Epilepsy & Behavior 71 (2017) 243–249

Contents lists available at ScienceDirect

Epilepsy & Behavior

journal homepage: www.elsevier.com/locate/yebeh

Wistar audiogenic rats display abnormal behavioral traits associated with artificial selection for seizure susceptibility

Gabriel Perfeito Castro [a], Daniel de Castro Medeiros [a,b], Leonardo de Oliveira Guarnieri [a], Flávio Afonso Gonçalves Mourão [a,b], Hyorrana Priscila Pereira Pinto [a], Grace Schenatto Pereira [a], Márcio Flávio Dutra Moraes [a,b,*]

[a] Núcleo de Neurociências (NNC), Departamento de Fisiologia e Biofísica, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais CEP 31270-901, Brazil
[b] Centro de Tecnologia e Pesquisa em Magneto-Ressonância, Escola de Engenharia, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais CEP 31270-901, Brazil

ARTICLE INFO

Article history:
Revised 25 August 2015
Accepted 30 August 2015
Available online 4 October 2015

Keywords:
Epilepsy
Seizure predisposition
Wistar audiogenic rat
Comorbidities
Psychiatric disorder

ABSTRACT

Accumulating evidence from different animal models has contributed to the understanding of the bidirectional comorbidity associations between the epileptic condition and behavioral abnormalities. A strain of animals inbred to enhance seizure predisposition to high-intensity sound stimulation, the Wistar audiogenic rat (WAR), underwent several behavioral tests: forced swim test (FST), open-field test (OFT), sucrose preference test (SPT), elevated plus maze (EPM), social preference (SP), marble burying test (MBT), inhibitory avoidance (IAT), and two-way active avoidance (TWAA). The choice of tests aimed to investigate the correlation between underlying circuits believed to be participating in both WAR's innate susceptibility to sound-triggered seizures and the neurobiological substrates associated with test performance. Comparing WAR with its Wistar counterpart (i.e., resistant to audiogenic seizures) showed that WARs present behavioral despair traits (e.g., increased FST immobility) but no evidence of anhedonic behavior (e.g., increased sucrose consumption in SPT) or social impairment (e.g., no difference regarding juvenile exploration in SP). In addition, tests suggested that WARs are unable to properly evaluate degrees of aversiveness (e.g., performance on OFT, EPM, MBT, IAT, and TWAA). The particularities of the WAR model opens new venues to further untangle the neurobiology underlying the co-morbidity of behavioral disorders and epilepsy.

This article is part of a Special Issue entitled "Genetic and Reflex Epilepsies, Audiogenic Seizures and Strains: From Experimental Models to the Clinic".

1. Introduction

Epilepsy is a neural disorder characterized by seizure predisposition associated with sustained brain abnormalities, either restricted or diffused [1,2]. Animal models for several epileptic conditions or convulsive disorders have been proposed in an attempt to mimic, if only partially, the myriad of causes, signs, and symptoms of human epilepsy. One such example, the Wistar audiogenic rat (WAR), comprises an audiogenic reflex epilepsy animal model obtained by inbreeding, from Wistar breeding stocks, of seizure-susceptible rats to high-intensity acoustic stimuli. As a consequence, sporadically presenting high-intensity acoustic stimuli to WARs triggers brainstem circuitry-dependent generalized tonic–clonic seizures [3–8]. When repeatedly stimulated (audiogenic kindling) [4,9,10], WARs become a two-in-one model as epileptogenic neural substrates gradually compromise, through on-demand seizures, more rostral prosencephalic structures [4,8,11]. Thus, depending on the hypothesis being tested, such particularities of WARs may work to the experimenter's advantage, when compared with other models that have spontaneously recurrent seizures, which are undoubtedly closer to human epilepsy. This work aimed to explore the bidirectional comorbidity associations between genetic seizure susceptibility and behavioral abnormalities before seizure occurrence interferes by inducing dynamic neuroplastic changes to underlying circuits. In fact, most studies in patients and rodents found throughout literature do not make this distinction [12–16].

