Learning and Motivation 80 (2022) 101855

Contents lists available at ScienceDirect

Learning and Motivation

journal homepage: www.elsevier.com/locate/l&m

Acute stress increases behaviors that optimize safety and decreases the exploration of aversive areas

Laura H. Ahumada [a], Silvio Morato [b], Marisol R. Lamprea [a,*]

[a] Laboratorio de Neurociencias, Departamento de Psicología, Facultad de Ciencias Humanas, Universidad Nacional de Colombia, Bogotá 111321, Colombia

[b] Departamento de Psicologia, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto 14040-901, Brazil

ARTICLE INFO

Keywords:
Exploratory behavior
Acute restraint stress
Anxiety
Security optimization
Elevated gradient of aversion

ABSTRACT

The avoidance-approach conflict has been proposed as the main drive of spontaneous exploration in rodents. Given this assumption, exploratory behavior has been interpreted as an anxiety index that can be measured in several paradigms. Although pharmacological manipulations predominate in anxiety and exploratory behavior studies, exposure to real stressful situations could be considered a more ecological approach. In the present study, we evaluated the immediate effect of 1-hour restraint stress on *Wistars* rats' exploratory behavior in the following models of anxiety: the light/dark box (L/D box), the elevated plus-maze (EPM), and the elevated gradient of aversion (EGA). Results showed that animals avoid risky areas according to the complexity of each apparatus and that acute stress increases that tendency. We propose that subjects tend to search and remain in safer areas when exposed to novel environments and that a single exposure to stress by motor restriction increases these behaviors which could be associated with a response related to anxiety but also to the optimization of a more complex survival system.

1. Introduction

Mammals, like rodents, need to explore their surroundings in the search for elements that contribute to their general fitness and survival, such as food sources or mating opportunities (Gibb, 2005). Sometimes when all basic needs are fulfilled, exploration appears as a spontaneous behavior, mainly when novelty occurs, a phenomenon known as neophilia (Barnett, 2005). The motivational force that leads a rodent to explore a new situation has been explained as a consequence of an internal conflict between the fear and the curiosity drive, the approach-avoidance conflict (Hughes, 1997; Montgomery, 1955). It seems that although fear can inhibit exploration (Hughes, 1997; Russell, 1973), there is a second factor, a sort of "behavioral need" for sensory change (Hughes, 1997) that pushes the animal to explore.

Given the approach-avoidance conflict, exploratory behavior can be interpreted as an anxiety index when it is assessed in animals models of anxiety (Campos et al., 2013; Harro, 2018; Lister, 1990; Pellow & File, 1986; Sanson & Carobrez, 1999; Takeda et al., 1998). These models include areas of an apparatus that represent a potential danger, and which reduce exploration. The elevated plus-maze (EPM), for example, has been one of the most used models to study anxiety, mainly because it is sensitive to anxiolytic and anxiogenic

* Correspondence to: Laboratorio de Neurociencias, Departamento de Psicología, Facultad de Ciencias Humanas, Universidad Nacional de Colombia, Carrera 30 # 45-03, Bogotá 111321, Colombia

E-mail addresses: lcahumadah@unal.edu.co (L.H. Ahumada), smorato@ffclrp.usp.br (S. Morato), mlamprear@unal.edu.co (M.R. Lamprea).

https://doi.org/10.1016/j.lmot.2022.101855
Received 14 July 2022; Received in revised form 28 October 2022; Accepted 4 November 2022
Available online 9 November 2022

drug effects (Carobrez & Bertoglio, 2005; Lister, 1990; Pellow & File, 1986; Rodgers & Dalvi, 1997; Tejada et al., 2009). Behavior in the EPM has been related to defensive behavior, since the neural activation is similar during both defense and exploration (Carobrez & Bertoglio, 2005). Also, it has been suggested that the typical anxiety-related behavior in this maze is caused by the contrast between the open and closed arms and not necessarily by the open arms' aversive feature (Salum et al., 2003); that is why the EPM is considered an ethological model of anxiety. Usually, anxiolytic-like substances increase the number of entries and the time animals spend in the open arms (Chaves et al., 2018; Garcia et al., 2005; Nin et al., 2012; Rogóz & Skuza, 2011), while anxiogenic-like drugs have the opposite effect (Bashiri et al., 2016; Rogerio & Takahashi, 1992; Setem et al., 1999).

Another widely used model of anxiety using exploratory behavior is the light/dark box (L/D box), a paradigm mainly based on the natural aversion rodents have to bright spaces (Hascoët et al., 2001). In general, anxiolytic-like drugs, such as diazepam or chlordiazepoxide, increase the number of transitions to and from the bright or light compartment (Chaouloff et al., 1997). This result is more salient when animals are initially placed on the light compartment (Chaouloff et al., 1997).

Although pharmacological manipulations predominate in the studies of anxiety and exploratory behavior in those environments, alternative forms to induce anxiety-related emotional states could be explored to evaluate how real stressful situations modify behavior and thus have a more ecological vision. Stress induction is one alternative that can be applied through foot-shock (MacNeil et al., 1997), forced swimming (Andreatini & Bacellar, 1999), or restraint stress (Buynitsky & Mostofsky, 2009). In particular, the latter has the advantage of being a mixed stressor with a physical component that limits the defensive response and a psychological component that makes the situation impossible to control (Bowman et al., 2003; McIntyre et al., 1999). Restraint stress has been shown to alter the stress circuitry through the change in the level of stress hormones such corticosterone and a reduced response of the Pituitary-Adrenal Axis (HPA) (Buynitsky & Mostofsky, 2009). These physiological changes ultimately affect a variety of behaviors.

However, the literature on the effects of restraint stress on exploration in animal models of anxiety is limited. Most works evaluate the late effect of restraint stress, *e.g.*, a day after the restraint exposure (Mendonça-Netto & Guimarães, 1996; Mendonça & Guimarães, 1998; Padovan et al., 2000), or in the case of the L/D box, most studies are focused on the consequences of chronic stress (Chotiwat & Harris, 2006; Li et al., 2016; Naert et al., 2011). In addition, a handful of the behavioral studies made using restrain stress had the objective of evaluating its effect on different stages of memory (Bowman et al., 2003; Li et al., 2012; Troncoso et al., 2010). Therefore, there is a lack of information about the effect of acute restraint stress on anxiety-related behaviors, in spite of the demonstration that even low intensity stressors used in some manipulation protocols are able to modify the behavior of animals in anxiety tests such as the EPM (Hurst & West, 2010).

