the Yellow River based on elasticity analysis. *Sci. World J.*, 2014, **2014**, 1–11.

15. Mann, H. B., Nonparametric tests against trend. *Econometrica*, 1945, **13**, 245–259.

16. Kendall, M. G., *Rank Correlation Methods*, Griffin, London, UK, 1975.

17. Zhang, Q., Xu, C. Y., Becker, S., Zhang, Z. X., Chen, Y. D. and Coulibaly, M., Trends and abrupt changes of precipitation maxima in the Pearl River basin, China. *Atmos. Sci. Lett.*, 2009, **10**, 132–144.

18. Wu, Z. H., Huang, N. E. and Chen, X. Y., The multi-dimensional ensemble empirical mode decomposition method. *Adv. Adapt. Data Anal.*, 2009, **1**, 339–372.

19. Huang, N. E. *et al.*, The empirical mode decomposition and the Hilbert spectrum for nonlinear and non-stationary time series analysis. *Proc. Math., Phys. Eng. Sci.*, 1998, **454**, 903–995.

20. Huang, N. E. and Wu, Z. H., A review on Hilbert–Huang transform: method and its applications to geophysical studies. *Rev. Geophys.*, 2008, **46**, RG2006.

21. Wu, Z. H. and Huang, N. E., Ensemble empirical mode decomposition: a noise-assisted data analysis method. *Adv. Adapt. Data Anal.*, 2009, **1**, 1–49.

22. Rehman, S., Study of Saudi Arabian climatic conditions using Hurst exponent and climatic predictability index. *Chaos Soliton. Fract.*, 2009, **39**, 499–509.

23. Sakalauskiene, G., The Hurst phenomenon in hydrology. *Environ. Res. Eng. Manage.*, 2003, **3**, 16–20.

24. Ding, Y. H., Wang, Z. Y. and Sun, Y., Inter-decadal variation of the summer precipitation in East China and its association with decreasing Asian summer monsoon. Part I: Observed evidences. *Int. J. Climatol.*, 2008, **28**, 1139–1161.

25. Su, B. D., Jiang, T., Shi, Y. F., Stefan, B. and Marco, G., Observed precipitation trends in the Yangtze river catchment from 1951 to 2002. *J. Geogr. Sci.*, 2004, **14**, 204–218.

26. Liu, Q., Yang, Z. F. and Ćui, B. S., Spatial and temporal variability of annual precipitation during 1961–2006 in Yellow River Basin, China. *J. Hydrol.*, 2008, **361**, 330–338.

27. Bai, L., Xu, J. H., Chen, Z. S., Li, W. H., Liu, Z. H., Zhao, B. F. and Wang, Z. J., The regional features of temperature variation trends over Xinjiang in China by the ensemble empirical mode decomposition method. *Int. J. Climatol.*, 2015, **35**, 3229–3237.

28. Wu, Z. H. and Huang, N. E., A study of the characteristics of white noise using the empirical mode decomposition method. *Proc. R. Soc. London, Ser. A.*, 2004, **460**, 1597–1611.

29. Sun, X. and Lin, Z. S., A new technology HHT and its diagnosis for ENSO. *Meteorol. Mon.*, 2006, **32**, 17–22.

Predatory stress paradigm to induce anxiety-like behaviour in juvenile male C57BL/6J mice

Abraão Tiago Batista Guimarães[1],
Joyce Moreira de Souza[1],
Wellington Alves Mizael da Silva[1],
Bruna de Oliveira Mendes[2],
Joice Gomes de Queiroz[2],
André Luis da Silva Castro[1,3] and
Guilherme Malafaia[1,2,4,*]

[1]Programa de Pós-Graduação em Conservação de Recursos Naturais do Cerrado, and
[2]Laboratório de Pesquisas Biológicas, and
[3]Laboratório de Zoologia, Instituto Federal Goiano – Câmpus Urutaí, GO, Brazil
[4]Programa de Pós-Graduação em Biodiversidade Animal, Universidade Federal de Goiás – Câmpus Samambaia, Goiânia, Brazil

The present study deals with a chronic stress paradigm to induce anxiety-like behaviour in male C57Bl/6J mice, using Wistar rats as predators. The predatory stress paradigm includes placing the mice in a cage protected by a metallic screen, which is placed inside a larger metallic cage, containing adult male Wistar rats. Male mice (21 days old) were put in indirect contact with Wistar male rats for 1 h daily for 12 days. The anxiety behaviour of mice was analysed by means of elevated plus-maze test, after 12 days of predatory stress daily (first behavioural assessment) and 12 days after the stress protocol (second behavioural assessment). We demonstrate that this predatory stress paradigm induces anxiety-like behaviour in male juvenile mice C57Bl/6J. We conclude that the predatory stress paradigm used is capable of inducing anxiety in male C57Bl/6J mice after a short duration (12 days) of predatory stress with Wistar rats.