The WARs are inherently prone to have seizures, as evidenced by their lower threshold to proconvulsant electrical stimulation [17] or pharmacological stimulation [18], such as pilocarpine and pentylenetetrazole. In addition, WARs and other audiogenic strains show abnormalities in neurochemical systems and anatomical structures that have also been suggested to modulate emotional, social, motivational, and mnemonic aspects of behavior. The WARs show evidence for altered cholinergic and GABAergic systems when compared with Wistar controls [19,20].

* Corresponding author at: Núcleo de Neurociências, Departamento de Fisiologia e Biofísica, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Av. Antônio Carlos, 6627, Campus Pampulha, Belo Horizonte, Minas Gerais CEP 31270-901, Brazil. Tel.: +55 31 3409 2930; fax: +55 31 3409 2924.
E-mail address: mfdm@ufmg.br (M.F.D. Moraes).

http://dx.doi.org/10.1016/j.yebeh.2015.08.039
1525-5050/

Moreover, WARs also have a hyperresponsive hypothalamic circuitry which leads to high activity of the posterior [21] and anterior [22] pituitary. The latter neuroendocrine circuitry is known to play a crucial role in emotional behavior [23], epilepsy [24,25], and depression [26]. Altogether, these inherited traits may account for interictal mood and emotional behavioral abnormalities that may partially model human epilepsy. In fact, it has long been observed that patients often present interictal cognitive deficits, e.g., memory impairment [27–30], as well as mood and anxiety disorders [31–35]. As an example, the prevalence of depression among people with recurrent seizures ranges from 20% to 80% (for review see [36]) with consistently negative impact in health-related quality of life [32].

In summary, the use of a genetically prone animal model of on-demand seizure elicitation may contribute to the understanding of the underlying neuronal mechanisms shared by epilepsies, mood disorders, and learning and memory impairments.

2. Materials and methods

2.1. Animals

The present work was conducted on male Wistar rats from the main breeding stock of the Institute of Biological Sciences (ICB) of the Federal University of Minas Gerais (UFMG) and male Wistar audiogenic rats (WARs) from an inbred colony maintained at the Department of Physiology and Biophysics—ICB—UFMG, weighing between 250 and 310 g. Five animals were housed per cage on a 14/10-hour light–dark cycle (lights on at 06:00 h), with room temperature at 22 ± 1 °C, and with food and water ad libitum. Different groups of animals were used in each experiment. Efforts were made to avoid any unnecessary distress to the animals, in accordance with the Federal University of Minas Gerais Guidelines for Animal Experimentation. All protocols used were approved by the ethics committee on animal testing of this institution (Protocol Nos: 141/10 and 151/06).

Audiogenic susceptibility of WAR was confirmed through a screening test that consisted of three presentations of the audiogenic stimulus (a school bell recorded on a compact disk at 120 dB SPL) once every other day, for 60 s or until the onset of the tonic seizure. Seizure severity was evaluated using a sequential behavioral severity index (SI) scale, as follows: no seizure (SI = 0.0), one running episode (SI = 0.11), one wild running (with jumping and atonic falling—SI = 0.23), two wild running episodes (SI = 0.38), tonic convulsion (SI = 0.61), clonic convulsions (SI = 0.85), ventral flexion of the head (SI = 0.90), forelimb extensions (SI = 0.95), and hindlimb extensions (SI = 1.0) [8,37]. Only WARs that demonstrated a seizure-prone behavior (severity seizure index superior to 0.23) at least in one of the three screening tests were used. Before all procedures, the animals were acclimated to the experiment room for 30 min. All experiments were conducted in the same well-lit room and under a constant white noise condition (55 dB SPL).

2.2. Sucrose preference

This test consists of a paradigm in which the choice for water or sucrose consumption is measured in order to evaluate the behavioral expression of anhedonia [38,39]. The animals (9 Wistar rats and 7 WARs) were isolated and habituated to drink from two bottles on the day before test. The animals were then exposed to sucrose (1%) and water for 48 h, when the final measurement as a total liquid consumed was performed. The preference for sucrose index, which ranges from 0 to 100% (sucrose intake divided by the sum of total water plus sucrose consumption: [sucrose/(sucrose + water)]), was used as a measure for the sensitivity to reward. The statistical measurement was performed by Student's t-test.