In order to have a broader understanding of how restraint stress elicits anxiety-related behaviors, and therefore affects exploratory behavior, the goal of the present study was to evaluate the immediate effect of a single exposure to restraint stress on the behavioral parameters of three different models of anxiety using exploratory behavior: the EPM, the L/D box and a new apparatus, the elevated gradient of aversion (EGA) (Rico et al., 2019). This test was included because it can be a useful model for screening exploratory, impulsive, and fear traits in rats exposed to a novel environment (Rico et al., 2019, p: 11/12). In the EPM, in addition to the conventional parameters, such as the time spent in the open arms, we also measured the stretched-attend posture (SAP), a parameter of risk assessment that complements our interpretation of the behavioral disturbances.

2. Materials and methods

2.1. Subjects

Seventy-two male *Wistar* rats (190 ± 10 gr.) aged between 55 to 66 days were used in the present study. Females were not included since this was a first attempt at comparing exploratory behavior on these apparatuses and the hormonal variation could yield confounding results. The animals were housed in polypropylene cages (40 ×34 x 17 cm), five to a cage, with food and water *ad libitum*. The cages were kept in a room with the temperature maintained between 24 and 27°C, under a light-dark cycle of 12 hours (lights on at 07:00 am.). All experimental procedures were carried out in accordance with the Guidelines of the Brazilian Society for Neuroscience and Behavior recommendations for animal care and with the U.K. Animals (Scientific Procedures) Act 1986, and associated guidelines.

2.2. Equipment

The apparatus we used to restrain the rats was a horizontally placed cylindrical metal tube (20 cm long and 6 cm in diameter) with small holes for breathing. Also, the tube extremities had holes that could fit both the animal's snout and tail. The tube was elevated 10 cm above a metallic base that supported the apparatus. A restrained animal could still execute small movements inside the restraining tube. Animals were restrained in this apparatus for one hour and then immediately taken to the test room.

For the behavioral tests, we used an EPM consisting of two opposite open arms (50 ×10 cm) crossed at a right angle by two arms of the same dimensions enclosed by 40-cm high wooden walls, except for the central area, the whole apparatus being elevated 50 cm above the floor. To prevent animals from falling, the open arms were surrounded by a 0.5 cm edge. The apparatus was made of wood lined with opaque black Formica. Light intensity inside the closed arms, open arms, and central area were 24, 34, and 30 lux, respectively.

We also used a L/D box, which was a wooden rectangular box (100 ×50 x 40 cm) divided into two equal sections by a wall with a small opening (9 ×9 cm) which allowed the animals to go from one compartment to the other. The dark compartment was lined with black opaque Formica while the light compartment was lined with white opaque Formica. Light intensity in the dark compartment was 25 lux and in the light one was 32 lux.

Finally, we also used the EGA, which consisted of an alley (210 ×20 cm) divided into three equal compartments of equal length, elevated 40 cm above the floor. The first compartment at one extremity of the apparatus was a tunnel enclosed by 25-cm high walls lined with black opaque Formica, with a transparent red Plexiglas plate on the top (which allowed observation and video recording). At the outer extremity, the tunnel had a sliding door (20 ×25 cm) through which subjects were introduced into the apparatus. The other end of the tunnel was open, allowing the animals to enter the next (middle) compartment, which was enclosed by 60-cm high walls lined with black opaque Formica, resembling the closed arms of an EPM, except at the end which gave access to the last compartment. The last compartment had the floor lined with white opaque Formica and was surrounded by a 1-cm high edge in order to prevent the animals from falling, and was like the open arm of an EPM (see the schematic representation of EGA at Fig. 1).

2.3. Procedure

All tests were conducted between 8:00 and 11:00 am. The subjects were randomly assigned to their groups. Twenty-four rats were assigned to the EPM testing, 24 to the L/D box, and 24 to the EGA. Twelve rats in each of these three groups were controls left undisturbed in the living cages except for the cleaning routine and the behavioral tests in the apparatus they were assigned to. The remaining twelve animals in each of the three groups were placed inside the restraining tube for 60 minutes in a separated room and immediately taken to the experimental room, where they were tested according to the apparatus assigned to each one. No habituation period before testing was included.

The tests were carried out in batches, according to each of the instruments used, starting with the light/dark test, then the elevated plus-maze and finally the elevated gradient. Control and stressed animals were tested alternately in each instrument. The experimental sessions with the apparatuses were carried out in a 3 ×2 m room with no windows and lit by a 60-W electric bulb 1.75 m above the floor. The room had no furniture and contained only the apparatus being used. A video camera placed 2 m above the floor recorded the sessions performed with each apparatus.

To start a test session in the EPM, the rats were placed on the center of the apparatus facing one of the closed arms and were left to explore the entire maze for 5 min. The recorded sessions were later analyzed in order to record the percentage of time spent in the open and in the closed arms, in the closed arm extremities and in the center of the EPM. We also recorded the distance run in the apparatus and the frequency of stretched-attend posture (SAP), a behavior characterized by the elongation of the body which allows exploring forward with the fore paws while not displacing the hind paws (Albrechet-Souza et al., 2007, Van der Poel, 1979).

To start a session in the L/D box, subjects were placed at the end of the light area away from the wall separating it from the dark compartment and left to explore the entire box for 5 min. The recorded sessions were later analyzed in order to record the following parameters: the percentage of time spent in each area, the percentage of time spent in the dark compartment's deep section, the percentage of time spent in the dark compartmentś closer zone to the dividing wall and the frequency doing the SAP. During the L/D box sessions one of the restrained rats was injured and had to be removed from the experiment.

Finally, to start a session in the EGA, subjects were placed into the apparatus through the tunnel's sliding door and left to explore the apparatus for 3 min. The recorded sessions were later analyzed in order to record the following parameters: the percentage of time spent in each area, the percentage of time spent in the last rectangle of the tunnel (T5), the percentage of time spent at the extremity of the tunnel (T1), the percentage of time spent in the last rectangle of the closed arm (C5), the total distance run, and the frequency SAP occurrence.