Keywords: Anxiety, elevated plus maze test, juvenile mice, predatory stress.

ONE of the psychiatric disorders associated with the damaging effects of stress is anxiety. Unfortunately, we know very little about how the changes in stress load with time are related to changes in anxiety prodromal symptoms and to the development of an anxiogenic disorder[1]. In order to understand the ethology of anxiety and its relationship with stress and stressors, laboratory animals have been used in different studies, whereby a probable situation suffered in various forms of discomfort. As noted by Nunes and Hallak[2], these studies provide important inputs for the treatment of stress and its effects.

*For correspondence. (e-mail: guilhermeifgoiano@gmail.com)

Some studies have used predatory stress with rats to induce anxiety-like behaviour in mice[3–5]. Miura et al.[3] studied the long-term effects of acute, inescapable predator stress (male Wistar rats) on the behaviour and brain tryptophan metabolism in juvenile mice. With regard to behavioural data, surprisingly, the authors observed that predator stress caused anxiolytic behaviour in mice (by elevated plus-maze (EPM)) rather than anxiogenic behaviour in the evaluations performed 1 and 4 weeks after exposure to stress[3].

Barnum et al.[4] studied the long-term outcomes of chronic psychological stressors (predatory stress – male Long Evans rats as stressors for 28 consecutive days) on the neuroinflammatory response in adult male C57BL/6J mice (3–4 months old). It has been found that predatory stress can elicit changes in neuroinflammation and behaviour.

More recently, Burgado et al.[5] observed that two weeks of predatory stress (male Long Evans rats as stressors) induces anxiety-like behaviour with co-morbid depressive-like behaviour in adult male C57Bl/6J mice.

These studies have provided interesting and useful results to study the factors or mechanisms involved in the anxiety and relationship diseases. This is because the study of the physiopathogeny of these diseases and the effectiveness of pharmacological therapies directly depend on the existence of proper model animals, genetically induced or not, as well as experimental protocols able to create or provide desirable conditions and/or situations.

However, most predatory stress paradigms use Long Evans rats as stressors[4,5]; this strain of exogamous rats was developed in 1915 by crossing various female albino rats from the Wistar Institute with male wild grey rats[6]. In practice, there is some difficulty in getting this lineage because it involves the crossing of distinct lineages. Miura et al.[3], on the other hand, utilized male Wistar rats, is one of the most used strains worldwide in laboratory studies[6]. Obtaining these rats does not depend on crossing different strains. However, Miura et al.[3] evaluated the effects of acute stress on predatory specific pathogen free (SPF) mice used in very specific studies. Truly 'germ-free' mice are referred to as 'axenic', meaning that they are free of all microorganisms, including those that are typically found in the gut. Axenic mice are produced by hysterectomy rederivation and must be maintained in isolators under strict handling procedures to keep them germ-free[7].

Another interesting aspect is the absence of a predatory paradigm stress that lasts only for a short duration, is reliable, validated, and, more importantly, is capable of inducing anxiety-like behaviour in juvenile mice. In the studies available in the literature, the predatory stress paradigm adopted can be questioned since the apparatus used is not familiar to animals (for example, a transparent hamster ball by itself can generate animal behaviours related to fear and anxiety, besides the effects of the agitated ball)[4,5]. Miura et al.[3] used the complex stress protocol that included both restraint and predator stress; this work is difficult to compare with other works and, surprisingly, was notable to induce anxiogenic behaviour in mice.

We wanted to determine whether male Wistar rats (commonly found and easily reared in several animal facilities around the world)[6] could be used as chronic stressors and for the development of innovative predatory paradigm stress and also whether they could efficiently induce anxiety in young C57Bl/6J mice. These mice are used as an in vivo model in the study of different areas such as cardiovascular biology, developmental biology, diabetes and obesity, genetics, immunology, neurobiology, oncology and behavioural biology, and are commonly used to develop transgenic animals[8].

