal personality, by exploring one of its possible mechanisms: the individual differences in emotional reactions, in two rodents of wild origin (the house mouse and the mound building mouse). To this aim, I first assessed the short-term consistency over time of three components of an emotional reaction (behavior: **Chapter 1**; cognition: **Chapter 2**; physiology: **Chapter 3**). Second, I tested for associations between consistent individual differences in exploration tendency and these three emotional components (see 5.2 for a complement to the **Chapter 1**). Moreover, rather than determining discrete emotions, such as joy or fear, I used different non-invasive techniques to evaluate the valence (judgement bias: **Chapter 2**) and the arousal (pups' ultrasonic vocalizations: **Chapter 1**; peripheral temperature via infrared thermography: **Chapter 3**) of the emotional experience.

Overall, we found that the individual differences in each emotional component were consistent over time, although on short time spans. Also, the house mouse pups' isolation calls were consistent across three distinct situations, that is, these calls could be used to phenotype the pups from an early age (**Chapter 1**). As expected, we could also phenotype our individuals based on their exploration tendency in repeated open field and novel object tests. Finally, we showed that more explorative individuals judged an ambiguous situation more negatively (house mouse: **Chapter 2**) and showed a stronger sympathetic reactivity in response to a mild stressor, particularly when considering differences within sibling groups (mound-building mouse: **Chapter 3**).

5.2. Complement of Chapter 1: Associations between individual differences in isolation call rate and exploration tendency

As a supplementary question to **Chapter 1**, with a developmental perspective, I asked whether and how individual differences in pups' isolation calls were associated with individual differences in exploration tendency measured during early adulthood. To this aim, from the 320 pups tested for their individual profiles in isolation call rate, a subsample of 118 males were tested for their individual exploration tendency through repeated open field and novel object tests (postnatal days 41 and 71). The individual exploration scores were calculated by running a PCA based on the total distance covered in the open field, the latency to first sniff the object and the percentage of time spent exploring the object; behavioral variables were averaged between the two times of test beforehand. All pairwise associations were tested using linear mixed-effects models with litter identity as a random factor. More details about the experimental procedure and the calculation of the exploration score are given in the Material and Methods of **Chapter 2** (p. 72).

***Results 1**: Associations between individual differences in exploration scores and in isolation calls (rates and increases) during the 2-min initial isolation*. Exploration scores were not significantly associated with the average number of isolation calls emitted (i.e., isolation call rate) during the first 2 min of separation each day (LMM: $pseudoR^2 = 0.017$; $F_{1,113} = 1.921$, $p = 0.168$) or with the average initial increase in vocalization, as measured by individual regression slopes during the same time period ($pseudoR^2 = 0.009$; $F_{1,113} = 1.179$, $p = 0.280$).

Results 2*: Associations between individual differences in exploration score and changes in isolation call rate in response to treatments.* None of the pairwise associations between exploration scores and the immediate or 1-min delayed responses to the treatments were significant (Table 1). That is, the exploration tendency in early adulthood could not be predicted by early vocalization profiles.

Table 1 Associations between exploration scores and changes in isolation call rates in response to three different treatments ($N = 118$ males). Responses to treatments were calculated as the difference in isolation call rate between 60 s before and (a) 20 s or (b) the 2nd minute after treatment (see details in Fig. 1 of **Chapter 1**). Exploration scores were calculated by running a PCA based on three behavioral variables averaged between the two times of test: the total distance covered in the open field, the latency to first sniff the object and the percentage of time spent exploring the object. Analysis by linear mixed-effects models with litter identity as a random factor; none of the tested associations was statistically significant.

		Changes in call rate [*Repeated isolation treatment*]			Changes in call rate [*Nest treatment*]			Changes in call rate [*Male treatment*]		
		$pseudoR^2$	F	p	$pseudoR^2$	F	p	$pseudoR^2$	F	p
(a)	Exploration score	0.001	0.090	0.764	0.020	2.485	0.118	0.004	0.509	0.477
(b)	Exploration score	0.000	0.058	0.810	0.000	0.026	0.872	0.022	2.920	0.090

Discussion. We did not find significant associations between the early vocalization profiles and the individual differences in exploration tendency evaluated during early adulthood. Our results are consistent with another study conducted in the mound building mouse which did not report significant correlations between the number of calls emitted during a short separation from the nest and behavioral responses in repeated open field and novel object tests (Rangassamy, 2016).

However, the latter author reported that pups emitting a higher number of early isolation calls showed less positive social behaviors within their sibling group. Contradictory results have been reported in laboratory mice, in which high vocalizer pups were more sociable when tested as adults (Yoshizaki et al., 2017). Although both studies labeled the variables quantified as 'sociability', the methods applied highly differed. Rangassamy (2016) assessed the social interactions (number of initiations of contacts and approaches) within the sibling group, composed of 4 individuals, in their home cage. Yoshizaki et al. (2017) confronted a single individual to an unfamiliar conspecific in a three-chamber apparatus and recorded the time spent with it. Isolation calls are considered as an expression of an anxiety state driven by the separation from the nest and caregivers (Caruso et al., 2018; Hofer et al., 2002) and may be an expression of the PANIC/SADNESS system (Panksepp, 2005). We suggest that individuals showing a higher sensitivity of this latter system, which is triggered by social separation, would spend more time near their siblings. Hence, quantifying other behavioral variables, such as time spent huddling and inter-individual distance, would indicate whether the individuals might have been phenotyped as less sociable (i.e., they initiated less contacts: Rangassamy, 2016) because they spent more time in close proximity to their siblings. Moreover, pups emitting a higher number of vocalizations may trigger more care behaviors (e.g., licking) and interactions with the mother (Brouette-Lahlou, 1992; Hahn &

Lavooy, 2005; Noirot, 1969), leading to an increased adult sociability, also expressed towards unfamiliar conspecifics (Lassi & Tucci, 2017; Yoshizaki et al., 2017). Investigating sociality in different situations within the same study would help to understand the mechanisms underlying its associations with early isolation calls. Our next steps will be to evaluate the social interactions among siblings, shortly after weaning (postnatal days 28 and 35, Fig. G), using a method adapted from Rangassamy (2016), to potentially highlight similar associations in the house mouse than the ones reported in the mound building mouse (Rangassamy, 2016). We will also test for association between isolation call rates and responses to the emotional contagion test (Appendix 6.2., p.179).

Based on our results reported in **Chapter 2** (more explorative mice showed a more negative judgement bias; see also 5.4.3., p.161), we would have expected that the number of isolation calls would be positively associated with exploration tendency, as both are assumed to be driven by negative affective states. The absence of associations may be due to different mechanisms driving the emission of early isolation calls and exploration tendency. Also, it may be a consequence of the crucial changes (both behavioral, physiological and neuronal), that the young undergo before and around weaning (Biro & Stamps, 2008; Stamps & Groothuis, 2010a,b; Trillmich & Hudson, 2007; Trillmich et al., 2018).

The study of ultrasonic vocalizations outside courtship (Musolf et al., 2010) remain recent in subadults and adult mice (Ferhat et al., 2016; Heckman et al., 2016). Such vocalizations have been shown to be expressed during affiliative encounters in subadults (Panksepp et al., 2007), during territorial interactions (Portfors & Perkel, 2014) or in response to pain (Williams et al., 2008) and fear (Ko et al., 2005). It was also reported that adult male laboratory mice vocalize when exploring a novel environment (Chabout et al., 2012; Mun et al., 2015). The acoustic features of the ultrasonic calls also depend on the context of emission and, in particular, low-frequency calls may be emitted in more stressful situations (Mun et al., 2015). We suggest that quantifying the number and type of ultrasonic vocalizations (Heckman et al., 2016), during social and non-social test situations in subadults and adults, may considerably contribute to the study of the ontogeny of early isolation calls and their underlying emotional state.

In conclusion, we suggest that individual isolation call rates, showing a high consistency across time and situations, may reflect an early personality trait. Further studies should emphasize on the contexts in which personality traits and emotional behaviors are assessed in adults, as it could explain the discrepancies reported across studies, and focus on the associations between early and adulthood ultrasonic vocalizations. It could help to understand whether early vocalization profiles might predict individual differences in personality traits and coping ability when confronted to challenging social and non-social situations in adulthood, and might shed light on the mechanisms of such associations.