After each session in each apparatus, subjects were returned to their home cages and the apparatus was cleaned with a cloth moistened with a 5% alcohol solution. Behavioral parameters were scored with a behavior scoring freeware (X-PloRat) developed at the Laboratory of Exploratory Behavior of the University of São Paulo at Ribeirão Preto, Brazil (Tejada et al., 2018).

2.4. Data analysis

Comparison between the control and the restrained groups was performed using a Student t-test. In addition, two-way analysis of variance (ANOVA) was performed to compare the effects of the group condition and the zones of the EGA (repeated measure) on the animal behavior followed, whenever appropriate, by pairwise multiple comparisons (Bonferroni t-test). One subject from the control group was excluded from the analysis in the EGA because of the absence of locomotor activity. In all cases, significance was considered whenever $P < 0.05$.

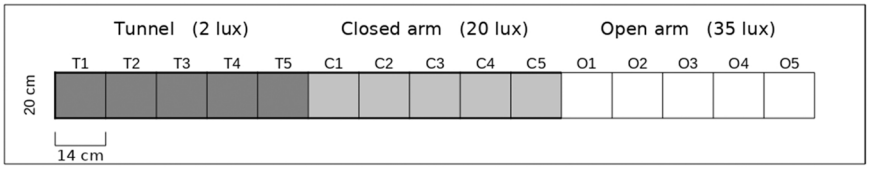

Fig. 1. Schematic representation of the Elevate gradient of aversion (EGA). Dark gray area represents the tunnel compartment (T1 – T5), light gray area represents the closed compartment (C1 – C5), and white area represents the open compartment (O1 – O2).

3. Results

3.1. Conventional parameters

Fig. 2 shows the percentage of time spent in the light and dark compartments of the L/D box. The Student t-test showed no significant differences between controls and restrained rats in the percentage of time spent in the light compartment ($t_{[21]} = 0{,}54$, $p = 0{,}59$, $d = 0{,}22$). The same figure shows the percentage of time spent in the open and closed arms of the EPM. The Student t-test shows that the restrained rats spent significantly less time in the open arms as compared to control rats ($t_{[2]} = 2{,}53$, $p = 0{,}02$, $d = 1{,}03$); there was no significant difference between control and restrained rats in the time spent in the closed arms ($t_{[22]} = 1{,}93$, $p = 0{,}07$, $d = 0{,}79$). ANOVA applied to the percentage of time spent in the EGA showed no significant effects of condition ($F_{[1,10]} = 1$, $p = 0{,}34$, $\eta^2p = 0{,}09$), a significant major effect of compartment ($F_{[2,20]} = 743{,}83$, $p < 0{,}001$, $\eta^2p = 0{,}98$) and an interaction between factors ($F_{[2,20]} = 17{,}64$, $p < 0.001$, $\eta^2p = 0{,}64$). Pos hoc analysis showed that, within the controls, animals spent significantly more time in the tunnel than in the open compartment ($t = 26{,}88$, $p < 0{,}001$, $d = 14{,}04$), more time in the tunnel than in the closed compartment ($t = 21{,}11$, $p < 0{,}001$, $d = 11{,}02$), and more time in the closed than in the open compartment ($t = 5{,}78$, $p < 0{,}001$, $d = 3{,}02$). Likewise, restrained rats spent more time in the tunnel than in the open compartment ($t = 30{,}40$, $p < 0{,}001$, $d = 15{,}88$), more time in the tunnel than in the closed compartment ($t = 28{,}17$, $p < 0{,}001$, $d = 14{,}71$), but there was no difference between the closed and open compartments ($t = 2{,}23$, $p = 0{,}48$, $d = 1{,}16$). Finally, in comparison to control rats, restrained rats spent more time in the tunnel ($t = -5{,}14$, $P < 0.001$, $d = -1{,}84$), less time in the closed compartment ($t = 5{,}15$, $p < 0{,}001$, $d = 1{,}85$) with no significant difference in the time spent in the open compartment ($t = 0{,}01$, $p = 1$, $d = -0{,}005$).

Figs. 3 and 4 shows the percentage of time spent in the extremity of each apparatus. First, the figure shows the percentage of time spent in the dark compartment's deep section of the L/D box. The Student t-test showed no significant difference between controls and restrained rats ($t_{[21]} = -1{,}39$, $p = 0{,}18$, $d = -0.58$). The figure in the middle shows the percentage of time spent in the closed arm extremities of the EPM and the Student t-test showed no significant difference between control and restrained animals ($t_{[22]} = 0{,}96$, $p = 0{,}35$, $d = 0{,}39$). Finally, the last figure shows the percentage of time spent at the bottom of the tunnel of the EGA (T1). The Student t-test showed that restrained rats spent more time in the extremity than control rats ($t_{[21]} = -3{,}63$, $p = 0{,}002$, $d = 1{,}51$).

Fig. 4 A shows the percentage of time spent in the transition zone of each apparatus. The figure shows the percentage of time spent in the dark compartments closer zone to the dividing wall of in the L/D box; the Student t-test showed no significant difference between controls and restrained rats ($t_{[21]} = 0{,}63$, $p = 0{,}54$, $d = 0{,}26$). Also, the figure shows the percentage of time spent in the central area of the EPM, and the Student t-test showed no significant difference between control and restrained rats ($t_{[21]} = 1{,}12$, $p = 0{,}28$, $d = 0{,}46$). In the case of the EGA, the Student t-test showed that restrained rats exhibited a significantly lower percentage of time spent in the last rectangle of the closed arm (C5) ($t_{[21]} = 2{,}27$, $p = 0{,}03$, $d = 0{,}95$) but no significant difference ($t_{[21]} = 1{,}17$, $p = 0{,}26$, $d = 0{,}49$) in the percentage of time spent in the last rectangle of the tunnel (T5).