Thus we modified the predatory stress paradigms to determine if a simple and new paradigm could produce anxiety-like behaviour in juvenile male C57BL/6J mice, exposed to Wistar rats in either or both the short and long term. We hypothesized that the stressor employed would induce anxiety in the short and long term in mice.

Male C57Bl/6J mice used in the study were obtained from the matrices at the Animal Facility of the Instituto de Patologia Tropical e Saúde Pública (IPTSP – Institute of Tropical Pathology and Public Health) of Goiás State (Goiânia, Goiás, Brazil), and were kept in the animal facility of the Laboratório de Pesquisas Biológicas (Laboratory for Biological Research) of the Instituto Federal de Educação, Ciência e Tecnologia Goiano – Urutaí Campus (Urutaí, Goiás, Brazil). During the whole experimental period, the animals were subjected to a natural light/dark cycle (approximately 12.12 h), and offered food and liquids ad libitum. The 21-day-old animals were distributed in two experimental groups: predatory stress (PS) group ($n = 6$), which remained in indirect contact with Wistar rats (3–5 months), and control (C) group ($n = 6$), which was not exposed to Wistar rats. The methodology adopted in this study was consistent with the ethical principles for animal experimentation and approved by the Ethics Committee for Animal Use of the Instituto Federal de Educação, Ciência e Tecnologia, GO, Brazil (protocol no. 18/2014). All efforts were made to minimize both the number of animals used and their degree of suffering.

To induce predatory stress in the PS group, the mice were placed in a 21.5 cm × 11.5 cm × 29 cm cage protected by a 5-mm mesh metallic screen, which was placed inside a bigger cage (39.5 cm × 34.0 cm × 58.0 cm), containing three adult male Wistar rats. These rats are natural mice predators and constituted the stress agents in the experiment. The apparatus was built so as to prevent the mice from being preyed by the Wistar rats (Figure 1). The mice of C group were subjected to the same experimental conditions, without the presence of Wistar rats.
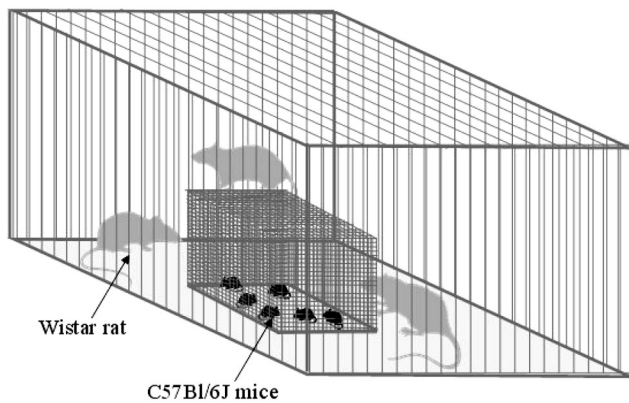
Figure 1. Sketch of the apparatus used in the exposure of C57Bl/6J mice to predatory stress (predatory stress group). The colours are merely illustrative.

The mice were kept in indirect contact with the Wistar rats for 12 consecutive days, during an hour each day (between 2 and 3 p.m.). The mice were exposed to sight, sound, smell and physical effects produced by the rats. The stress test took place in a controlled environment (a dark room, where external noise and human movement during the stress test were avoided). To enhance the predatory instinct of the rats, they were subjected to 9 h of fasting before indirect contact with the mice. It is to be emphasized that to avoid familiarity and possible habituation, chronically stressed mice were paired with three different Wistar rats for each predatory stress session.

The anxiety behaviour of the mice was analysed by means of EPM test, after 12 days of predatory stress daily (first behavioural assessment – 13th day) according to the schedule presented in Figure 2. The EPM test was chosen because it is adequate for the analysis of anxiety predictive behaviours[9]. To evaluate the permanent effects of the predatory stress, the mice were once again analysed on the 25th day, which represented 12 days after the stress protocol (second behavioural assessment).