2.3. Social preference

The modified sociability test from Crawley et al. has been successfully employed to study social affiliation in rodents [40]. The main principle of this test is based on the free choice of the animal in exploring a new object and a new juvenile animal. The social behavior apparatus was adapted from previous studies [40,41]. The device used was a rectangular acrylic box (100 × 100 cm). The animal (13 Wistar rats and 11 WARs) was placed in the center of the apparatus and allowed to explore the novel environment containing an empty cylinder for 5 min. After the time for habituation, the animal was removed from the apparatus, it was cleaned with a 70% alcohol solution, an object was placed in one of the corners of the apparatus, and the juvenile rat (male—21 days old) was placed within a transparent acrylic cylinder (10-centimeter diameter—60 evenly spaced holes) in another corner of the apparatus. The animal was then returned to the center of the apparatus and allowed to explore freely for 10 min. The sessions were video recorded with a digital camera linked to a computer in an adjacent room. The recordings were analyzed by a well-trained researcher using X-PLO-RAT (version 3.3), an ethological analysis software package developed at the Laboratory of Exploratory Behavior USP/Ribeirao Preto [42]. The total time of exploratory behavior (nose touching the object or the juvenile container) was measured. The sociability index was calculated using: [juvenile exploration time/(object exploration time + juvenile exploration time)]. Through this mathematical transformation, it was possible to compare the social behavior in the different groups by analyzing the index, which ranges from 0 to 100%. The statistical analysis was performed by the Student's t-test.

2.4. Open-field

The open-field test was conducted in a black circular arena (diameter: 1.0 m) enclosed by a 40-centimeter high wall under uniform illumination. Each rat (13 Wistar rats and 8 WARs) was allowed to freely explore the arena for 10 min. The whole experiment was recorded on VHS, digitalized, and processed offline by an automated homemade position detector. The open-field arena was divided in three circular areas: inner, inter, and outer as a gradient of aversiveness. The results are expressed as mean ± SEM for each circular area, and the analyses were performed by two-way ANOVA and Bonferroni's post hoc test in order to determine statistical significance. Distance crossed, number of visits, and time spent in each circular area on each visit were the variables measured by the automated position detector. Number of grooming and rearing episodes were also counted by a well-trained researcher who is blinded to the study.

2.5. Elevated plus maze

The elevated plus maze (EPM) consists of a device with two opposite open arms (50 × 10 cm) and two closed arms (50 × 10 cm), also opposing, elevated 45 cm from floor level, and a central platform (10 × 10 cm) [43]. The animals (6 Wistar rats and 5 WARs) were placed individually in the center of LCE with the head turned to one of the closed arms, and their behavior was evaluated for 5 min. Each trial was video recorded by digital camera linked to a computer in an adjacent room. The recordings were analyzed offline by a highly-trained researcher using X-PLO-RAT (version 3.3). The behavioral parameters evaluated in this test were the number of entries and length of stay of the animal in the open arms, closed arms, and center area of the equipment. The results are expressed as mean ± SEM for each area, and the analyses were performed by two-way ANOVA and Bonferroni's post hoc test in order to determine statistical significance.

2.6. Marble burying

This test is based on the trend of laboratory animals to hide potentially aversive objects [44]. Anxiolytic and antidepressant drugs reduce

or suppress this behavior [45]. Animals (9 Wistar rats and 9 WARs) were placed in Plexiglas boxes containing sawdust and 16 glass marbles randomly distributed across the surface. After 5 min, the number of balls hidden (minimum 2/3 their depth) by the animal was quantified. The statistical measurement was performed by Student's t-test.

2.7. Forced swim test

This test assesses the response from a rodent to a drowning threat when exposed to an inescapable stress situation. The animals (15 Wistar rats and 13 WARs) were tested twice, during which they were forced to swim in an acrylic cylinder (50-centimeter height and 30-centimeter diameter) filled with water (28 °C), and from which they could not escape. The first trial, for habituating the animal to the protocol, lasted 15 min. Then after 24 h, a second test was performed lasting 5 min. The time spent by the animals at swimming, climbing, and immobility behavior was measured and statistically analyzed by two-way ANOVA and Bonferroni's post hoc test. The latency to first episode of immobility was also quantified.