5.3. About the difficulty to use the elevated plus maze data to evaluate anxiety in the house mouse

It was initially planned to assess anxiety, through repeated elevated plus maze tests (EPM, at postnatal days 40 and 70; Fig. G), and to test for associations with the pups' isolation calls (**Chapter 1**) and the adults' judgement bias (**Chapter 2**). I finally decided to exclude the EPM test from analysis, hence to not assess these associations, for several reasons.

First, it was surprising that the percentage of time spent in the open arms and the number of entries in the open arms, two common variables evaluated in the EPM for anxiety in rodents (Carobrez & Bertoglio, 2005; Harro, 2018; Lecorps et al., 2016; Rangassamy et al., 2016; Rodgers & Cole, 1994), were not associated with any of the behavioral variables assessed in the open field and novel object tests. Yet, numerous studies reported that the behavior of rodents in these three standard tests, although the name given to the variables (exploration, boldness, anxiety, etc.) may differ between researchers, can form together a behavioral syndrome (e.g., mound-building mouse: Rangassamy, 2016; laboratory rat: Rödel & Meyer 2011; guinea pig: Guenther et al. 2014; house mouse: Lecorps et al., 2016). Typically, less anxious individuals in the EPM are also more active in the open field and explore more the novel objects. Moreover, the behavior in EPM was not significantly associated neither with the pups isolation call rates (**Chapter 1**) nor with judgement bias (**Chapter 2**), where we expected that more anxious individuals would judge more negatively the ambiguous cue (results not shown).

Second, the EPM is assumed to be aversive and to lead to unconditioned fear responses (Arabo et al., 2014; Carobrez & Bertoglio, 2005; Roy et al., 2009). Indeed, due to the fear of rodents for open spaces leading to thigmotaxis behavior (that is, animals remain close to vertical surfaces in a defensive purpose: Grossen & Kelly, 1972), it is expected that individuals will prefer the closed arms and avoid the open ones (Carobrez & Bertoglio, 2005; Pellow et al., 1985; Treit et al., 1993). When I assessed the overall time spent in open and closed arm during the 5 min of test, I found that the individuals spent on average more time in the open than in the closed arms (Table 2).

Table 2. Average time spent in open and closed arms of the elevated plus maze, over the total 5 min of the test, for (a) the first test session T[1] at postnatal day 40 and (b) the second test session T[2] at postnatal day 70 (*N* = 110 male house mice). The percentage of average time is also presented. 13 individuals, which repeatedly jumped out of the apparatus, were excluded. Description of the apparatus is given in Rangassamy et al. (2016). The individual was counted as being entered in the arm when at least half of its body crossed the entrance.

		Average (s)	% Average
(a) T^1	Time spent in open arms	134.6	45.1
	Time spent in closed arms	106.8	35.8
(b) T^2	Time spent in open arms	101.8	34.2
	Time spent in closed arms	112.1	35.5

Thus, instead of analyzing the overall 5 min of test, I performed a more detailed temporal analysis of the time spent in open and closed arms, for each minute, in a subsample of 67 individuals during the first test session (Table 3), as it has been advised by Carobrez & Bertoglio (2005).

Table 3. Repartition of the time spent in open and closed arms of the elevated plus maze during each minute of the first test session at postnatal day 40 (*N* = 67 male house mice). The average time and percentage of average time per minute are also presented. The trial lasted for 5 min. Description of the apparatus is given in Rangassamy et al. (2016). The individual was counted as being entered into an arm when at least half of its body crossed the entrance.

	Time of the test						
	1st min	2nd min	3rd min	4th min	5th min	Average	% Average
Time spent in open arms (s)	35.7	30.3	26.8	23.6	23.7	28.0	46.7
Time spent in closed arms (s)	15.1	19.9	20.7	23.7	23.0	20.5	34.1

I found that the mice exhibited an unexpected pattern of exploration in the EPM: a higher initial time spent in the open arms that decreased over time and an opposite pattern for the closed arms. The individuals did not show a clear preference for the closed arms.

Thus, our individuals did not exhibit the expected anxiety-related behavior which is typically displayed in such test (that is, an avoidance of the open arms; Pawlak et al., 2012; Rodgers & Johnson, 1995). In other studies, the arms of the EPM are most often illuminated with white lights, which may directly increase anxiety-related behavior (Albani et al., 2015; Griebel et al., 1993b; Hogg, 1996; Post et al., 2011). Furthermore, arms are either exposed with different light intensities with the open arms being brighter (Pereira et al., 2005) or with a similar illumination (Walf & Frye, 2007). Our experiment was conducted under red light conditions, to imitate the night period. Thus, we tested the individuals during their activity period, which might have led to higher arousal and risk-taking behavior. However, our EPM method has been previously applied with success by others within our group to assess individual differences in anxiety in the house mouse (Lecorps et al., 2016) and the mound-building mouse (Rangassamy et al., 2016). However, they did not report an analysis detailed per minute, which makes it difficult a direct comparison of these results with our current experiment.

Other researchers have conducted this type of minute-by-minute analysis and reported a similar or a higher exploration of the open arms at the beginning of the trial, before shifting towards a preference for the closed arms from the third minute on (Arabo et al., 2014; Carobrez & Bertoglio, 2005; Casarrubea et al., 2013). Anxiety may arise from the conflict between avoiding and exploring a threatening stimulus (Gray and MacNaughton, 2000; Ohl, 2003). Behavioral variables used to quantify anxiety should not be confounded with escape or avoidance responses; anxiety should persist when there is no possibility to escape from the threat (Ennaceur et al., 2010). In the EPM, the open arms constitute such a threat and typically should lead the animals to prefer the closed arms. However, it has been suggested that the time spent in open arms within the first two minutes could be motivated by curiosity towards the novelty of the situation (Carobrez & Bertoglio, 2005). From the third minute on, the approach/avoidance conflict has been suggested to lead to a shift in behavior and the closed arms are preferred (Carobrez & Bertoglio, 2005). Hence, anxiety due to the approach/avoidance conflict would arise only after a familiarization to the apparatus and the learning of its spatial configuration (Arabo et al., 2014; Rodgers & Shepherd, 1993; Roy, 2009). Some entries into the open arms within the first minutes might also be considered as attempts to escape from the maze (Arabo et al., 2014), which would be coherent with other studies reporting an increase in exploration of the open arms by individuals of a laboratory mouse strain selected for high anxiety levels (Brinks et al., 2007).

As an alternative to ‘forced exposure’ EPM, it has been suggested to allow the individuals to freely explore the maze from a familiar box connected to the extremity of a closed arm (Roy et al., 2009). Studies comparing ‘free’ to ‘forced exposure’ EPM reported that laboratory rats and mice avoided the open arms from the beginning of the trial (Arabo et al., 2014; Roy et al., 2009). Although it was argued that the anxiety state should decrease rapidly when there is a possibility to escape from the threat (Ennaceur et al., 2010), laboratory mice exposed to ‘free exposure’ EPM showed behaviors indicating an approach/avoidance conflict, such as a higher number of returns to the closed arms and risk-assessment behaviors, and the animals spent less time in the open arms during the total duration of the trial (Arabo et al., 2014).

However, in our EPM experiment, the individuals did not show any preference for the closed arms, even during the last minute of the trial (Table 3). Therefore, it is not possible to interpret their anxiety level based solely on the time spent in each type of arms. Indeed, spending more time in the open arms could result from low anxiety levels (Pellow et al., 1985; Post et al., 2011), but it could also be interpreted as attempts to escape due to high anxiety levels induced by the aversive situation (Roy et al., 2009). Other variables, as the number of feces, number of rearing in open arms (Arabo et al., 2014) or other risk assessment behaviors (Rodgers et. al., 1996; Sorregotti et al., 2013), may be integrated into the analysis. Such variables might allow us to clarify the intensity of the emotional state driving the individuals to spend more time in the aversive, open arms. Furthermore, the number of individuals jumping out of the apparatus is rarely reported (e.g., Frynta et al., 2018) and might be interpreted as a way to escape from the maze. In our experiment, 12% of the tested individual had to be excluded because they repeatedly jumped out of the apparatus within the first 3 min of testing, which is higher than what has been reported by others (e.g., Frynta et al., 2018).