The EPM used in this study was made of light grey acrylic walls, having two open arms (30 cm × 5 cm × 0.25 cm) and two closed arms with opaque walls (30 cm × 5 cm × 15.5 cm), joined by a central platform (5 cm × 5 cm) and elevated 45 cm above the ground. In the testing room, illumination was kept at 100 lux. The mice were placed individually at the central zone of the maze, facing an open arm, and they were allowed 5 min of free exploration. All mice were tested just once. The following parameters were evaluated: number of open and closed-arm entries, time spent in open and closed arms, and total number of entries (number of open-arms entries + number of closed-arm entries). These parameters were used to calculate the anxiety index, according to Estrela et al.[10] as follows: Anxiety index = 1 − [([Open arm time/test duration] + [Open arms entries/total number of entries])/2].

Entries in the open and closed arms were counted only when the four legs of the mice passed from an open arm to the closed arm and vice versa, with no need for the whole tail to be inside the arm[9]. The maze was carefully cleaned with ethanol solution after each animal, thus avoiding any influence of chemical communication between them[9]. The tests were recorded by a video camera placed vertically over the EPM. The animals were transported to the test room in boxes familiar to them. The animals were taken to the testing room by their tail (ref. 9) 30 min before the beginning of the test, so that they could get used to the testing room[11].

Initially the residual normality of the data was checked using Shapiro–Wilk's test. Bartlett's test was used to check the residual homoscedasticity using software R, version 3.0.3. Then, the data were subjected to Student's t-test at 5% probability using the software ASSISTAT, version 7.7 beta (freeware).

It is known that the typical approach–avoid conflict occurs in the EPM test, because the animal has to face an unknown environment, and it is its natural tendency to avoid open spaces, which are potentially dangerous and conflicting[9]. According to Pinto et al.[12], the stress classically produced by feline odours by exposure to water or by the swim test and by electrical shocks in the legs, significantly decreases the rodents' exploration of the EPM open arms; that is, the behaviour of the rodents is compatible with high anxiogenic indices. Therefore, lower frequencies and time spent in the open arms correspond to higher anxiety.

The present study demonstrates that this predatory stress paradigm induces anxiety-like behaviour in male juvenile C57Bl/6J mice in the short term (12 days), but that the behaviour does not last in the long term (12 days after the end of the stress). Our data indicate that mice evaluated after exposure to predatory stress paradigm (first behavioural assessment) had the highest anxiety index compared to the control group ($t_{1,12} = 6.427$, $P = 0.021$) (Figure 3 a). In addition, mice exposed to the predatory stress paradigm showed higher percentage of entries into the open arms of the EPM ($t_{1,12} = 7.141$, $P = 0.020$) (Figure 3 b) and a higher ratio of the number of open-arm entries/number of closed-arm entries ($t_{1,12} = 6.147$, $P = 0.029$) (Figure 3 c), which constitute index of primary anxiety[13–16]. Thus, our data demonstrate that the period of only 12 days exposure to rat stimuli was sufficient to induce anxiety-like behaviour in mice; these findings corroborate previous studies on exposure to predatory stress (with rats) that demonstrate significant increases of anxiety behaviour in mice[4,5].

On the other hand, this anxiogenic behaviour was not observed in the second evaluation performed 12 days after exposure to predatory stress paradigm when comparing the C and PS groups. Thus, our data differ from studies which show that exposure to potentially traumatizing stressful experiences in early life can cause
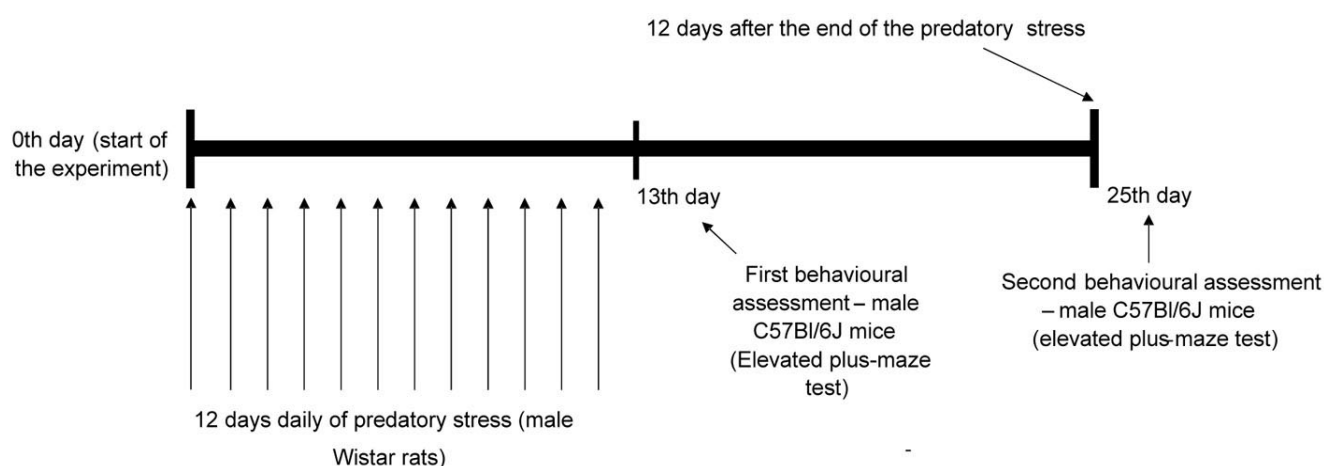
Figure 2. Time distribution of the test performed with C57Bl/6J mice subjected to predatory stress.