Finally, restrained rats traveled lesser distances in the EPM as compared to control rats ($t_{[22]} = 3.19$, $p = 0{,}004$, $d = 1.3$. Control: 8.96 m ± 0.92; Restrained: 5.35 m ± 0.65). Similarly, restrained rats traveled lesser distances in the EGA as compared to control rats ($t_{[21]} = 4.32$, $p < 0.001$, $d = 1.8$. Control: 10.31 m ± 0.53; Restrained: 6.24 m ± 0.76).

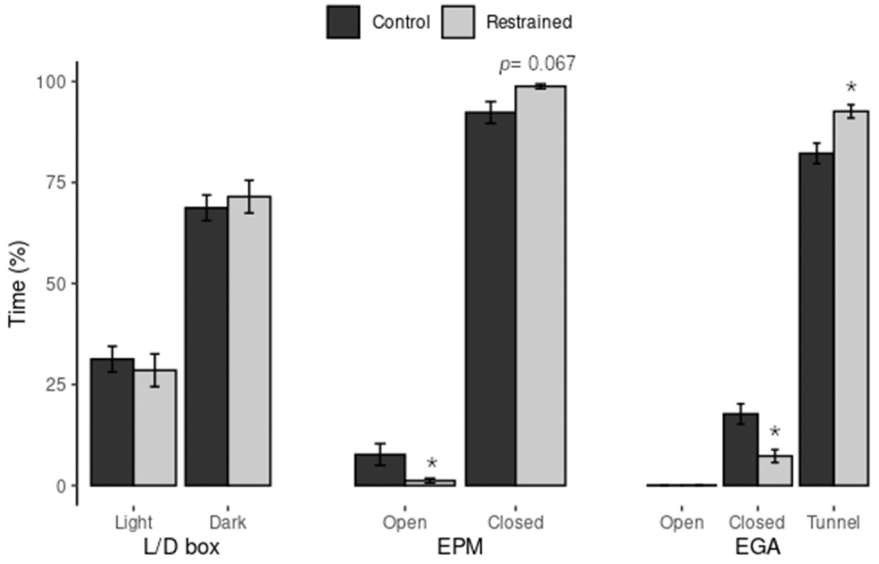

Fig. 2. Percentage of time spent in the areas of each apparatus. Means ± SEM.
(*), Significantly different from controls ($p < 0.05$).

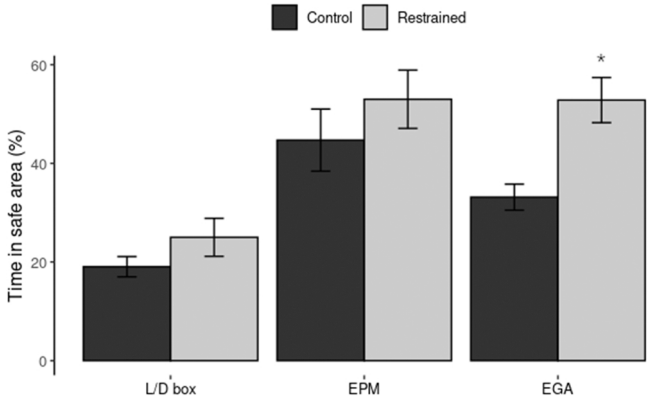

Fig. 3. Percentage of time spent in safe areas. (1) dark compartment's deep section of the L/D box, (2) closed arms' extremities of the EPM, and (3) T1 in the tunnel of the EGA. (means ± SEM) (*), Significantly different from controls ($p < 0.05$).

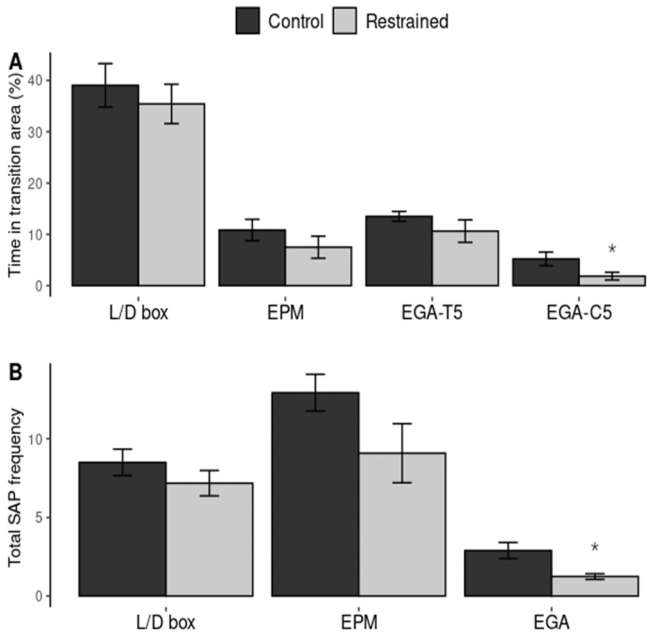

Fig. 4. Risk assessment measures. A: Percentage of time spent in the transition areas: (1) dark compartment's closer zone to the dividing wall of the L/D box, (2) central area of the EPM, (3) T5 and (4) C5 area of the EGA (means ± SEM). B: Total SAP frequency (means ± SEM). *, Significantly different from controls ($p < 0.05$).

3.2. Stretched-attend posture (SAP) frequency

Fig. 4B shows the total SAP frequency in all apparatuses. The Student t-test showed no significant difference between controls and restrained rats in the L/D box ($t_{[21]} = 1.13$, $p = 0,27$, $d = 0,47$) nor in the EPM ($t_{[22]} = 1,738$ $P = 0,1$, $d = 0,71$). On the other hand, restrained animals decreased the frequency of SAP inside the EGA as compared to control animals ($t_{[21]} = 2,16$ $P = 0,005$, $d = 1,32$).

4. Discussion

Rodent motivation to explore new environments has long been related to the existence of a conflict between curiosity, which allows collecting information from the novel environment, and the fear of being exposed to potential dangers – the so-called approach-avoidance conflict (Hughes, 1997; Montgomery, 1955). In spite of this theory having predominated in studies of behavior (Campos et al., 2013; Sanson & Carobrez, 1999), another emphasis on the function of exploratory behavior has appeared as a complement to the first approach. Whishaw et al. (2006) have proposed the theory of security optimization while exploring, according to which the subjects are motivated to seek and remain in the safest areas of an environment in order to minimize risk, particularly when there is little to be gained by exploring. These authors have shown that the safer the area where the animal begins to explore, the less

exploration it will exhibit (Whishaw et al., 2006).