Finally, it has been reported that individuals from the subspecies *Mus musculus domesticus* of wild origin, as we used in this thesis, were bolder (Frynta et al., 2018), more aggressive (Frynta et al., 2005) and exhibited a lower thigmotaxis (Hiadlovská et al., 2013) than the subspecies *Mus musculus musculus* of wild origin. We suggest that the EPM, at least in its 'forced exposure' configuration, might not be a suitable test to study anxiety in the house mouse of wild origin. Comparing individual differences in short-term thermal responses as a measure of anxiety, using infrared thermography, in 'free' and 'forced exposure' EPM within the same individuals might help clarifying the intensity of the emotional state experienced by house mice. In particular, the 'free exposure' EPM would avoid to induce a stress reaction by handling the individuals (as we reported in **Chapter 3**), which might have been a potential confounding effect in a previous study using infrared thermography to assess anxiety in a 'forced exposure' EPM (Lecorps et al., 2016).

5.4. Relationships between individual differences in emotions and personality

5.4.1. Consistency of emotional responses over time

The existence of a consistency over time of the emotional components (behavior, physiology and cognition) would support that differences in emotional systems may result in distinct personality traits. Indeed, as personality is considered stable over reasonably long periods of time, its causal mechanisms can be expected to also show a certain temporal consistency. Although we applied in this research project a correlational, and not a causal, approach, our first aim was to assess the consistency over time of each emotional component.

Individual differences in isolation call rate (**Chapter 1**) and judgement bias (**Chapter 2**) were highly repeatable (respectively, $R_{\mathrm{ICC}} = 0.577$ and $R_{\mathrm{ICC}} = 0.711$). However, the maximal peripheral temperatures showed only low to moderate individual-level repeatabilities in adults (body: $R_{\mathrm{ICC}} = 0.122$; tail 1st pixel: $R_{\mathrm{ICC}} = 0.046$; tail 5th pixel: $R_{\mathrm{ICC}} = 0.061$; tail 10th pixel: $R_{\mathrm{ICC}} = 0.065$; **Chapter 3**). We found a moderate repeatability in pups maximal peripheral body temperature ($R_{\mathrm{ICC}} = 0.315$; **Chapter 1**) but the measurements were done on three consecutive days whereas 10 days separated the two test sessions in **Chapter 3**, which may explain the difference in repeatability estimates. A meta-analysis pointed out that hormones levels (e.g., glucocorticoids, under the control of HPA axis) showed only low repeatability estimates compared to behavior (Holtmann et al., 2017). However, repeatability estimates of heart rate variability, controlled by the autonomic nervous system which also drives peripheral thermal changes, were reported to be moderate to high in humans (Schroeder et al., 2004) and domestic horses (Pérez Manrique et al., 2019). Further studies should investigate to what extent short-term changes in peripheral temperature may be consistent over time.

Consistent behavioral responses over time when animals are confronted to emotionally-challenging situations have been widely reported (Boissy & Erhard, 2014; Mazurek et al.,2011). However, personality traits may modulate such behavioral responses and explain why individuals react differently to an identical stimulus. For instance, in the same fear-eliciting test, some individuals may immobilize ('freezing' behavior) while others may increase their locomotor activity (Harro, 2018; Koolhaas et al., 2010). Yet, the tendency to express certain emotions is rarely considered as a trait in animals and it has been suggested that researchers should focus on the distinction between emotions as a trait or a state (de Vere & Kuczaj et al., 2016). For instance, anxiety can been seen as a trait (that is, a basal characteristic of an individual) or as a state (that is, the anxiety level experienced in a specific situation) (Lister, 1990). Individuals with a higher trait anxiety may also experience more intense states of anxiety in challenging situations (Spielberger et al., 1984). Therefore, evaluating the consistency over time of emotional responses may help to make the distinction between trait and state, as a trait should be stable across reasonable periods of time (Carter et al., 2013; Strelau, 2001).

Furthermore, numerous studies reported that environmental conditions can modify the expression of emotional reactions. For instance, sheep exposed to unpredictable and uncontrollable negative stimuli during a prolonged time period became more fearful (Destrez et al., 2012). Interestingly, sheep exposed to such living conditions were also found to react more strongly to emotional situations, both positively and negatively (Reefmann et al., 2012). Hence, when aiming to assess the consistency of emotional reactions, it is crucial to maintain stable environmental (e.g., housing) conditions and to take into consideration potentially unexpected changes between test sessions.

Studies on affective styles (that is, variation between individuals in the intensity and quality of emotional responses; Davidson, 1998) mainly focus on how individuals differ in their expression of emotions, when conducted in animals. However, individual differences in the regulation of emotions by secondary (via learning) and tertiary (via cognitive executive functions) processes might bring new insights into the study of personality traits (Montag & Panksepp, 2017; Pankepp, 2011; Paul et al., 2005; see an example in Braw et al., 2008).

5.4.2. Assessment of early emotional profiles using isolation calls and infrared thermography

In **Chapter 1**, we focused on isolation call rates as a way to behaviorally phenotype young individuals. Isolation calls also represent communicative signals of a negative emotional state (Granon et al., 2018). The participation of emotion-linked brain regions in the control of production of ultrasonic vocalizations (Hofer, 1996), the reduction of calling rate by anxiolytics drugs while anxiogenic drugs increase it (Simola & Granon, 2018; Wöhr & Gaalen, 2018; Wöhr et al., 2015), as well as the selection of rodent pups for high vocalizations leading to stronger emotional behavior during adulthood (Burgdorf et al., 2009; Zimmerberg et al., 2005) are some of the arguments that led to consider the isolation-induced vocalizations as “distress calls”, a behavioral measure of negative emotional reactions (Branchi et al., 2001; Granon et al., 2018; Panksepp, 2005; Wöhr & Schwarting, 2013).

Surprisingly, some of the pups did not emit any vocalization during at least one of the three days of test. It could be interesting to evaluate in more details what differentiates these individuals from the ones vocalizing. The absence of calls might be due to a low body mass, as the body temperature of lighter individuals decreases faster (Zepeda et al., 2018). Hence, calling might be too costly for pups with a low temperature. In our study (**Chapter 1**), we tested for potential effects of body mass and body temperature, assessed via infrared thermography, only on the changes in call rate in response to the treatments, but not on the initial isolation call rate, as it was not the main goal of the study. Therefore, we cannot exclude this potential explanation. Moreover, the maximal peripheral body temperature was quantified as a proxy of the internal core temperature, and not to measure an emotional reaction. A next step will be to conduct a more detailed analysis of the temperature on all the individuals instead of the subsample of 70 pups. In addition to the maximal peripheral temperature, the temperature of proximal and distal parts of the tail will be analyzed. Furthermore, a 30-s detailed temporal analysis of the body and tail peripheral temperatures, as in **Chapter 3**, may shed light on subtle temporal changes in the emotional reaction. Such analysis could help to understand why our pups reacted more strongly (by decreasing more their call rate) to the *nest* than to the *male treatment* during the 2nd min after treatment, and whether the *nest treatment* led to a more calming effect (**Chapter 1**). Moreover, a precise spectral analysis may also highlight the use of different call types depending on the situation (Caruso et al., 2018). For instance, harmonic isolation calls that increase during the first days of life may communicate a negative emotional state (Grimsley et al., 2011).

