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Assessment of anxiety in open field and elevated plus maze using infrared thermography

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HIGHLIGHTS

- We assessed the changes in temperature of mice during unconditioned fear tests.
- Changes in tail and eye temperatures suggest a fear-like response during the tests.
- Changes were also related to anxiety-related behaviors expressed by individuals.
- Initial temperatures predicted individual differences in behavioral responses.
- We stress the link between physiology and behaviors in those fearful situations.

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ABSTRACT

Due to their direct inaccessibility, affective states are classically assessed by gathering concomitant physiological and behavioral measures. Although such a dual approach to assess emotional states is frequently used in different species including humans, the invasiveness of procedures for physiological recordings particularly in smaller-sized animals strongly restricts their application. We used infrared thermography, a non-invasive method, to assess physiological arousal during open field and elevated plus maze tests in mice. By measuring changes in surface temperature indicative of the animals' emotional response, we aimed to improve the inherently limited and still controversial information provided by behavioral parameters commonly used in these tests. Our results showed significant and consistent thermal responses during both tests, in accordance with classical physiological responses occurring in stressful situations. Besides, we found correlations between these thermal responses and the occurrence of anxiety-related behaviors. Furthermore, initial temperatures measured at the start of each procedure (open field, elevated plus maze), which can be interpreted as a measure of the animals' initial physiological arousal, predicted the levels of activity and of anxiety-related behaviors displayed during the tests. Our results stress the strong link between physiological correlates of emotions and behaviors expressed during unconditioned fear tests.

1. Introduction

Fear and anxiety and their associated behavioral response have been extensively studied using animal models and can be defined as negative emotional states induced by the presence or the anticipation of a threat [1]. Thus, anxiety allows animals to prepare coping with a potentially dangerous situation. Fear responses of animals have been mainly assessed during unconditioned tests, where fear is induced through a spontaneous aversion of the apparatuses and conditioned procedures, where fear is induced through learning that a signal or an apparatus is associated with a painful/anxiogenic stimulus. Unconditioned fear tests using laboratory rodents, especially the open field (OF) and the elevated plus maze (EPM) became fundamental tools in the fields of psychopharmacology and behavioral neurosciences over the years. These tests, usually applied in laboratory rodents, make use of the animals' natural aversion for unfamiliar open and elevated space to induce anxiety [2]. Simplicity of the apparatuses, of their use and of behavioral measurements has certainly contributed to their widespread use [3]. Through decades of utilization, different behaviors have been described as anxiety-related behaviors that can be divided into classical spatio-temporal measures, such as open arms exploration of the EPM and the time spent close to the wall of the OF [4–6], and behavioral measures referring to the defensive repertoire of rodents [7].

Unconditioned fear tests and their associated anxiety-related behaviors have mostly been validated through pharmacological and cross-study validation [8,9]. Substantial support comes, for example, from drug screening studies, where the use of anxiogenic and anxiolytic substances typically leads to an increase or decrease in the occurrence of anxiety-related behaviors, respectively [4–6,10–12]. Although

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behaviors expressed both in the EPM and the OF indicate a two-way response to some drugs [11], consistent questions referring to what is really measured during these tests are frequently found in the literature (for a review see [1]). Besides, studies have frequently led to inconsistent results, questioning the inherent validity of these tests [1–3,7,9,13]. This underlines the great difficulty to infer about what is happening at the emotional level, while only using behavioral markers. Indeed, the need to integrate concomitant behavioral and physiological responses, the two main accessible components of emotions, has been frequently emphasized in order to improve the validity of emotional assessment [14,15].

So far, physiological measurements taken during OF and the EPM testing have mostly focused on serum or plasma levels of glucocorticoids and/or adrenocorticotropic hormone (ACTH) that once again provided controversial results. For instance, exposure to unconditioned fear tests was found to increase levels of corticosterone (for a review see [1]). However, while some studies questioned the relationship between corticosterone levels and open field behaviors (for a review see: [3]), others found significant relationships between corticosterone levels and the expression of risk assessment behaviors [16] or inter-individual differences in anxiety [17]. Few studies used simultaneous recording of autonomic responses (i.e. blood pressure, heart rate and heart rate variability) during unconditioned fear tests, while they have shown their usefulness in the assessment of emotions [14,18,19]. Some failed to provide convincing support for direct associations between simultaneously recorded autonomic physiological response and the occurrence of anxiety-related behaviors [8,20–22]. Yet, methods mostly used to assess physiological response in rodents frequently involve highly invasive procedures, including the necessity of surgical procedures (i.e. heart-rate, heart-rate variability, blood pressure) [23,24]. Other methods such as hormonal sampling are less invasive, but imply delayed measurements (e.g., measures in feces) and some of them still require handling animals during sampling (e.g., measures taken from blood, saliva or hair).