In the present study, the particular characteristics of each apparatus caused exploration to start in different conditions, which modified exploration, particularly in the areas that potentially could present greater risk. In the L/D box the rats started exploration in the extremity of the light compartment, the area most exposed to light. In the EPM, exploration started in the center, an area of transition between the closed and the open arms. Finally, in the EGA, the rats started in the tunnel, the safest area of the apparatus. Thus, our results show that, as the starting point of our experiments constituted safer places, the exploration of the more exposed areas decreased, which coincides with the results of Whishaw et al. (2006). Also, in general, those results coincide with those of Roy et al. (2009) showing that, when not forced to explore the maze, the animals reduce the exploration of the open arms almost completely, suggesting it is rather an innate tendency than a conditioned learning. In the same sense, Arabo et al. (2014), showed a reduction of exploration in the open arms of the EPM when the animals started the test from a familiar and safe box in comparison to the conventional forced exploration starting in the center of the maze. These results suggest that while exploring the animals acquire information about the characteristics of the apparatus and attribute greater safety to the closed arms, inside which they remain longer. The classic experiments by Eilam & Golani (1989) show that, when exposed to a new environment, rats remain longer in certain places considered "home base", from which they stablish a characteristic exploration pattern (Golani et al., 1993; Tchernichovski & Golani, 1995).

As to the exposure to acute restraint stress, our results show a reduction in the exploration of the more aversive areas, an effect which proves more significant in function of the complexity of the apparatuses. In the L/D box, stress produced a small reduction in the exploration of the more illuminated compartment as well as a small increase in the time spent in the safe area. As far as we know, there are no studies evaluating the effect of acute restriction stress on exploration in the L/D box; there are only studies on the effects of chronic stress exposure in studies with contradictory results (Chotiwat & Harris, 2006; Li et al., 2016). This makes the present study the first to investigate the effects of acute restriction on exploration in the L/D box.

In the EPM, acute exposure to motor restriction produced a decrease in the total distance traveled, suggesting a general reduction in the exploratory drive, an effect in accordance with previous studies reporting a reduction in locomotor activity in the EPM by rats exposed to different forms of stress (Padovan & Guimarães, 2000; Martinez et al., 2007). Additionally, motor restriction produced a significant decrease in open arm exploration, which is considered an area of maximum risk to the animals (Rodgers & Dalvi, 1997), as well as a tendency to remain longer in the closed arms, particularly in the extremities, the safest area of the maze. These results are in contrast with those of a previous study in which short term effects on EPM open arm exploration were not found after two hours of motor restriction (Padovan & Guimarães, 2000). The difference with the results we obtained may be related to the stress duration. It has been shown that exposure to different stressors produces a dynamic response of the hypothalamus-hypophysis-adrenal axis, which is characterized by a peak of glucocorticoid liberation 30 min after the start of the stress, followed by a gradual decrease until reaching basal levels (Herman, 2013). In our study the animals were taken to be tested immediately after the 1-hour restriction. In the study of Padovan & Guimarães (2000), on the other hand, the subjects were tested in the EPM three hours after starting the restriction. It is possible that, at this point, corticosterone levels had returned to basal levels, resulting in little or no stress effect on behavior in the EPM.

In the EGA, we observed that the subjects exposed to restriction remained longer in the tunnel area and less in the closed arm. Since this apparatus was recently developed, the present study is the first to demonstrate stress effects due to motor restriction. Using this apparatus in a study evaluating different drugs, Rico et al. (2019) have shown that the administration of anxiogenic drugs, such as pentylenetetrazole, decreases closed arm exploration, while Bonuti et al. (2020) have shown that iron deficiency during gestation, a condition that causes severe alterations in brain development, caused an increase in the time remaining in the tunnel. Such data shows the sensitivity of this new apparatus to external and internal factors that alter rodent exploratory behavior. Our study has shown that the stressed subjects spent approximately half of the test time in the first quadrant of the EGA tunnel. According to Rico et al. (2019), remaining in this place could be motivated by anxiety and fear. Yet it could be considered a self-protection behavior minimizing risk, especially in animals that are not deprived or that have no other motivation to explore. It is possible that stress has driven the animals to remain longer in the initial part of the EGA tunnel since this is the point in which they were placed to start exploration. Previous reports, like that of Nemati & Whishaw (2007), show that rats exhibit a greater number of visits and remain longer in the places where they start the exploration, which allows them to optimize safety and make a spatial representation of the environment.

On the other hand, the results concerning risk assessment, as measured by the SAP and the behavior of remaining in the areas of transition between a safer area to a less safe one, show the tendency of stressed subjects to exhibit less risk assessment than control ones. Just like the above results, this tendency is significant in the EGA. Such a reduction in the occurrence of this behavior by stressed subjects is in accordance with results reported by Ortolani et al. (2011) with the EPM after applying foot electric shocks as stressor. But it is not in accordance with the results of Mikics e al. (2005), who reported SAP increases in the EPM and in the open-field after systemic corticosterone injections. In spite of not being conclusive, our results together with those of Ortolani et al. (2011) and of Mikics et al. (2005) suggest that risk evaluation is not altered in function of the exploration of aversive areas. Ortolani et al. (2011) reported, in comparison with control subjects, an increase in the EPM open arms exploration while Mikics et al. (2005) report no difference in the exploration of these arms after corticosterone treatment. If risk evaluation starts when the approach impulse and the avoidance impulse are in conflict, as suggested by Van der Poel (1979) and by McNaughton & Corr (2018), then solving the conflict would result in either exploring or retreating, which would confirm that risk evaluation does not depend on exploration. As far as our data are concerned, we considered that stressed subjects, specifically in the EGA tests, remain longer in the safe area, therefore not showing an approach impulse which, in turn, does not generate the conflict that leads to risk evaluation.