However, assessing emotional responses via the measurement of thermal responses is challenging in pups, as such responses may be strongly impacted by developmental changes. In adults, the maximal peripheral body temperature is usually related to eye temperature, and this latter parameter has been shown to increase in reaction to a stressor in adult rodents (e.g., in **Chapter 3**). In pups, during the early days of life before the eyes open, the maximal peripheral body temperature corresponds to either the pups' peripheral temperature in the ear or the neck (Maurer et al., 2015; Zepeda et al., 2018), the latter being related to the activity of brown adipose tissue that allows the thermogenesis by young pups confronted to cold challenges (Cannon & Nedergaard, 2004). Thus, it is crucial to not confound the measures of emotional reaction and of thermogenesis activity. In our experiment, the pups were subjected to a light cold challenge to improve the measurements taken by infrared thermography. The room temperature was maintained at 20.0 ± 2.0°C and the pups were placed on a heating plate kept at 20.0°C. Furthermore, we tested the pups between postnatal days 9 and 11, when they start acquiring the ability to keep a stable body temperature outside their nest (Zepeda et al., 2018). Thus, it is likely that the tested pups were not at the same stage of development, leading to considerable individual variation. As a consequence, the assessment of the tail temperature would prevent such confounding effect of thermogenesis activity. Moreover, the time window to assess the emotional reaction through both isolation calls and temperature on pups already capable to maintain their body temperature is very short. Our preliminary tests conducted in house mouse pups (not shown here but see similar results in Elwood & Keeling, 1982) indicated that the number of isolation calls drastically drops from postnatal day 13 on, when the eyes start to open.

Moreover, we showed in **Chapter 3** that handling led to a physiological stress reaction in adult mound-building mice. It has also been shown that handling can provoke the emission of ultrasonic vocalizations in laboratory mouse pups (Hennessy et al., 1980; Okon, 1970). In our tested pups, the assessment of both ultrasonic vocalizations and body temperature started as soon as the pups were placed on the heating plate, to minimize the separation time from mother and litter siblings. It is possible that the very first ultrasonic calls were due to both isolation from the nest and handling of the pups. For instance, Branchi and co-authors (1998) started the recording of the calls after a delay of 10 s following the transfer of the pup into the experimental apparatus. If the effect of handling on the vocalizations may disappear rapidly, the physiological reaction may last longer and mask the effect of the isolation per se.

Finally, similarly than in **Chapter 3** in which some results were found only when considering individual differences within sibling groups, inter-individual variation in early emotional profiles, both in physiology and behavior, may be better highlighted by applying the same approach in house mouse pups. Other studies have also successfully used this method to report individual differences in behavior and body temperature in pups (Hudson et al., 2015; Rödel et al., 2017; Zepeda et al., 2018).

5.4.3. Judgement bias test as a tool to assess the emotional state driving exploration tendency

The motivation to explore new environments and items is crucial for animals, to meet basic needs such as finding sexual partners or food, but also to seek out general information. From a psychological perspective, information seeking behavior may be motivated by curiosity, broadly defined as a “drive state for information” that stimulates learning (Kidd & Hayden, 2015). Yet, curiosity has been poorly studied in animals (Byrne, 2013), except in a comparative early work from Glickman and Sroges (1966) and by psychologists of the 20th century who built their theories based on studies in both human and non-human animals (e.g., Berlyne, 1966). Curiosity can also be considered as “a desire for new information aroused by novel, complex, or ambiguous stimuli” (Litman & Jimerson 2004). Such curiosity state that leads to exploration may be motivated by either positive or negative affective states. The *optimal arousal* model theorizes curiosity as a positive emotional experience, arguing that exploration aims to increase and maintain an optimal level of arousal (Berlyne, 1967; Hebb, 1955). In particular, when individuals are bored, i.e., under-aroused, they may explore to seek out novel or complex stimulations that will increase their level of arousal. Thus, the induction of curiosity is thought as inducing positive feelings of interest (Berlyne, 1967; Hebb, 1955; Leuba, 1955). On the contrary, the *curiosity-drive* theory suggests that exploration arises to reduce unpleasant feelings of uncertainty when confronted to complex, novel or ambiguous information in the environment (Berlyne, 1950, 1955). Once the uncertainty is diminished or suppressed, curiosity is reduced and exploration should stop. The reduction of curiosity is here seen as rewarding.

Litman (2005, 2007) proposed a theory to combine both induction and reduction of curiosity as a motivation for exploration, suggesting that both were rewarding: the Interest/Deprivation (I/D) model of curiosity. The author defined two types of curiosity, elicited depending on the situation. On the one hand, the *interest* (I-type) curiosity may be aroused when individuals are confronted to an opportunity to acquire new information, inducing positive feelings of interest with a rewarding value. On the other hand, the *deprivation* (D-type) curiosity would arise from uncomfortable feelings due to uncertainty in the environment or lacking information, the gain of new information would have a rewarding value by decreasing the negative affective state. Others have also reported that exploring to reduce uncertainty or ambiguity has a rewarding value (Anselme, 2010; Franks et al., 2013; Inglis, 2000).

Based on the conceptual approach involving the primary-process brain emotion systems as foundational for personality (David & Panksepp, 2011), we expected that individual exploration tendency was strongly linked with an individual's emotional profile. We then used a judgement bias test in **Chapter 2** to better understand the valence of the emotional state underlying exploration tendency. Our results showed that more explorative house mice judged ambiguous information (represented by an ambiguous spatial cue) more negatively. According to the theory presented above (the I/D model of curiosity), the behavioral responses in repeated open field, novel object and judgement bias tests might be driven by D-type curiosity (Litman, 2005, 2007). In the standard tests we applied, the novelty of the environment and of the object may have induced negative affective states leading to an increase in exploratory behavior (greater distance covered in the open field, shorter latency to approach and to sniff the object, and more time exploring it). In the judgement bias test, this would also be consistent with D-type curiosity, activated by the confrontation with the novel, ambiguous stimulus. In such uncertain situation, the individual would be driven by D-type curiosity to obtain the lacking information by exploring its environment. This would lead to an increased latency to consume the ambiguous reward, interpreted as a pessimistic bias, as individuals would spend more time exploring their environment, to reduce their negative affective state before consuming. Hence, the negative judgement bias would be representative of a negative emotional state – but see the discussion of **Chapter 2** for an alternative explanation regarding the behavioral inflexibility of more explorative individuals.

In humans, scaling systems have been created to assess individual differences in these two types of curiosity (e.g., Day, 1971; Naylor, 1981), considering it as a personality trait. For Litman and Jimerson (2004), following the theory of Loewenstein (1994), individuals exhibit consistent differences in experiencing curiosity as a ‘feeling of interest’ (CFI, based on the I-type curiosity) or as a ‘feeling of deprivation’ (CFD, based on the D-type curiosity) when confronted to stimuli that can evoke curiosity. This would be consistent with the high repeatability we reported in individual differences in exploration tendency ($R_{\text{ICC}} = 0.511$) and judgement bias ($R_{\text{ICC}} = 0.711$) and which might both be supported by curiosity as a ‘feeling of deprivation’ (**Chapter 2**). Moreover, searching for associations with the personality traits anxiety, depression and anger, Litman and Jimerson (2004) reported that the tendency to express D-type curiosity was associated, although moderately, with a negative affectivity.

The proximal mechanisms underlying curiosity became more evident by recent advances in neuroscience reporting associations between curiosity-driven behaviors and the reward system (Kidd & Hayden, 2015). Two studies investigated, through functional MRI in humans, the neural correlates of curiosity as a negative state due to uncertainty or a gap in knowledge, according to the psychological theory of Loewenstein (1994; similar to the D-type curiosity of Litman, 2005). Jepma and co-authors (2012) presented ambiguous (blurry) visual stimuli to their subjects before presenting clear, non-ambiguous pictures. They reported that curiosity, induced by the presentation of blurry images, stimulated two brain regions usually activated during arousal and aversive conditions such as conflict (anterior insula and anterior cingulate cortex). The presentation of the clear images activated the striatum circuits, associated with rewarding processes. Their results support the theory that the deprivation-type curiosity, which typically occurs when the individual is confronted to an ambiguous stimulation, leads to negative affective states whose relieving is rewarding (Litman, 2005). Kang and co-authors (2009) found that self-reports of curiosity while reading trivia questions were associated with the activation of caudate regions (caudate nucleus and inferior frontal gyrus) that are involved in the anticipation of a reward. They also found that subjects were willing to use resources (a token or waiting time) to receive the answer to the questions they were more curious about, i.e., when they were more uncertain about it, highlighting the rewarding value of the acquisition of missing information. This latter result was confirmed by another study which reported that a high level of curiosity activated the brain dopaminergic circuits (midbrain and nucleus accumbens; Gruber et al., 2014). Although the neural correlates of curiosity remain to study in non-human animals, recent progresses in the application of functional MRI in awake and unrestrained dogs (Andics, 2014, 2018; Thompkins et al., 2016) might bring new opportunities to the field and would help to disentangle the associations between individual differences in I- and D-type curiosity and exploration tendency.