long-term effects on the still-developing neurobiological system[17–19], which may increase the vulnerability of animals to stressors encountered during adulthood.

However, it is important to emphasize that the depth and duration of the effects of exposure to potentially traumatizing stressful experiences in early life are related to several factors, including stressor agents, predatory stress paradigm, juvenile age at which the behavioural tests are performed, strains studied rodents, applied behavioural tests, etc. In summary, these factors may explain the absence of anxiogenic behaviour in the animals evaluated 12 days after exposure to predatory stress paradigm.

Besides the fact that lasting effects of exposure to a predatory stress paradigm in anxiogenic behaviour of the studied mice were not observed, our stress protocol presents interesting advantages over existing protocols. First, we used as stressors a rat strain used worldwide in laboratory research and easily maintained at animal facilities (Wistar rats)[6]; this is different from other studies that used Long Evans rats[4,5].

Second, we evaluated the effect of predatory stress on a mouse strain that has also been used worldwide in various biomedical studies (C57Bl/6J mice)[8], and we could induce anxiogenic behaviour in these animals in a period of only 12 days of exposure to predatory stress. In the study by Burgado et al.[5], which we consider to be the most similar to our work, the authors showed that adult male C57Bl/6J mice exposed to predatory stress for 15 days, using male Long Evans rats, presented anxiogenic behaviour when compared to the non-stressed group.

Third, our predatory stress paradigm consisted of apparatus (metallic cages) familiar to the mice, that were simpler than those used in other studies[4,5]. It is important to emphasize that the use of unfamiliar apparatus during predatory stress sessions can alone cause animal behaviour-linked to fear.

Fourth, we used the EPM test, which is considered to be one of the classic tests to evaluate anxiety in rodents[9,12,16]. Burgado et al.[5] used several behavioural tests to assess anxiety-like behaviour in mice (social interaction, marble burying, and open-field tests), interspersed with a test to assess anhedonia, a central symptom of depression in humans and novel object recognition tasks to assess learning and memory in rats and mice. However, there were some major problems. Note that the EPM test was not used to assess behaviour. According to Carola et al.[20], the open field test is more appropriate to assess motor activity indices (mechanical component), but is less sensitive to assess psychomotor behaviours in rodents when compared to the EPM test. As discussed by Komada et al.[11], even if the open field test assesses behaviours related to anxiety, it assesses anxiety in open spaces (open-space anxiety-like behaviour), which is not the case with the EPM test.

The social interaction test, developed by File and Hyde[21], provided the first test of anxiety-like behaviour that focused on ethologically relevant concepts. However, manipulating environmental conditions allows the researcher to induce varying levels of anxiety in the test subject[22]. In the study of Burgado et al.[5], there is no characterization of the environment in which the test was performed, which compromises interpretation of the results.

On the other hand, the marble burying test is used to measure repetitive and anxiety-related behaviour in rodents[23]. However, a recent study has criticized how data from this test are analysed, raising questions about the forms commonly used, which also creates biases that hinder interpretation of the data[24].