2.8. Step-down inhibitory avoidance

The test was performed in a transparent acrylic box (50 × 25 ×25 cm) whose floor was a grill of metal bars 0.5 cm thick with 1.0–centimeter spacing. A wooden platform (25 × 10 × 5 cm) was positioned at the leftmost extreme of the box. There was no habituation to the task apparatus before the behavioral procedure. All rats were acclimatized to the experiment room for 1 h for three days before the experiment day. Animals (5 Wistar rats and 6 WARs) were gently put on the platform to evaluate the step-down latency on the acquisition trial (maximum of 40 s) and on the two memory tests (maximum of 600 s) performed 1.5 and 24 h after training. When the animal stepped down the platform placing its four paws on the metal grid, a 0.8-milliampere, 2-second scrambled footshock was delivered, after which the rat was immediately removed from the box. No shock was administered to the rat during the two memory tests. The latency to step down was used as the behavioral measure of aversive memory retention. Data were analyzed using the two-way ANOVA and Bonferroni's post hoc test.

2.9. Two-way active avoidance

The two-way active avoidance test was performed by another eight ($n = 8$) Wistar rats and nine ($n = 9$) WARs in the same transparent acrylic box used for step-down inhibitory avoidance with the wooden platform removed. An opaque barrier was placed at the center of the box, parallel to and 1.5 cm above the bars in order to differentiate both sides of the shuttle box. All rats were acclimatized to the experiment room for 1 h for three days before the experiment day. Each rat was habituated for 5 min to the apparatus on each of the two days before and on the first day of the experiment, immediately previous to the first training session. On the second day of the experiment, rats were submitted to a second training session with a 60-second habituation period. A training session consisted of 50 trials that started when the rat's body was completely on one side of the box. On each trial, rats received the conditioning stimulus (4 kHz, 80 dB SPL tone presented for 25 s or until the animal crossed to the opposite side of the box), and five seconds after the beginning of the CS, rats received the unconditioned stimulus (0.8-milliampere scrambled footshock delivered for 20 s or until the animal crossed to the opposite side of the apparatus). The tone used for conditioning had a sound intensity of at least 25 dB SPL below seizure threshold levels. Thus, it is important to highlight that the animals did not present any seizure-like behavior during the TWAA test. The intertrial intervals varied randomly between 14 and 26 s. A correct avoidance response was counted when the rat crossed to the opposite side of the box during the first 5 s of the presentation of the auditory CS, without receiving the foot shock. The number of avoidance responses in consecutive blocks of ten trials was taken as the behavioral measure of learning and memory. To compare the number of avoidance reactions between groups, the two-way ANOVA and Bonferroni's post hoc test were used.

3. Results

The forced swim test revealed distinct behavior patterns regarding WAR and Wistar controls. Both strains remained for a similar percentage of time at swimming behavior (Fig. 1A), showing that WARs have no motor system impairment. Nevertheless, WARs presented lower climbing (Fig. 1A) and longer immobility behaviors (Fig. 1A). Furthermore, WARs' latency to immobility was significantly shorter than resistant rats (Fig. 1B). In the assessment of anhedonia, a greater preference for sucrose by WARs compared with Wistar rats after 48 h was observed (Fig. 1C). In relation to total fluid intake, an increase in the amount of sucrose ingested by WARs (Fig. 1D) and no significant difference in water consumption compared with resistant rats was observed.

In the open field, although both strains remained in the inner circle for the same amount of time (highest aversiveness), WARs spent significantly more time in the inter circle and less in the outer one compared with resistant rats (referring here and throughout the paper as the Wistar rats that are resistant to audiogenic seizures) (Fig. 2A) [56]. Regarding the number of entries in each of the areas (Fig. 2B), an increase in the number of entries in the intermediate area for WAR compared with controls was observed. Despite this fact, the distance crossed by both strains in the circle areas was similar (Fig. 2C) which suggests that WAR had no limitation in their motor system. No significant difference was noted regarding the number of rearing episodes ($p = 0.25$—Student's t-test) and grooming ($p = 0.74$—Student's t-test) expressed during the 600 s of open-field test (Fig. 2D). In the elevated plus maze test, susceptible animals spent more time in the central area and less time in the closed arms compared with controls, with no significant difference regarding the open arms (Fig. 2E). As for the number of entries, WARs significantly approached only the central area more frequently compared with resistant rats, and no difference regarding the remaining areas was noted (Fig. 2F). In the marble burying test, WARs buried significantly less marbles compared with controls (Fig. 2G).