Finally, the positive association we found between exploration tendency and negative judgement bias (**Chapter 2**) might be due to a negative mood (i.e., the sum of short-term emotional states) of our animals. For instance, the housing conditions imposed by captivity may have led to boredom, i.e., "a negative [emotional] state induced by barren conditions that causes an increased, generalized interest in diverse stimuli" (Meagher et al., 2017). Boredom can arise from a deprived, i.e., spatially and/or temporally monotonous, environment (Burn, 2017; Wemelsfelder, 2005). When bored, individuals may experience aversive suboptimal levels of arousal (Burn, 2017) and new stimulations will induce exploratory behaviors (see the *optimal arousal* model of curiosity described above: Berlyne, 1960, 1967; Hebb, 1955). For instance, sheep may experience boredom as a consequence from an invariable and highly predictable environment (Veissier et al., 2009). Bored minks *Neovison vison*, due to deprived raising conditions, engaged in interactions with all kinds of stimuli, both novel and familiar (Meagher & Mason, 2012). Despite the presence of cardboard rolls and nest material as enrichment in the housing cages of our house mice, their environment was kept stable and predictable to avoid a modification of the affective state that could bias their responses in the different experiments conducted. Some individuals may have experienced boredom and, when given the chance to interact with new environment (open field test) and objects (novel object test), we suggest that this would most probably lead to more exploratory behaviors, driven by a curiosity feeling to increase their arousal level and end boredom (Berlyne, 1960, 1967; Lilley et al., 2017). When tested for their judgement bias, bored individuals might have negative expectations about future outcomes, hence exhibiting a negative judgement bias

(Burn, 2017). Evaluating the behavior of the animals in their home cage might increase our understanding of their mood, as more explorative individuals might exhibit more abnormal or stereotypic behaviors or, on the contrary, a prolonged inactivity when they are bored (Burn, 2017; Wemelsfelder, 1984, 2005).

It has also been recently suggested that free-choice exploration paradigms, allowing the individuals to retreat to safe or familiar places and/or explore voluntarily the devices, would avoid some issues when interpreting exploratory behavior in forced-choice tests (Franks, 2018). Individuals in a good welfare state should evaluate their environment more positively, thus voluntarily exploring it more, in search for new cognitive stimulations (Franks, 2018). For instance, as an alternative to the forced-choice open field test used in this thesis, a 'free exploration' open field may be applied, in which a safe area is connected to the arena and the individual is free to enter and explore the apparatus (Fonio et al., 2009; Frynta et al., 2018; Griebel, 1993a; Perals et al., 2017). Studies in laboratory rats (Franks et al., 2013) and laboratory mice (Novak et al., 2015), using an 8-arm radial maze with positive (safe and/or with food) arms, also showed that the exploration tendency in such apparatus may be associated with a more positive affective state. Further studies should investigate which personality traits may facilitate boredom and whether house mice of wild origin are more prone to it in standard laboratory conditions than laboratory mice (Latham & Mason, 2004; Meagher, 2019).

5.4.4. The use of infrared thermography to investigate individual emotional profiles underlying personality traits

The usefulness of infrared thermography to remotely assess emotional reactions is increasingly recognized in human and non-human animals (Clay-Warner & Robison, 2015; Pereira et al., 2018; Stewart et al., 2005; Topalidou & Ali, 2017). This method allows the measurement, using a specific camera, of infrared radiations due to the animals' body heat loss at the surface of their skin. Short-term changes in the peripheral temperature reflect variations of the cutaneous blood flow (i.e., by vasoconstriction or vasodilatation), under the control of the sympathetic nervous system (Vianna & Carrive, 2012; von Holst, 1998). One of the main reasons for the recent development of that technique is its non-invasive aspect, as the temperature can be assessed remotely without restraining the individual or implementing telemetry devices.

In **Chapter 3**, we demonstrated that infrared thermography can be used to assess short-term thermal changes in response to a brief handling, a procedure commonly used in laboratory conditions. The responses we reported (decrease in tail surface temperature and increase in the maximal peripheral body temperature most probably corresponding to the eyes) in our tested mound-building mice were consistent with previous studies in the laboratory rat (Ågren et al., 2009; Vianna & Carrive, 2005), the laboratory mouse (Miyazono et al., 2018) and the wild-origin house mouse (Lecorps et al., 2016) confronted to stressful or fearful situations. In other species, an increase in eye temperature has also been reported after the exposition to a negative stimulus (e.g., in horses: Dai et al., 2015; in calves *Bos taurus*: Lecorps et al., 2018a; Stewart et al., 2010; in dogs: Rigterink et al., 2018; Travain et al., 2015). However, the time course of changes in eye temperature requires a detailed analysis, as a decrease may precede the usually reported increase (Edgar et al., 2013; Stewart et al., 2008).

Such decrease can also been observed in the Fig. 1 of **Chapter 3** (p.105), also we did not analyze it. This initial drop in eye temperature might be due to a vasoconstriction in the posterior border of the eyelid and the lacrimal caruncle, as a result of the sympathetic nervous system reactivity (Stewart et al., 2008). Moreover, the localization of the decrease in peripheral body part temperatures, similar to the drop in tail temperature in laboratory rats and mice, is species-specific. For instance, after a confrontation with a negative event, cows showed a drop in nasal temperature (Proctor & Carder, 2016). Dogs (Riemer et al., 2016) and rabbits *Oryctolagus cuniculus* (Ludwig et al., 2007) showed a decrease in the ear temperature and chickens *Gallus domesticus* in the comb temperature (Edgar et al., 2013).

Although infrared thermography has mainly been applied to investigate negative emotional reactions after a fearful or painful stimulus, some recent studies investigated whether this technique might be useful to distinguish the valence of the emotional reaction (see also our side project in the Appendix 6.3., p.182). Studies first reported similar thermal changes in response to positive stimuli than after a negative event, as reported above. In cows, a drop in nasal temperature was reported after eating a favored food (Proctor and Carder, 2016) or being stroked in their preferred body part (Proctor and Carder, 2015). Anticipation and consumption of a positive food reward also led to a decrease in the comb of chickens (Moe et al., 2012). In dogs, the eye temperature increased after receiving a food treat (Travain et al., 2016). However, contradictory results were found in sheep: a positive brushing induced an increase in nasal temperature (Tamioso et al., 2017) whereas a similar gentle touch (stroking) was followed by a decrease in nose and eye temperature (Cyprinus et al., 2017).

It has been suggested that drops in the temperature of peripheral body parts may only be due to a high arousal emotional state induced by a stimulus of high intensity, independently from its positive or negative valence (e.g., humans: Kosonogov et al., 2017; cows: Stewart et al., 2010; laboratory rat: Vianna & Carrive, 2005). Yet, positive stimuli of low (Proctor and Carder, 2015) and high (Proctor and Carder, 2016) intensity both provoked a decrease in cows' nasal temperature. Further studies with varying emotional arousal and valence are needed, although it remains difficult to find adequate stimuli to separate valence from arousal. For instance, male common marmosets showed distinct thermal responses after the confrontation with positive and negative stimuli but the positive stimuli were assumed to have induced a low arousal, relaxed emotional state while the negative ones may have led to a higher arousal (Ermatinger et al., 2019).

More detailed analysis may also help to distinguish between positive and negative emotions when using infrared thermography. In a study on several species of monkeys and apes, the increase of the upper lip temperature was associated with the induction of a negative emotional state whereas a decrease in nose temperature was related with a positive emotional state (Chotard et al., 2018). In humans, distinct patterns of temperature related to specific emotions have been described (joy, disgust, anger, fear and sadness: Cruz-Albarran et al., 2017; happiness and sadness: Goulart et al., 2019b; disgust, fear, happiness, sadness and surprise: Goulart et al., 2019a) and may be used to diagnose emotions (Cruz-Albarran et al., 2017; Goulart et al., 2019a; Fu & Frasson, 2016). Hence, analyzing detailed thermal changes in animals' faces might help to determine distinct thermal patterns to differentiate positive and negative emotions.