In spite of these apparatuses having being designed to evaluate anxiety, our results indicate that exploratory behavior in these areas respond to factors which are not exclusively related to an anxiety state but are part of a more complex survival system. This opens the

door to continue investigating rodent exploratory behavior from distinct theoretical perspectives aiming at a more parsimonious approach to its biological function, which is in accordance with more modern propositions (see Ennaceur, 2014; Kalueff et al., 2007; Whishaw et al., 2006). As far as risk evaluation is concerned, we suggest it is necessary to distinguish between this group of measures from those associated with exploration *per se*, since they could be responding to different processes. On the other hand, physical stress has been demonstrated to be a useful method for studying behavior in different environments, since it can evidence behavioral intrinsic trends of the animals which are manifested when they are exposed to novel potentially dangerous environments, as we could observe in our results. Finally, we may state that the structural advantages of the EGA allow a gradual transition from safe environments to potentially dangerous ones, which makes this apparatus appropriate to study exploration. The data is gathered after a short 3-min session, since the original paper reported that from the 4th min on (up to the 60th min of session), there are no entries into the open arms and very little exploration in the rest of the apparatus (Rico et al., 2019, p: 3/12).

In conclusion, our study shows that the subjects prioritize safety when exposed to novel environments and tend to search for and remain in safer areas, which was evidenced in the complexity of each apparatus. As to the effects of acute stress on exploration, our data show that a single exposure to stress by motor restriction increases behaviors that optimize safety and decrease the exploration of more aversive areas, which could be associated with a response related to anxiety but to other factors as well.

Funds

This work was supported by a grant of the División de Investigaciones de la Sede Bogotá, Universidad Nacional de Colombia (DIB-16459).

CRediT authorship contribution statement

Laura H. Ahumada: Investigation, Writing – original draft; **Silvio Morato:** Conceptualization, Resources, Writing – review & editing; **Marisol R. Lamprea:** Conceptualization, Resources, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors report no declarations of interest.

References

Albrechet-Souza, L., Carvalho, C. M., Franci, R. C., & Brandão, M. L. (2007). Increases in plasma corticosterone and stretched-attend postures in rats naive and previously exposed to the elevated plus-maze are sensitive to the anxiolytic-like effects of midazolam. *Hormones and Behavior, 52*(2), 267–273. https://doi.org/10.1016/j.yhbeh.2007.05.002

Andreatini, R., & Bacellar, L. F. S. (1999). The relationship between anxiety and depression in animal models: A study using the forced swimming test and elevated plus-maze. *Brazilian Journal of Medical and Biological Research, 32*(9), 1121–1126. https://doi.org/10.1590/S0100-879X1999000900011

Arabo, A., Potier, C., Ollivier, G., Lorivel, T., & Roy, V. (2014). Temporal analysis of free exploration of an elevated plus-maze in mice. *Journal of Experimental Psychology. Animal Learning and Cognition, 40*(4), 457–466. https://doi.org/10.1037/xan0000031

Barnett, A. (2005). Ecology. In I. Q. Whishaw, & B. Kolb (Eds.), *The behavior of the laboratory rat: a handbook with tests* (pp. 15–24). Oxford university press.

Bashiri, H., Rezayof, A., Sahebgharani, M., Tavangar, S. M., & Zarrindast, M. R. (2016). Modulatory effects of the basolateral amygdala α2-adrenoceptors on nicotine-induced anxiogenic-like behaviours of rats in the elevated plus-maze. *Neuropharmacology, 105*, 478–486. https://doi.org/10.1016/j.neuropharm.2016.02.010

Bonuti, R., Horiquini-Barbosa, E., & Morato, S. (2020). Late effects of iron deficiency during gestation and lactation followed by iron replacement on anxiety-related behaviors and brain morphology of young adult rats. *Psychology and Neuroscience, 13*(4), 516–530. https://doi.org/10.1037/pne0000239

Bowman, R. E., Beck, K. D., & Luine, V. N. (2003). Chronic stress effects on memory: Sex differences in performance and monoaminergic activity. *Hormones and Behavior, 43*(1), 48–59. https://doi.org/10.1016/S0018-506X(02)00022-3

Buynitsky, T., & Mostofsky, D. I. (2009). Restraint stress in biobehavioral research: Recent developments. *Neuroscience and Biobehavioral Reviews, 33*(7), 1089–1098. https://doi.org/10.1016/j.neubiorev.2009.05.004

Campos, A. C., Fogaça, M. V., Aguiar, D. C., & Guimarães, F. S. (2013). Animal models of anxiety disorders and stress. *Revista Brasileira de Psiquiatria, 35*(SUPPL.2), 101–111. https://doi.org/10.1590/1516-4446-2013-1139

Carobrez, A. P., & Bertoglio, L. J. (2005). Ethological and temporal analyses of anxiety-like behavior: The elevated plus-maze model 20 years on. *Neuroscience and Biobehavioral Reviews, 29*(8), 1193–1205. https://doi.org/10.1016/j.neubiorev.2005.04.017

Chaouloff, F., Durand, M., & Mormède, P. (1997). Anxiety- and activity-related effects of diazepam and chlordiazepoxide in the rat light/dark and dark/light tests. *Behavioural Brain Research, 85*(1), 27–35. https://doi.org/10.1016/S0166-4328(96)00160-X

Chaves, E. M. C., Honório-Júnior, J. E. R., Sousa, C. N. S., Monteiro, V. S., Nonato, D. T. T., Dantas, L. P., Lúcio, A. S. S. C., Barbosa-Filho, J. M., Patrocínio, M. C. A., Viana, G. S. B., & Vasconcelos, S. M. M. (2018). The anxiolytic-like effect of 6-styryl-2-pyrone in mice involves GABAergic mechanism of action. *Metabolic Brain Disease, 33*(1), 139–149. https://doi.org/10.1007/s11011-017-0139-5

Chotiwat, C., & Harris, R. B. S. (2006). Increased anxiety-like behavior during the post-stress period in mice exposed to repeated restraint stress. *Hormones and Behavior, 50*(3), 489–495. https://doi.org/10.1016/j.yhbeh.2006.06.007

Eilam, D., & Golani, I. (1989). Home base behavior of rats (*Rattus norvegicus*) exploring a novel environment. https://doi *Behavioural Brain Research, 34*(3), 199–211. https://doi.org/10.1016/S0166-4328(89)80102-0.