The social preference test revealed a significantly higher preference of WARs for the juvenile cospecific than for the object, compared with Wistar controls (Fig. 3A). In fact, WAR animals showed an important decrease in object exploration compared with resistant rats with no statistical difference regarding juvenile exploratory behavior (Fig. 3B).

The aversive memory tests were performed by two different paradigms, inhibitory and active avoidance. In the former (Fig. 4A), WARs demonstrated significantly higher latencies to step down the platform than resistant rats (1.5 and 24 h after training). It is important to highlight that only one WAR animal stepped down at the 1.5 h test. Nevertheless, WARs demonstrated a significantly inferior performance at the two-way active avoidance test (Fig. 4B) compared with controls. The conditioned avoidance responses performed by each animal in 10 blocks of 10 trials are shown in Fig. 4B. The resistant animals demonstrated significantly better performance compared with WARs in the majority blocks (except the 2 and 6 blocks). In the second training session, although not significantly different from the first day, the rats demonstrated an improvement of corrected responses, especially in the seventh block, suggesting a positive effect on the long-term memory learning. The WARs, in turn, showed no improvement in performance from the first to the second day.

4. Discussion

This work employed a series of behavioral tests in order to investigate possible common underlying mechanisms between WAR seizure

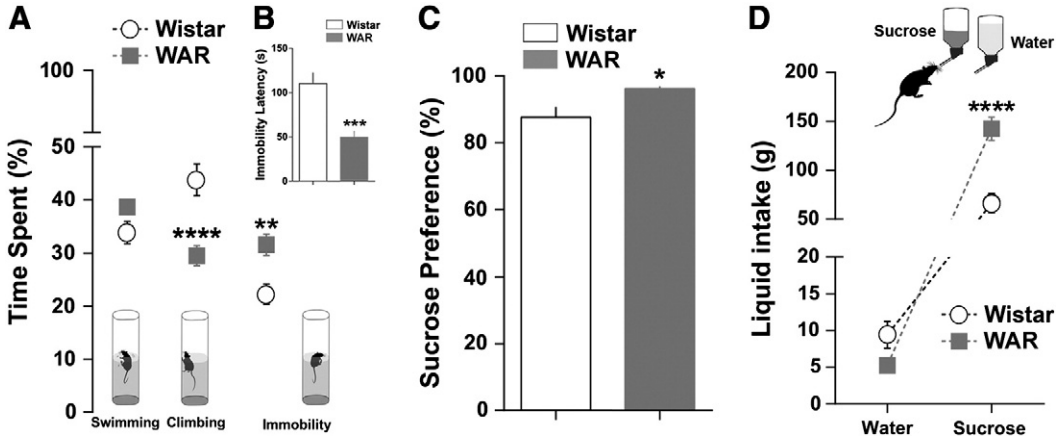

Fig. 1. Forced swim test. A: Time spent in swimming, climbing, and immobility behaviors (**p < 0.01 and ****p < 0.0001—two-way ANOVA–Bonferroni's post hoc test, mean ± SEM). B: Latency for the first immobility behavior (***p < 0.001—Student's t-test, mean ± SEM). Anhedonic behavioral assessment by sucrose consumption. C: Sucrose preference measured as percentage over total fluid consumed (*p < 0.05—Student's t-test, mean ± SEM). D: Liquid intake (water and sucrose solution) (****p < 0.0001—Student's t-test, mean ± SEM).

susceptibility and traits regarding mood disorders and learning and memory impairments. Results suggest that circuits involved in the emergence of emotional and cognitive behaviors [46] may correlate with the dysfunctional known epileptogenic circuits from WAR's inherited genetic susceptibility. Although the WARs showed behavioral abnormalities that clearly correlate with depressive-like symptoms, e.g., increased despair response, results also show some apparently paradoxical behavioral traits that do not mimic the psychiatric comorbidities found in human epilepsy. Before discussing each finding in more detail, some considerations must be made regarding the "classical" animal tests used in order to correlate with human psychiatric disorders. First, some behavioral tests might be unsuitable for evaluating animals subject to the particular "brain-state" imposed by the epileptic condition. Second, depending on the particular animal model of epilepsy, a progressive evolution of the epileptic condition may gradually compromise different brain circuitries that, even though not necessarily affecting the behavioral manifestation of the seizure itself during ictus, may have a profound effect on proper brain function during interictal