Coming back to the main goal of my thesis, we demonstrated in **Chapter 3** that specific short-term thermal responses were associated with two distinct exploration types. That is, fast explorers had a higher sympathetic reactivity than slow explorers, as showed by their consistently lower maximum tail surface temperature during 5 minutes following the handling procedure. However, it might be difficult to infer their affective, internal state from the physiological component alone. For instance, in humans, some individuals did not verbally reported changes in their subjective feeling after the exposure to negative stimuli, although changes in physiology were measured (e.g., skin conductance, heart rate: Stone & Nielson, 2001).

We suggest that associating the evaluation of personality traits with individual thermal profiles would help to interpret thermal reactions. Such relationships between individual variation in behavior and physiology have been the focus of the study of coping styles, investigating the neuroendocrinological mechanisms at the origin of behavioral variation in response to stress challenges (Koolhaas et al., 2010). However, researchers in that field consider that individual variation in coping styles (that is, the behavioral reaction per se) is not induced by changes in the hypothalamic pituitary adrenocortical (HPA) axis or sympathetic-adrenomedullary (SAM) system (de Boer et al., 2017). These physiological changes, such as changes in the peripheral temperature, would not be a consequence of emotional arousal but rather of the physical activity associated with the different coping styles (de Boer et al., 2017). Indeed, physical activity has the potential to critically affect measurements via infrared thermography (Fukuzawa et al., 2016; Kano et al., 2016; Vianna & Carrive, 2005; see also Pereira et al., 2018 for the assessment of activity patterns in an open field using infrared thermography). Yet, several studies applying infrared thermography to investigate peripheral thermal changes, under the control of the SAM system and in response to negative or positive stimuli, excluded the general motor activity as a potential confounding effect of the thermal changes observed (Boileau et al., 2019; Chotard et al., 2018; Ermatinger et al., 2019; Travain et al., 2015). While controlling for physical activity, other parameters under the control of the SAM system might also be evaluated to complement thermal measurements (e.g., eye white exposure: Sandem et al., 2002).

Integrating the evaluation of personality traits when assessing peripheral thermal responses might shed light on the discrepancies across studies, allowing the description of specific thermal profiles depending on the personality type but also reducing the unexplained variation, hence increasing the robustness and reliability of the findings. So far, it has been shown that more extravert dogs showed higher pain scores and a greater right eye temperature, which might be explained by a dominance of the right hemisphere in processing and expressing emotional responses, in particular during painful or negative stimuli (Lush & Ijichi, 2018). Personality differences might also be predictive of stress sensitivity, assessed through eye temperature in cats (Foster & Ijichi, 2017). Finally, calves *Bos taurus* phenotyped as more fearful and pessimistic showed a higher eye temperature after a short transportation challenge (Lecorps et al., 2018a).

Moreover, it is neScessary to investigate associations between physiological responses and personality traits in various situations, to study more diverse emotions (e.g., play or joy; Ahloy-Dallaire et al., 2018) and capture the whole emotional lives of animals (de Vere & Kuczaj, 2016; Ha & Campion, 2019). For example, infrared thermography has been used to assess peripheral thermal reactions during agonistic, negative social encounters in the domestic pig (Boileau et al., 2019), but positive situations might also be studied (e.g., during play in piglets: Held & Špinka, 2011; Newberry et al., 1988), in association with, for instance, vocalizations (Friel et al., 2019). We suggest that it might allow to categorize specific thermal patterns for different emotions of varying valence and arousal, similar to the heat facial patterns described in humans (Cruz-Albarran et al., 2017; Goulart et al., 2019a,b).

In turn, such thermal profiles, which would have been assessed in a variety of situations, might be applied to better interpret personality traits. For instance, emotions driving exploration tendency might not be positive in all situations, as discussed in section

5.4.3. (p.161) and in **Chapter 2** (p.72). We suggest that comparing peripheral thermal responses in exploration and judgement bias tests might clarify whether negative emotions were driving behavioral responses during these tests. We filmed both experiments with an infrared thermal camera, thus such analysis will be conducted in a further step. Whether exploration tendency and judgement bias were driven by negative emotions (e.g., due to deprivation-type curiosity: Litman & Jimerson, 2004), we hypothesize that more explorative individuals should display (*i*) stronger thermal changes (i.e., decreases in tail temperature and increases in eye temperature) when confronted to the ambiguous cue than during the training days, and (*ii*) that such individuals should show similar patterns of thermal responses during exploration and judgement bias tests. On the contrary, whether the association we reported was related to a greater inflexibility of higher explorative individuals, these individuals should show (*i*) similar thermal changes during the days of training and testing for judgement bias and (*ii*) different patterns of thermal responses during exploration and judgement bias tests. This latter hypothesize might also challenge the ability of judgement bias tests to detect changes in emotional states.

5.4.5. Emotions are multimodal: associating several components to improve our understanding of emotions and personality

The foremost goal of emotion research in animals is to be able to infer the internal, subjective state of the individual. It is particularly important as well-being is not only the absence of prolonged negative emotions but also the presence of positive ones (Désiré et al., 2002; Fraser, 1995, 2009), leading to the concept of a 'life worth living' (Wathes 2010; Webster 2016). Therefore, it is crucial to develop quantifiable and reliable cues based on the different components constitutive of an emotional reaction: behavior, physiology and cognition (Désiré et al., 2002; Mendl et al., 2010; Panksepp, 1998).

The interpretation of the affective state of an individual based on a single emotional component is rarely straightforward. Therefore, we may reach a far better understanding of the animals' emotional life by combining measurements from different components (e.g., risk-assessment behaviors, cortisol concentration and attention bias) and/or measuring different variables within each component (e.g., heart rate, cortisol concentration and peripheral temperature within the physiological component) (Baciadonna, 2017; Briefer et al., 2015; de Vere & Kuczaj, 2016; Paul et al., 2005). In particular, the same behavioral or physiological variable can be found to be involved in various emotional reactions (Ede et al., 2019; Kreibig, 2010; Paul et al., 2005). For instance, an increase in plasma concentrations of corticosteroids can occur after the confrontation with a negative stimulus (Cockrem, 2013) or when expecting a positive event (sexual partner: Colborn et al., 1991). Similarly, a decrease in general activity might be due to high fear levels (e.g., freezing) or to a lack of motivation to explore (Guesgen & Bench, 2017). By combining different emotional components, and various variables within each one, we might be able to describe distinct patterns that could allow us to diagnose emotions in animals. In this context, integrating the study of individual differences is essential to understand why and how each individual perceives, interprets and reacts differently to a same situation, leading to various emotional responses.

It has been suggested that integrating the individuality into the study of emotions might also help to dissociate different mechanisms and systems involved in emotional reactions (Paul et al., 2005). This is consistent with the theory developed by Panksepp and co-workers: individual differences in primary emotional systems form the basis of individual differences in personality (Montag & Panksepp, 2017). In this perspective, each emotional system organizes, by activating or inhibiting, various behavioral and autonomic-hormonal changes (Panksepp, 1998). Hence, we should find coherent patterns of behavioral, physiological, cognitive and neuronal changes characteristic of specific, short-term emotions. Evaluating the consistency over time of such patterns might also broaden our understanding of animal personality by taking into account more than behavioral changes.

5.5. General conclusion

This research project aimed to assess the consistency over time of behavioral, cognitive and physiological emotional responses and their associations with personality traits, although we focused on exploration tendency. We could successfully phenotype our individuals according to their emotional characteristics (isolation call rate: **Chapter 1**; judgement bias: **Chapter 2**; peripheral thermal changes: **Chapter 3**) and to their exploration tendency (assessed in open field and novel object tests). Although the pups' early emotional profiles were not related to adults' exploration tendency, they might constitute an appropriate tool to phenotype the young, before the personality is stable. We also showed that more explorative individuals judged an ambiguous situation more negatively (house mouse: **Chapter 2**) and had a stronger decrease in tail temperature, indicative of a higher sympathetic reactivity (mound-building mouse: **Chapter 3**).