Since the majority of physiological measurements are invasive per se and as invasiveness should be avoided as much as possible in animal experimentation, infrared thermography (IRT) attracts growing attention. Allowing the detection of changes in peripheral blood flow, this method has shown a great potential to assess, non-invasively and easy-to-use, the physiological correlates of emotions. As short-term temperature responses are associated with the activation of the autonomic nervous system [25,26] this method has proven its efficiency to detect blood flow changes during alertness [25], conditioned fear [27], aversive procedures [28–31], fear tests [32] and positive emotional situation [33]. Besides, it has been used to assess positive as well as negative emotions in humans as well [34,35].

We used IRT as novel, non-invasive approach to assess physiological responses during affective states. In our study conducted on house mice (*Mus musculus domesticus*), we first explored the evolution of peripheral body temperatures during two established unconditional fear tests, frequently used in biomedical sciences, the open field and the elevated plus maze test [1,2]. In particular, we aimed to verify whether these tests would generally induce changes in peripheral temperature, similar to those associated with fear. That is, we expected that IRT measures such as the tail temperature will tend to decrease [25,27], whereas the eye temperature will tend to increase during the stressful situation induced by these tests [32,36]. In a second step, we investigated individual-level associations between the animals' anxiety-related behaviors and their temperature profiles during the two tests, respectively. On the one hand, we (*i*) tested for relationships between anxiety-related behaviors and changes in the animals' body temperature profiles. We expected that more anxious individuals, as assessed by their behavioral responses, will also show more pronounced changes (either increases or decreases) in their temperature profiles during the course of the tests. On the other hand, we (*ii*) explored potential relationships between the animals' initial body temperatures and their subsequent behavioral profiles during the tests. To this end, we quantified different parameters such as temperature of the eye and the tail, and we developed a way to quantify the animals' global thermal image.

2. Methods

2.1. Study animals

A total of 26 domestic mice *Mus musculus domesticus* of wild origin were used in this study. They were all adult sexually naïve males not older than 3 months. After weaning, on postnatal day 35, animals were housed in single sex groups of two to four individuals in $26 \times 14 \times 13$ cm polycarbonate cages (Iffa Credo, Lyon, France). Animals were maintained in a reverse light:dark cycle of 14:10 h and were provided with food (type M20, Special Diet Services, Witham, Essex, U.K.) and water ad libitum.

2.2. Behavioral tests

Subjects were tested using an open field and an elevated plus maze. A detailed description of the apparatuses used in this study can be found in [37]. Both tests were performed on the same day during the activity period of the animals (dark, red light phase). The OF test was always carried out first, one hour prior to the EPM test. Tests were recorded by an infrared video camera (T650sc, Flir Systems USA, Boston, MA) mounted over the OF and EPM, respectively. Behavioral analysis was carried out using a computerized animal observation system (EthoVision XT 10, Noldus, The Netherlands).

2.2.1. Open field test

Individuals were put in the center of the circular open field arena. The total arena had a diameter of 60 cm, and for analysis we defined a central zone with a diameter of 15 cm. The test was performed and recorded for 10 min. During video analysis, we quantified the time spent in the center and distance covered in this area as measure of boldness. The total distance covered was used as a measure of the animals' locomotor activity, and the time spent freezing during the test was used as a measure of anxiety.

2.2.2. Elevated plus maze test

The apparatus, which was elevated 60 cm above the floor, consisted of four arms of 30 cm length and 5 cm width, two of them surrounded by walls of 30 cm. Subjects were entered on the central platform, facing an open arm. The test was performed for 7 min. Some animals jumped from the EPM after this time, thus we were not able to perform longer tests. We quantified the percentage of open arms entries and the percentage of time spent in the open arms as measures of boldness. The total distance covered in the maze was used to measure the locomotor activity, and the time spent freezing was considered as anxiety-related behavior. Behaviors were quantified during the first 5 and 7 min of testing.

Animals were always entered into the test apparatuses (OF, EPM) by using a tunnel box. No tail handling was performed according to animal welfare purposes [38] and to avoid blocking the blood flow in the tail.

2.3. Infrared thermography

Infrared thermal videos (resolution: 640×480 pixels, sensitivity $= 0.03$ °C, with an emissivity fixed at 0.95) were recorded during the tests and used for later analysis.

Analysis was done according to 3 different times during the tests. Frames of the videos were selected for measurement of tail and eye temperature at minute 0, 5 and 10 (± 15 s) for the OF and at minute 0, 5 and 7 (± 15 s) of the EPM test. Tail temperature was assessed by drawing a line along the tail from its basis using FLIR ResearchIR analysis software. The temperature at the point located at a distance of 5 pixels