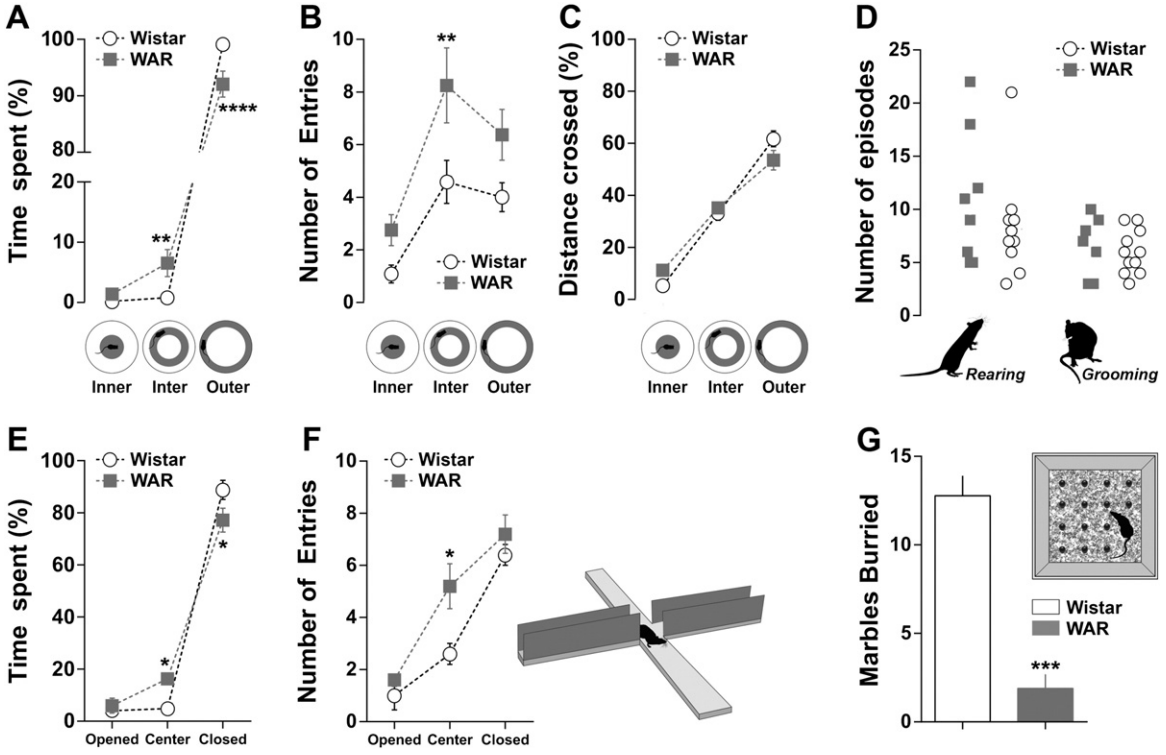

Fig. 2. Distinct anxiety behavior tests performed Wistar and WAR rat strains. A, B, C, D: Spontaneous behaviors expressed in open-field arena during the 600-second test. A: Percentage of time spent in each of the circular areas (**p < 0.01 and ****p < 0.0001—two-way ANOVA–Bonferroni's post hoc test, mean ± SEM). B: Number of entries in each of the areas (**p < 0.01—two-way ANOVA–Bonferroni's post hoc test, mean ± SEM). C: Percentage of distance crossed over each of the circular areas of open-field arena (mean ± SEM, no significant difference detected—two-way ANOVA–Bonferroni's post hoc test). D: Total number of grooming and rearing episodes (no significant difference detected—Student's t-test). E, F: elevated plus maze expressed behaviors. E: Percentage of time spent in each area of the apparatus (*p < 0.05—two-way ANOVA–Bonferroni's post hoc test, mean ± SEM) F: Total number of entries into open and closed arms and central area of the elevated plus maze (*p < 0.05—two-way ANOVA–Bonferroni's post hoc test, mean ± SEM). G: Defensive anxiety behavior expressed as number of marbles buried (***p < 0.001—Student's t-test, mean ± SEM).