Further research in emotions would gain by focusing on the individuality, in close relationship with the individual's developmental and current environment. Evaluating the relationships between emotions and personality through the lifespan of the individuals would also increase our understanding of the emergence and development of personality. A causal approach may be considered, for instance by modifying the postnatal environment (e.g., by providing cognitive enrichment) at different age classes and assessing the potential effects in emotional responses and personality traits later in life (Chapillon et al., 2002). Furthermore, the “if-then” approach of personality from human psychology (Mischel, 2004; Mischel & Shoda, 1995) theorizes that personality may be expressed differently depending on the situation: individuals should behave consistently when confronted to similar context but they may react differently in different situations. That is, an individual should not show more exploratory behaviors in all situations, but only under certain circumstances. This approach has been rarely applied to animals (see an example in Franks et al., 2012) but it might also bring new insights into the understanding of animal personality.

Moreover, it should by aimed to determine objective measurements of positive emotions, by associating behavioral, physiological and cognitive variables. Applying appraisal theories to a higher number of species, by giving an insight in how an individual perceive a situation, might also help to characterize the perceived intensity and valence of an event and therefore of the individual emotional reaction (Boissy & Erhard, 2014). We would expect that different personality types would perceive and interpret a similar event differently. Panksepp (2007a) argued that appraisal theories and affective neuroscience study the same emotions but at a different level, hence it would be interesting to study how primary-process emotions and cognitive evaluation of the situation interact together (e.g., Denson et al., 2009). More subtle emotional states, such as frustration or boredom, but also more complex, as inequity or empathy, would also deserve a strong interest.

Finally, although a consensus may never be reached about whether animals subjectively experience emotions (see also Špinka, 2019 for a review about different types of awareness to consider), it might be reasonable to act ‘as if’ and continue our efforts to have a positive impact on the animals under human care by reducing negative events and promoting positive experiences. To this aim, it is necessary to continue developing objective measures of emotions and to focus on their individuality.

6. Appendix (Ongoing projects)

I will present in the following sections a brief theoretical background and a short description of material and methods used, for three ongoing projects conducted during the thesis.

6.1. Motivational Conflict Test

Background

A motivational conflict typically arises when the animal is confronted to an event that induces, at the same time, a drive to explore and to avoid the situation (Pereira et al., 1999) or to remain at a comfortable place (Cabanac, 1999). Elevated plus-maze and open field tests are two widely used experimental paradigms leading to such approach/avoidance conflict that is assumed to induce anxiety (Carobrez & Bertoglio, 2005; Ennaceur et al, 2010), but their validity has been questioned (Ennaceur, 2014).

In our study, we intended to create a motivational conflict situation with a higher ecological significance. We had three main goals: assessing behavioral and thermal changes in response to odor cues stemming from females in estrus (i.e., sexual arousal) and in response to such cues simultaneously presented with a strong light placed on top of them (i.e., motivational conflict). Moreover, we asked whether exploration tendency would be associated with the intensity of sexual arousal and with thermal changes and risk-taking behaviors during the motivational conflict situation.

Material and Methods

38 male house mice, previously phenotyped for their exploration tendency (Fig. G), were used for this study. The apparatus was a square open field with a starting box in a corner and a small opening in the wall of the opposite corner of the starting box, to enter pieces of paper. A strong flashlight was fixed on the wall, on top of the latter opening. A thermal camera recorded peripheral temperature and behavior.

During the first four days, individuals were habituated to the apparatus and experimental protocol. They were first entered in the starting box and allowed to freely explore the open field during 10 min. Then pieces of clean paper (Whatman paper) were introduced in the opening and individuals were given another 10 min. On the fifth day, individuals were confronted to a first “sexual stimuli” situation: a mix of odor cues (urine collected on Whatman paper) from three females in estrus was presented after the first 10 min. Individuals were then given 10 min to explore it. On the next day, I simultaneously introduced odors from three new females in estrus and lighted up the flashlight (motivational conflict situation). Finally, a second “sexual stimuli” situation was presented to the mice on the last day, with odors of three other females.

Thermal and behavioral data have already been analyzed. Similarly to **Chapter 3**, we assessed the peripheral maximal body (corresponding to the eyes) and tail temperatures. Furthermore, activity has been analyzed by quantifying the latency to enter, the distance covered and the time spent in each zone (whole arena, starting box, corners, odor zone, light zone).

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6.2. Emotional Contagion Test

Background

Emotional contagion can be defined as a spontaneous imitation of the emotional behavior expressed by another individual which leads the observer to share the affective state of the demonstrator (Dezecache et al., 2015). In particular, studying emotional contagion may help to understand the origin of empathy, which is crucial in social interactions in humans

(Panksepp & Panksepp, 2013). It has been shown that laboratory mice observing a conspecific receiving electric foot shocks displayed freezing behavior, that is, they may be capable of emotional contagion (Jeon et al., 2010). However, although associations between personality traits and emotional contagion have received attention in humans (e.g., Lundqvist, 2008), such studies are still lacking in non-human animals.

In this study, we aimed to investigate whether and how emotional contagion (assessed through behavioral and physiological correspondences between observers and demonstrators) could be modulated by exploration tendency of individuals observing conspecifics receiving light electric foot shocks. We also investigated whether exploration tendency was related to a stronger emotional reaction to such stressors in the demonstrators, which may in turn induce a stronger emotional contagion in the observers.

Material and Methods

We tested 45 male house mice (hereafter: observers), that were previously phenotyped for their exploration tendency (Fig. G). Other 45 individuals (23 males and 22 females) were used as demonstrators and were also tested for their exploration tendency (Fig. G). The apparatus was constituted of two chambers separated by a transparent Plexiglass plate drilled of little holes to allow visual and olfactory, but not tactile communication between the two individuals. A thermal camera, placed on top of the apparatus, recorded peripheral temperature and behavior of both individuals. Ultrasonic vocalizations were also recorded in the observer’s chamber.

Each observer was paired with the same demonstrator for the whole procedure. Individuals were first habituated to each other, as well as to the apparatus and experimental protocol, during three consecutive days: the observer was first placed in its chamber for 10 min, then the demonstrator was placed in the adjacent chamber for 19 min. The first day of test was conducted the next day, individuals were placed following the same procedure than during habituation but after 10 min in the apparatus, the demonstrator received one light electric foot shock (0.3 mA) every 10 s during 4 min. After 5 min of recuperation period, the test was ended. Three days of rest, without experiment, were given to the individuals. Then, a second habituation period was carried out, identically to the first one. On the following day, 3 h before the second test, demonstrators were placed in their chamber for 10 min, without observers, then received light shocks (one every 10 s) during 4 min, followed by a 5 min recuperation period. Then, 3 h after this procedure, a second test was carried out following the same protocol than the first test.

For the behavioral analysis, each chamber was separated in three zones (close, middle and distant to the central Plexiglas plate) and I quantified the latency to enter, the distance covered and the time spent in each zone for each individual. Thermal data are currently being analyzed.

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6.3. Short-term thermal response to consumption of positive and negative food items in association with exploration tendency

This project was conducted in collaboration with Andrea Urrutia, PhD student, and Pr. Robyn Hudson (Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México (UNAM), Mexico). We conceived and planned the study with Andrea Urrutia, under the supervision of Robyn Hudson, Heiko G. Rödel and Christophe Féron. We carried out the experiments in November 2016. The behavioral and thermal data were analyzed in November 2016 and December 2017. However, as the thermal data were analyzed manually with the Flir software, we plan to re-analyze the data using the R script developed by CF.

Background

The necessity to study positive emotions has been outlined due to the increasing interest in animal sentience and welfare (Proctor, 2012), as a good welfare is not only characterized by the absence of prolonged negative emotions, but also by the presence of positive ones (Boissy et al., 2007; Mellor, 2015). However, it is still necessary to develop quantifiable and reliable indices of positive emotional experiences, as it has been done regarding negative emotions (see for instance the intensive study of animal models of depression and anxiety: Borsini et al., 2002; Kumar et al., 2013; Neumann et al., 2011).

Using a multimodal approach by evaluating facial expressions, behaviors and peripheral temperature (via infrared thermography), we had three main goals. First, we aimed to describe patterns of emotional reactions that might be used to differentiate positive and negative emotions, in the house mouse. Second, we aimed to assess individual emotional profiles to determine whether the intensity (arousal) of emotional responses might be consistent across situations. Third, we investigated the associations between individuals differences in emotional reactions and in exploration tendency.