Ennaceur, A. (2014). Tests of unconditioned anxiety - Pitfalls and disappointments. *Physiology and Behavior, 135*, 55–71. https://doi.org/10.1016/j.physbeh.2014.05.032

Garcia, A. M., Martinez, R., Brandão, M. L., & Morato, S. (2005). Effects of apomorphine on rat behavior in the elevated plus-maze. *Physiology and Behavior, 85*(4), 440–447. https://doi.org/10.1016/j.physbeh.2005.04.027

Gibb, R. L. (2015). Ecology. In I. Q. Whishaw, & B. Kolb (Eds.), *The behavior of the laboratory rat: a handbook with tests* (pp. 321–331). Oxford university press.

Golani, I., Benjamini, Y., & Eilam, D. (1993). Stopping behavior: Constraints on exploration in rats (Rattus norvegicus. *Behavioural Brain Research, 53*(1-2), 21–33. https://doi.org/10.1016/S0166-4328(05)80263-3

Harro, J. (2018). Animals, anxiety, and anxiety disorders: How to measure anxiety in rodents and why. *Behavioural Brain Research, 352*(October), 81–93. https://doi.org/10.1016/j.bbr.2017.10.016

Hascoët, M., Bourin, M., & Nic Dhonnchadha, B. A. (2001). The mouse light-dark paradigm: A review. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 25*(1), 141–166. https://doi.org/10.1016/S0278-5846(00)00151-2

Herman, J. P. (2013). Neural control of chronic stress adaptation. *Frontiers in Behavioral Neuroscience, 7*(MAY), 1–12. https://doi.org/10.3389/fnbeh.2013.00061

Hughes, R. N. (1997). Intrinsic exploration in animals: Motives and measurement. *Behavioural Processes, 41*(3), 213–226. https://doi.org/10.1016/S0376-6357(97)00055-7

Hurst, J. L., & West, R. S. (2010). Taming anxiety in laboratory mice. *Nature Methods, 7*(10), 825–826. https://doi.org/10.1038/nmeth.1500

Kalueff, A. V., Wheaton, M., & Murphy, D. L. (2007). What's wrong with my mouse model?. Advances and strategies in animal modeling of anxiety and depression. *Behavioural Brain Research, 179*(1), 1–18. https://doi.org/10.1016/j.bbr.2007.01.023

Li, J., Li, H. X., Shou, X. J., Xu, X. J., Song, T. J., Han, S. P., Zhang, R., & Han, J. S. (2016). Effects of chronic restraint stress on social behaviors and the number of hypothalamic oxytocin neurons in male rats. *Neuropeptides, 60*, 21–28. https://doi.org/10.1016/j.npep.2016.08.011

Li, S., Fan, Y. X., Wang, W., & Tang, Y. Y. (2012). Effects of acute restraint stress on different components of memory as assessed by object-recognition and object-location tasks in mice. *Behavioural Brain Research, 227*(1), 199–207. https://doi.org/10.1016/j.bbr.2011.10.007

Lister, R. G. (1990). Ethologically-based animal models of anxiety disorders. *Pharmacology and Therapeutics, 46*(3), 321–340. https://doi.org/10.1016/0163-7258(90)90021-S

MacNeil, G., Sela, Y., McIntosh, J., & Zacharko, R. M. (1997). Anxiogenic behavior in the light-dark paradigm following intraventricular administration of cholecystokinin-8S, restraint stress, or uncontrollable footshock in the CD-1 mouse. *Pharmacology Biochemistry and Behavior, 58*(3), 737–746. https://doi.org/10.1016/S0091-3057(97)00037-3

Martinez, R. C. R., Garcia, A. M. B., Lamprea, M. R., & Morato, S. (2007). Thermal stress decreases general motor activity of rats in the elevated plus-maze but does not alter aversion to the open arms. *Behavioural Brain Research, 182*(1), 135–139. https://doi.org/10.1016/j.bbr.2007.04.015

McIntyre, D. C., Kent, P., Hayley, S., Merali, Z., & Anisman, H. (1999). Influence of psychogenic and neurogenic stressors on neuroendocrine and central monoamine activity in fast and slow kindling rats. *Brain Research, 840*(1–2), 65–74. https://doi.org/10.1016/S0006-8993(99)01771-0

McNaughton, N., & Corr, P. J. (2018). Survival circuits and risk assessment. *Current Opinion in Behavioral Sciences, 24*, 14–20. https://doi.org/10.1016/j.cobeha.2018.01.018

Mendonça-Netto, S., & Guimarães, F. S. (1996). Role of hippocampal 5-HT1A receptors on elevated plus-maze exploration after a single restraint experience. *Behavioural Brain Research, 77*(1–2), 215–218. https://doi.org/10.1016/0166-4328(95)00211-1

Mendonça, F. H., & Guimarães, F. S. (1998). Intra-hippocampal administration of cycloheximide attenuates the restraint-induced exploratory deficit of an elevated plus-maze. *Behavioural Brain Research, 91*(1–2), 207–211. https://doi.org/10.1016/S0166-4328(97)00129-0

Mikics, É., Barsy, B., Barsvári, B., & Haller, J. (2005). Behavioral specificity of non-genomic glucocorticoid effects in rats: Effects on risk assessment in the elevated plus-maze and the open-field. *Hormones and Behavior, 48*(2), 152–162. https://doi.org/10.1016/j.yhbeh.2005.02.002

Montgomery, K. C. (1955). The relation between fear induced by novel stimulation and exploratory drive. *Journal of Comparative and Physiological Psychology, 48*(4), 254–260. https://doi.org/10.1037/h0043788

Naert, G., Ixart, G., Maurice, T., Tapia-Arancibia, L., & Givalois, L. (2011). Brain-derived neurotrophic factor and hypothalamic-pituitary-adrenal axis adaptation processes in a depressive-like state induced by chronic restraint stress. *Molecular and Cellular Neuroscience, 46*(1), 55–66. https://doi.org/10.1016/j.mcn.2010.08.006

Nemati, F., & Whishaw, I. Q. (2007). The point of entry contributes to the organization of exploratory behavior of rats on an open field: an example of spontaneous episodic memory. *Behavioural Brain Research, 182*(1), 119–128. https://doi.org/10.1016/j.bbr.2007.05.016