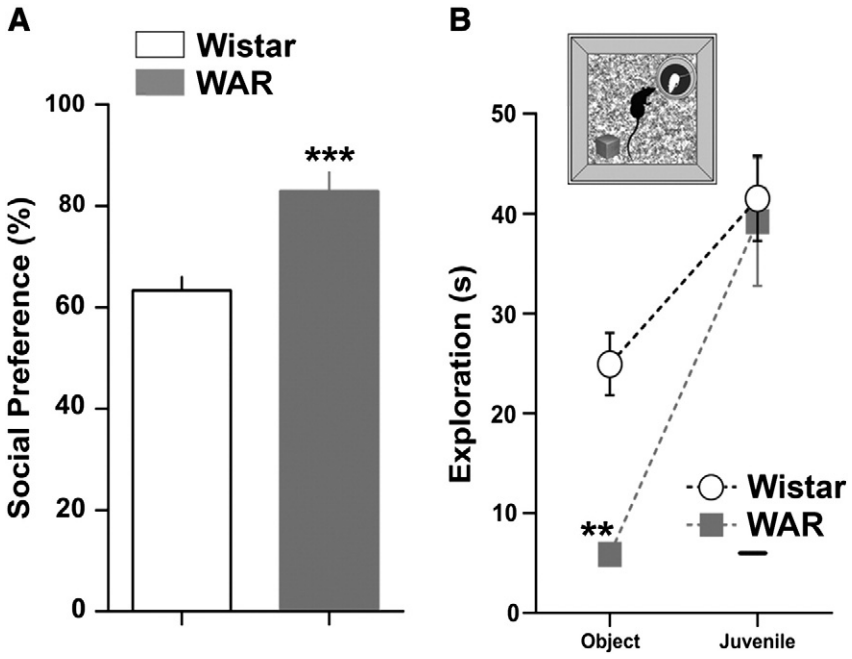

Fig. 3. A: Social preference measured as percentage over total exploratory time (***p < 0.001—Student's t-test, mean ± SEM). B: Exploration time for object and juvenile stimulus during the test (**p < 0.001—Student's t-test, mean ± SEM).

behaviors. And third, the fact that a specific animal model of epilepsy lacks the perfect parallelism with cardinal expressions of human depression (e.g., anhedonia, despair, and anxiety) does not necessarily imply that the model is an invalid tool for epileptology. In fact, the differences regarding the neural substrates recruited during the epileptogenic process among the various animal models is a very insightful way to address compromised circuitry during interictal behavioral challenges.

The shorter latency for immobility found in the FST (Fig. 1A) indicates increased behavior despair or lower threshold to evaluate a helplessness situation, since the animal is submitted to a no-escape paradigm [47]. The apparent paradox between these findings and the prohedonic results from the SPT (Fig. 1C–D) may be explained by the fact that these two behavioral traits, although forming the pillars of depressive-like behavior, do not necessarily share common neural substrates. Lim et al. [48] demonstrated that blocking MC4R synaptic mediated changes in the nucleus accumbens prevents the behavioral manifestation of anhedonia without significant changes to FST immobility measurements. The acute audiogenic seizure of WARs is mediated by the brainstem, i.e., primarily mesencephalic [3] but also recruits hypothalamic circuitry [21], only progressing to more rostral prosencephalic structures after audiogenic kindling (AK). In fact, different animal models of epilepsy have been shown to present one trait without the other, and vice versa (for review see [49]). In addition, studies designed to evaluate how developmental and social factors may influence affective disorders have shown, through Factor Cluster analysis [50], that behavioral markers may display competing regression coefficients extracted from forced swim test (FST), sucrose preference test (SPT), and open-field test (OFT). Sáenz et al. [50] suggest that increased immobility in FST correlate with increased sucrose preference in the SPT in order to better predict behavioral despair traits from animals subject to different environmental/social conditions. The idea of competing behaviors is also evident in the AK, where WAR's brainstem severity index [51] and a limbic index [52] respectively decrease and increase throughout seizure repetition [53]. The apparent linear expression of a simple bidirectional negative feedback network is certainly an oversimplification, as evidence suggests that continuing the AK paradigm eventually results in a bidirectional positive feedback between the midbrain and forebrain structures. The apparent initial "endogenous anti-epileptic" mechanism, that later becomes part of a different integrated epileptogenic circuit, may share common network architectural principles with the FST and SPT underlying neuronal circuitry, especially if evidence from ongoing studies shows that SPT performance of WARs reverses after AK. In other words, circuits involved in the behavioral despair and hedonic responses might also reflect the midbrain nature of the acute audiogenic seizure of WARs, which as argued before, could change during AK. Furthermore, considering that hippocampal lesions have been shown to reduce burying behavior [54] and that WARs performed poorly in the MBT, behavioral data further correlate with the initial brainstem nature of the WAR seizure. Altogether, these considerations highlight a possible advantageous aspect of using a model where seizures can be triggered repeatedly on demand, since such a model would make it easier to investigate the contribution of both genetic susceptibility and seizure occurrence factors in the development of behavioral abnormalities, in spite of the contrast to the obvious clinical disparity of not presenting spontaneous seizures.