Material and Methods

We tested the same individuals (17 adult male house mice) in three situations, using food stimuli expected to elicit distinct emotions. Each individual underwent 12 consecutive days of testing, consisting of four experimental conditions, each repeated during three successive days. The apparatus was constituted of two chambers: individuals were placed during 5 min in the first chamber then a sliding door was opened in the middle to allow the individual to enter the second chamber where the food item was presented in a Petri dish, for another 5 min. A thermal camera placed on top of the apparatus recorded their peripheral temperature and behavior. Another camera was placed on the floor, behind the Petri dish containing the food, and filmed the second chamber to record facial expressions when coming to eat and while eating.

The first three days constituted habituation days to the apparatus and experimental protocol and an empty Petri dish without food item was presented. Then, during three days, considered as the 'Neutral' condition, a piece of their usual food pellet was provided. This condition allowed us to obtain the basal temperature of the individuals and served as a control for the next two conditions. During the following three days ('Positive' condition), a piece of sugary cornflake was presented and expected to elicit a more positive emotional reaction than the usual food pellet. Finally, the last three days constituted a 'Negative' condition as the individuals were presented a piece of cornflake sprayed with quinine that was expected to induce a more negative emotion.

Moreover, we were particularly interested by the modifications of reactions when changing the valence of the food, as surpassing the individual's expectations by giving a food of higher quality than expected (that is, from 'Neutral' to 'Positive' condition) or frustrating the animals by giving a food of lower palatability (that is, from 'Positive' to 'Negative' condition) may lead to, respectively, more positive or negative emotional reactions (Flaherty, 1982).

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8. Education and scientific contributions

Education

Doctoral thesis, from **September 2016**.

Master thesis (January – June 2016): "Individual differences in behaviour in standard tests and in a transport task in the mound-building mouse (*Mus spicilegus*)". Laboratoire d'Ethologie Expérimentale et Comparée E.A. 4443, F-93430, Paris.

Internship (April – May 2015): "Personality and behavioural and thermal responses to repeated stressful situations in the mound-building mouse (*Mus spicilegus*)". Laboratoire d'Ethologie Expérimentale et Comparée E.A. 4443, F-93430, Paris.

Publications in international peer-reviewed journals

Verjat, A., Rödel, H. G., & Féron, C. (2019). Isolation calls in house mouse pups: Individual consistency across time and situations. *Developmental Psychobiology*, *00*, 1–11. https://doi.org/10.1002/dev.21884

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Contributions at scientific conferences

Oral presentations

Invited talk

Verjat, A. (2018). Assessment of early emotional profiles in domestic mice (*Mus musculus domesticus*). Centro Tlaxcala de Biología de la Conducta, Tlaxcala, Mexico.

International conferences

Verjat, A., Rödel, H. G., & Féron, C. (2019). Isolation calls in house mouse pups: Individual consistency across time and situations. Behaviour 2019, Chicago, USA.

National conferences

Verjat, A., Rödel, H. G., & Féron, C. (2019). Individual differences in judgement bias in the house mouse: consistency across time and influence of personality traits. SFECA 2019, Lille, France.

Poster presentations

International conferences

Verjat, A., Surreault, A., Carere, C., Gouat, P., & Rödel, H. G. (2017). Group-level differences in a transport task emerge from individual differences in personality: a study in a small rodent. Behaviour 2017, Estoril, Portugal.

Féron, C., Verjat, A., Lecorps, B., Bocquet, C., & Rödel, H. G. (2017). Analysis of infrared thermography recordings to assess physiological responses of mice during behavioural tests: Some tricks with R. Behaviour 2017, Estoril, Portugal.

National conferences

Duparcq, M., Jean, O., Verjat, A., Féron, C., & Rödel, H. G. (2019). Do fast and slow explorers differ in their short-term tail temperature responses to handling?, SFECA 2019, Lille, France.

Duparcq, M., Féron, C., Rödel, H.G., & Verjat, A. (2018). Could early emotional profiles predict personality traits in adulthood in male domestic mice (*Mus musculus domesticus*)?, IFE 2018, Villetaneuse, France.

Verjat, A., Surreault, A., Carere, C., Gouat, P., & Rödel, H. G. (2017). Personality is associated with individual and group-level differences in a transport task in the mound building mouse. SFECA 2017, Gif-sur-Yvette, France.

Attendance without presentation

2017: UFAW International Animal Welfare Symposium, Surrey, UK.

9. Declaration of authorship

I hereby declare that the thesis submitted, titled “Emotional characteristics and personality traits: a study in mice of wild origin” is my own unaided work. All direct or indirect sources used are given as references. All contributions of co-authors are acknowledged.

This thesis has not been submitted to another university or institute for acquiring a higher degree.

Paris, 17 November 2019

Aurélie VERJAT

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Emotional characteristics and personality traits:
a study in mice of wild origin

Abstract: The mechanisms driving animal personality (i.e., consistent individual differences in behavior across time and contexts) are still poorly understood. Recently, it has been proposed that personality traits may emerge from individual differences in emotional reactions. This thesis aimed to investigate how exploration tendency, one of the most frequently studied personality traits, is related to consistent individual differences in emotions in different age classes, using two rodent species of wild origin. In each chapter, we focused on one component of an emotional reaction (respectively, behavior, cognition and physiology), to assess either valence (i.e., positive or negative) or arousal (i.e., high or low) of the emotional experience. First, we showed that isolation call rate could be used to phenotype emotional profiles of young house mice, as pups' call rate was consistent over days and across three stressful situations. However, call rates were not associated with exploration tendency during adulthood. Second, our results suggested that a higher exploration tendency might be associated with a higher tendency to express negative affective states (i.e., a more negative judgement bias). Third, using infrared thermography, we found that fast explorative mound-building mice were characterized by a stronger sympathetic reactivity, as expressed by lower peripheral tail temperatures, than slow explorers shortly after a brief handling procedure. Overall, the findings of this research project contribute to the understanding of the emotional basis of personality traits and highlight the importance of considering individuality, through personality traits, when assessing emotions.

Keywords: *exploration tendency; affective state; isolation calls; cognitive judgement bias; infrared thermography; house mouse; mound-building mouse*

Caractéristiques émotionnelles et traits de personnalité :
une étude chez des souris d'origine sauvage

Résumé : Les mécanismes qui sous-tendent la personnalité animale (c.-à-d., les différences individuelles de comportement stables à travers le temps et les contextes) sont encore mal compris. Il a été suggéré que la personnalité pourrait émerger à partir de différences individuelles dans les réactions émotionnelles. Cette thèse a pour objectif d'étudier comment la tendance à l'exploration, l'un des traits de personnalité les plus étudiés, est liée aux différences individuelles d'émotions, à différentes classes d'âge chez deux rongeurs d'origine sauvage. Chaque chapitre aborde un composant d'une réaction émotionnelle (comportement, cognition et physiologie), afin d'évaluer la valence ou l'intensité de l'expérience émotionnelle. Tout d'abord, nous avons montré que le taux d'appels d'isolement pouvait être utilisé pour caractériser les profils émotionnels de jeunes souris domestiques, celui-ci étant stable durant trois jours et dans trois situations stressantes. Cependant, ce taux n'était pas associé avec la tendance à l'exploration durant l'âge adulte. Deuxièmement, nos résultats ont suggéré qu'une tendance plus forte à l'exploration pourrait être liée à une plus grande tendance à exprimer des états affectifs négatifs (c.-à-d., un biais de jugement plus négatif). Troisièmement, nous avons constaté que les souris glaneuses plus exploratrices étaient caractérisées par une réactivité plus forte du système sympathique, exprimée par des températures périphériques de la queue plus basses, peu de temps après une procédure de manipulation brève. Dans l'ensemble, les résultats de ce projet de recherche contribuent à la compréhension de la base émotionnelle des traits de personnalité et soulignent l'importance de prendre en compte l'individualité lors de l'évaluation des émotions.

Mots-clés : *tendance à l'exploration ; état affective ; appels d'isolement ; biais de jugement cognitif ; thermographie infrarouge ; souris domestique ; souris glaneuse*

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