Fifth, in our study, the mice were subjected to predatory stress in a juvenile stage (after weaning – 21 days of life), which differs from other studies that evaluated the effects of predatory stress in older animals[4,5]. Differences in behaviour and performance assessed by the EPM test
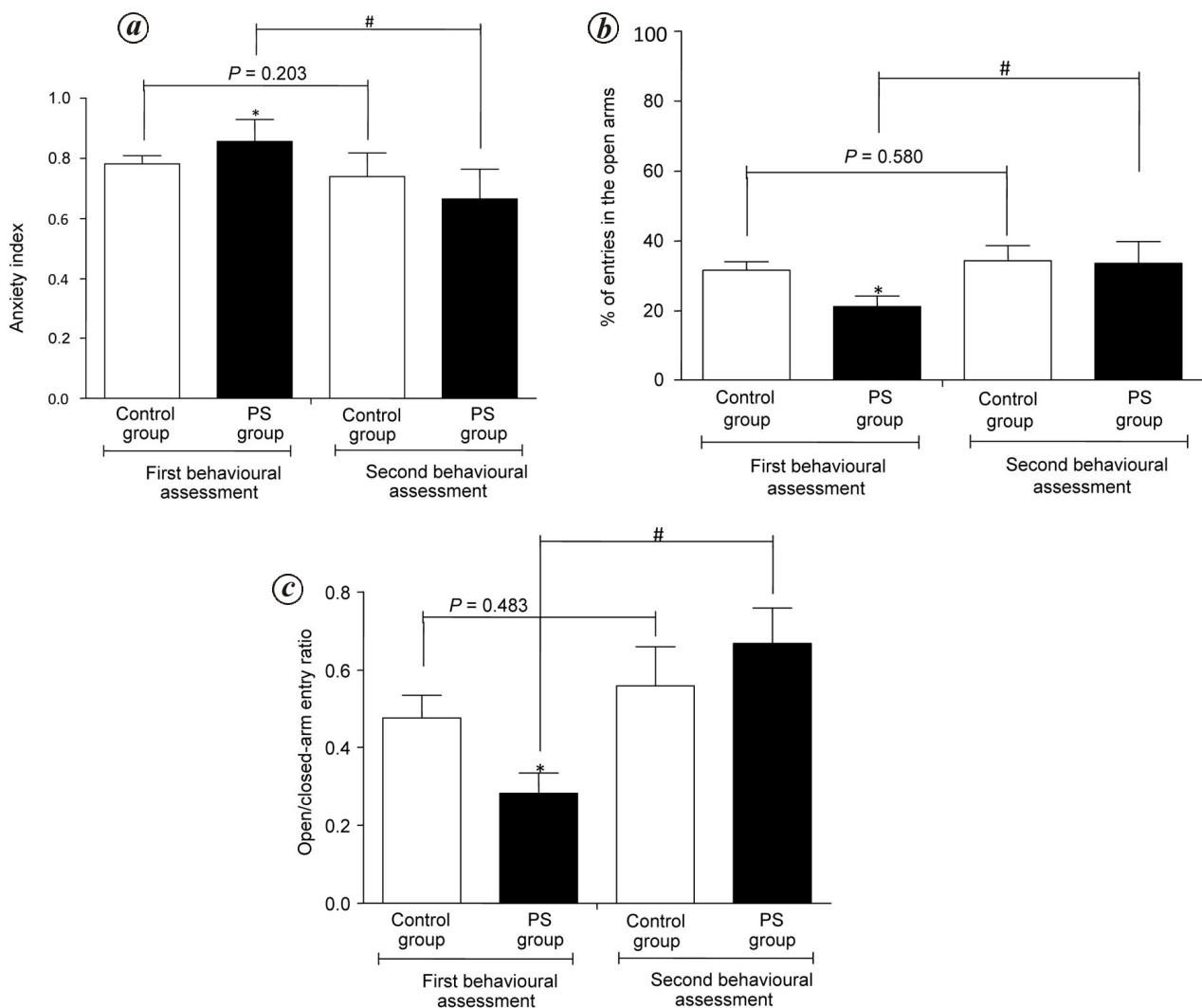
Figure 3. (*a*) Anxiety index, (*b*) percentage of entries into the open arms, and (*c*) open/closed-arm entry ratio of C57BL/6J male mice subjected to stress predatory with male Wistar rats for 12 consecutive days. Asterisk indicates significant difference between experimental groups by Student's *t*-test at 5% probability, in the first behavioural assessment. The lines compare the first and second behavioural assessment for each group. # indicates significant difference by Student's *t*-test at 5% probability.