Nin, M. S., Couto-Pereira, N. S., Souza, M. F., Azeredo, L. A., Ferri, M. K., Dalprá, W. L., Gomez, R., & Barros, H. M. T. (2012). Anxiolytic effect of clonazepam in female rats: Grooming microstructure and elevated plus-maze tests. *European Journal of Pharmacology, 684*(1–3), 95–101. https://doi.org/10.1016/j.ejphar.2012.03.038

Ortolani, D., Oyama, L. M., Ferrari, E. M., Melo, L. L., & Spadari-Bratfisch, R. C. (2011). Effects of comfort food on food intake, anxiety-like behavior and the stress response in rats. *Physiology and Behavior, 103*(5), 487–492. https://doi.org/10.1016/j.physbeh.2011.03.028

Padovan, C. M., Del Bel, E. A., & Guimarães, F. S. (2000). Behavioral effects in the elevated plus-maze of an NMDA antagonist injected into the dorsal hippocampus: Influence of restraint stress. *Pharmacology Biochemistry and Behavior, 67*(2), 325–330. https://doi.org/10.1016/S0091-3057(00)00361-0

Padovan, C. M., & Guimarães, F. S. (2000). Restraint-induced hypoactivity in an elevated plus-maze. *Brazilian Journal of Medical and Biological Research, 33*, 79–83. https://doi.org/10.1590/S0100-879X2000000100011

Pellow, S., & File, S. E. (1986). Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: A novel test of anxiety in the rat. *In Pharmacology, Biochemistry and Behavior* (Vol. 24,(Issue 3), 525–529. https://doi.org/10.1016/0091-3057(86)90552-6

Rico, J. L., Bonuti, R., & Morato, S. (2019). The elevated gradient of aversion: A new apparatus to study the rat behavior dimensions of anxiety, fear, and impulsivity. *Brazilian Journal of Medical and Biological Research, 52*(11), 1–12. https://doi.org/10.1590/1414-431x20198899

Rodgers, R. J., & Dalvi, A. (1997). Anxiety, defence and the elevated plus-maze. *Neuroscience and Biobehavioral Reviews, 21*(6), 801–810. https://doi.org/10.1016/S0149-7634(96)00058-9

Rogerio, R., & Takahashi, R. N. (1992). Anxiogenic properties of cocaine in the rat evaluated with the elevated plus-maze. *Pharmacology, Biochemistry and Behavior, 43*(2), 631–633. https://doi.org/10.1016/0091-3057(92)90203-R

Rogóz, Z., & Skuza, G. (2011). Anxiolytic-like effects of olanzapine, risperidone and fluoxetine in the elevated plus-maze test in rats. *Pharmacological Reports, 63*(6), 1547–1552. https://doi.org/10.1016/S1734-1140(11)70719-8

Roy, V., Chapillon, P., Jeljeli, M., Caston, J., & Belzung, C. (2009). Free versus forced exposure to an elevated plus-maze: Evidence for new behavioral interpretations during test and retest. *Psychopharmacology, 203*(1), 131–141. https://doi.org/10.1007/s00213-008-1378-2

Russell, P. A. (1973). Relationships between exploratory behaviour and fear: A review. *British Journal of Psychology, 64*(3), 417–433. https://doi.org/10.1111/j.2044-8295.1973.tb01369.x

Salum, C., Roque-Da-Silva, A. C., & Morato, S. (2003). Conflict as a determinant of rat behavior in three types of elevated plus-maze. *Behavioural Processes, 63*(2), 87–93. https://doi.org/10.1016/S0376-6357(03)00034-2

Sanson, L. T., & Carobrez, A. P. (1999). Long-lasting inhibitory avoidance acquisition in rats submitted to the elevated T-maze model of anxiety. *Behavioural Brain Research, 101*(1), 59–64. https://doi.org/10.1016/S0166-4328(98)00140-5

Setem, J., Pinheiro, A. P., Motta, V. A., Morato, S., & Cruz, A. P. M. (1999). Ethopharmacological analysis of 5-HT ligands on the rat elevated plus-maze. *Pharmacology Biochemistry and Behavior, 62*(3), 515–521. https://doi.org/10.1016/S0091-3057(98)00193-2

Takeda, H., Tsuji, M., & Matsumiya, T. (1998). Changes in head-dipping behavior in the hole-board test reflect the anxiogenic and/or anxiolytic state in mice. *European Journal of Pharmacology, 350*(1), 21–29. https://doi.org/10.1016/S0014-2999(98)00223-4

Tchernichovski, O., & Golani, I. (1995). A phase plane representation of rat exploratory behavior. *Journal of Neuroscience Methods, 62*(1-2), 21–27. https://doi.org/10.1016/0165-0270(95)00050-X

Tejada, J., Bosco, G. G., Morato, S., & Roque, A. C. (2009). Characterization of rat behavior in the elevated plus-maze using a directed graph. *Journal of Neuroscience Methods, 184*(2), 251–255. https://doi.org/10.1016/j.jneumeth.2009.08.009

Tejada, J., Chaim, K. T., & Morato, S. (2018). X-PloRat: A software for scoring animal behavior in enclosed spaces. *Psicologia: Teoria e Pesquisa, 33*, 1–4. https://doi.org/10.1590/0102.3772e3322

Troncoso, J., Lamprea, M., Cuestas, D. M., & Múnera, B. A. (2010). El estrés agudo interfiere con la evocación y promueve la extinción de la memoria espacial en el laberinto de Barnes. *Acta Biologica Colombiana, 15*(1), 207–222.

Van der Poel, A. M. (1979). A note on 'stretched attention', a behavioural element indicative of an approach-avoidance conflict in rats. *Animal Behaviour, 27*, 446–450. https://doi.org/10.1016/0003-3472(79)90181-7

Whishaw, I. Q., Gharbawie, O. A., Clark, B. J., & Lehmann, H. (2006). The exploratory behavior of rats in an open environment optimizes security. *Behavioural Brain Research, 171*(2), 230–239. https://doi.org/10.1016/j.bbr.2006.03.037