are evident for both rats and mice of the same lineages but in different stages of development[9,12]. In these cases, the immaturity of the telencephalic mechanisms that regulate anxiety is pointed out as an important factor that explains why adolescent rodents are less affected by variations in pre-test conditions than adult rodents[25]. Boguszewski and Zagrodzka[26], when comparing a group of 4-month-old rats with a 24-month-old group of the same isogenic lineage, observed a significant decrease in the motor activity indices (shown by a decrease in the number of entries into the closed arms) and in the number of entries into the open arms in relation to the total number of entries of the older group. The authors proposed that the lower motor activity and higher anxiety in older animals, when compared to the younger ones, would be the explanation for such results.

It is the predatory stress factor to which the mice are exposed, which makes the results obtained in the present study different from the earlier studies. The same is applied to stress intensity and duration. For example, Calvo-Torrent *et al.*[27] while placing male mice of the lineage CDL and rats in the same room (inducing stress by odours and sounds emitted by the predators), the acute stress did not cause behavioural changes in the mice. However, when the mice were subjected to chronic predatory stress, lower frequency and less time spent in the EPM open arms than the closed arms showed indicative of anxiety behaviour.

Finally, a limitation of our study is the use of only male mice. Several studies have already evidenced effects distinct from the predatory stress when the same protocol is applied to female and male animals[28–30]. Therefore, our

results cannot be generalized for both sexes of mice; this limitation should be studied further. Despite this, the current dataset extends previous findings to establish predatory stress as a valid model of chronic stress exposure in juvenile male C57Bl/6J mice.

We conclude that the predatory stress paradigm used here is capable of inducing anxiety in male C57Bl/6J mice after a short duration (12 days) of predatory stress with Wistar rats and that the stress does not last in the long term (12 days after the stress). Mice of the lineage C56Bl/6J are model animals compatible with the study of different human diseases, and therefore more research is needed to elucidate the physiopathogenic mechanisms associated with such diseases.

It is worth noting that the proposed experimental protocol can be tested with different mice lineages and variants, such as the period of behavioural analysis, time of exposure to the stressor, number of animals (mice and rats), age of animals and the behavioural parameters to be assessed. The use of the predatory stress protocol proposed for the analysis of anxiety predictive behaviours in mice allows for low-cost, short-time studies, with additional easy breeding and maintenance of the animals used as stressors in the laboratory.

1. Basowitz, H. *et al.*, *Anxiety and Stress*, McGraw Hill, New York, 1955.
2. Nunes, E. A. and Hallak, J. E. C., Modelos animais em psiquiatria: avanços e desafios. *Rev. Latino Am. Psicopatol.*, 2014, **17**(3), 528–543.
3. Miura, H. *et al.*, Long-lasting effects of inescapable-predator stress on brain tryptophan metabolism and the behavior of juvenile mice. *Stress*, 2011, **14**(3), 262–272.
4. Barnum, C. J. *et al.*, Psychological stress in adolescent and adult mice increases neuroinflammation and attenuates the response to LPS challenge. *J. Neuroinflamm.*, 2012, **9**, 9.
5. Burgado, J. *et al.*, Two weeks of predatory stress induces anxiety-like behavior with co-morbid depressive-like behavior in adult male mice. *Behav. Brain Res.*, 2014, **275**, 120–125.
6. Andrade, A., Pinto, S. C. and Oliveira, R. S., *Animais de laboratório – criação e experimentação*, Rio de Janeiro, FIOCRUZ, 2002.
7. Fox, J. G. *et al.*, *The Mouse in Biomedical Research*, Elsevier, Burlington, MA, USA, 2007.
8. Beck, J. A. *et al.*, Genealogies of mouse inbred strains. *Nature Genet.*, 2000, **24**(1), 23–25.
9. Walf, A. A. and Frye, C. A., The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. *Nature Protoc.*, 2007, **2**, 322–328.
10. Estrela, D. C. *et al.*, Predictive behaviors for anxiety and depression in female Wistar rats subjected to cafeteria and stress. *Physiol. Behav.*, 2015, **151**, 252–263.
11. Komada, M., Takao, K. and Miyakawa, T., Elevated plus maze for mice. *J. Vis. Exp.*, 2008, **22**, 1–3.
12. Pinto, W. B. V. R. *et al.*, Teste de labirinto em cruz elevado: aplicações e contribuições no estudo de doenças neuropsiquiátricas em modelos animais. *RESBCAL*, 2012, **1**(1), 102–120.
13. Adamec, R., Walling, S. and Burton, P., Long-lasting, selective anxiogenic effects of feline predator stress in mice. *Physiol. Behav.*, 2004, **83**(3), 401–410.
14. Adamec, R. *et al.*, Lasting anxiogenic effects of feline predator stress in mice: sex differences in vulnerability to stress and predicting severity of anxiogenic response from the stress experience. *Physiol. Behav.*, 2006, **88**(1–2), 12–29.
15. Armario, A., Escorihuela, R. M. and Nadal, R., Long-term neuroendocrine and behavioural effects of a single exposure to stress in adult animals. *Neurosci. Biobehav. Rev.*, 2008, **32**(6), 1121–1135.
16. Rodgers, R. J. and Dalvi, A., Anxiety, defence and the elevated plus-maze. *Neurosci. Biobehav. Rev.*, 1997, **21**(6), 801–810.
17. Maslova, L. N., Bulygina, V. V. and Markel, A. L., Chronic stress during prepubertal development: immediate and long-lasting effects on arterial blood pressure and anxiety-related behavior. *Psychoneuroendocrinology*, 2002, **27**(5), 549–561.
18. Avital, A. and Richter-Levin, G., Exposure to juvenile stress exacerbates the behavioural consequences of exposure to stress in the adult rat. *Int. J. Neuropsychopharmacol.*, 2005, **8**(2), 163–173.
19. Avital, A. *et al.*, Effects of early-life stress on behavior and neurosteroid levels in the rat hypothalamus and entorhinal cortex. *Brain Res. Bull.*, 2006, **68**(6), 419–424.
20. Carola, V. *et al.*, Evaluation of the elevated plus-maze and open-field tests for the assessment of anxiety-related behaviour in inbred mice. *Behav. Brain Res.*, 2002, **134**(1–2), 49–57.
21. File, S. E. and Hyde, J. R., Can social interaction be used to measure anxiety? *Br. J. Pharmacol.*, 1978, **62**(1), 19–24.
22. File, S. E. and Seth, P., A review of 25 years of the social interaction test. *Eur. J. Pharmacol.*, 2003, **463**(1–3), 35–53.
23. Njung'e, K. and Handley, S. L., Evaluation of marble-burying behavior as a model of anxiety. *Pharmacol. Biochem. Behav.*, 1991, **38**(1), 63–67.
24. Lazic, S. E., Analytical strategies for the marble burying test: avoiding impossible predictions and invalid *p*-values. *BMC Res. Note*s, 2015, **8**, 141; http://bmcresnotes.biomedcentral.com/articles/10.1186/S13104-015-1062-7
25. Doremus, T. L., Varlinskaya, E. I. and Spear, L. P., Age-related differences in elevated plus maze behavior between adolescent and adult rats. *Ann. N. Y. Acad. Sci.*, 2004, **1021**, 427–430.
26. Boguszewski, P. and Zagrodzka, J., Emotional changes related to age in rats – a behavioral analysis. *Behav. Brain Res.*, 2002, **133**(2), 323–332.
27. Calvo-Torrent, A. P., Brain, F. and Martinez, M., Effect of predatory stress on sucrose intake and behavior on the plus-maze in male mice. *Physiol. Behav.*, 1999, **67**(2), 189–196.
28. Bourke, C. H., Harrell, C. S. and Neigh, G. N., Stress-induced sex differences: adaptations mediated by the glucocorticoid receptor. *Horm. Behav.*, 2012, **62**(3), 210–218.
29. Harrell, C. S. *et al.*, Sex and lineage interact to predict behavioral effects of chronic adolescent stress in rats. *Behav. Brain Res.*, 2013, **248**, 57–61.
30. Pyter, L. M. *et al.*, Sex differences in the effects of adolescent stress on adult brain inflammatory markers in rats. *Brain Behav. Immunity*, 2013, **30**, 88–94.