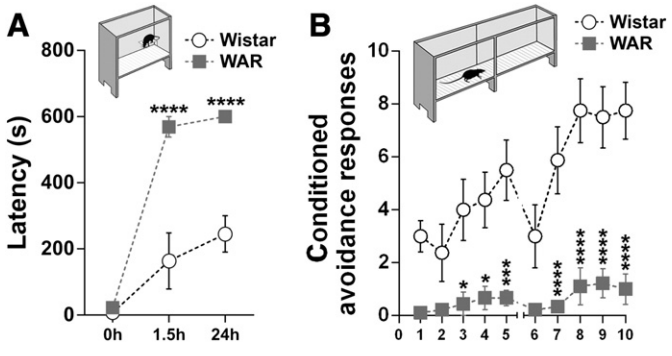

Fig. 4. Aversive memory tests performed on Wistar and WAR rat strains. A: Latency to step down the wooden platform during step-down inhibitory avoidance test (****p < 0.0001—two-way ANOVA–Bonferroni's post hoc test, mean ± SEM). B: Number of conditioned avoidance responses executed by Wistar and WAR during two-way active avoidance grouped in ten blocks of ten trials across two training/testing sessions, one session per day, 50 trials per session. Results plotted as mean and SEM. Dashed line separates results of both sessions (*p < 0.05, ***p < 0.001, ****p < 0.0001—two-way ANOVA–Bonferroni's post hoc test).

The marble burying test is also a measure of anxiety. According to MBT results, WARs could be considered less anxious than resistant rats. But the EPM and OFT results were not conclusive, despite showing a tendency of nonkindled WARs being less anxious than resistant animals. The WARs spent significantly less time on the margins of the arena than resistant controls, spending more time on inner sections, i.e., more aversive areas of the arena. Indeed, susceptible animals not only spent more time, but also approached the intermediate area circle significantly more often than resistant rats. In the EPM, WARs visited more often and stayed longer in the central area (Fig. 2A and B). Traditionally, permanence in the central area of the elevated plus maze is considered an ambiguous measure [55]. The ambiguity of the central area can, on the other hand, be considered an important context contingency of the EPM paradigm, possibly uncovering a decision-making issue regarding the strain, for it is a region where, in comparison with the closed arms, the animal becomes more exposed to a potential threat. Moreover, if one considers that the open/center/closed arm regions convey intrinsically different degrees of aversiveness; WARs perceived the open arm's high aversive component as do controls, but have a potentiated anxiety response to the lower aversive scenario of the closed arms; as in Garcia-Cairasco et al. [56]. In addition, data showing that WARs approached more times the central area of the EPM, an intermediate degree of aversiveness, favors the interpretation that WARs show compromised ability to evaluate mild-to-intermediate aversiveness situations. Comparing our results with those obtained by Garcia-Cairasco et al. [56], in our study, both resistant controls and WARs visited the enclosed arms about 6–7 times, as many times as Garcia-Cairasco et al. [56] resistant Wistar rats, favoring an interpretation that regarding this contingency, Garcia-Cairasco et al.'s [56] nonkindled rats would be less anxious than nonkindled WARs from the UFMG breeding stock. But the percentage of time spent in open arms and the number of entries in the distal half of the open arms performed by their susceptible animals favor the opposite interpretation, that WARs are more anxious than resistant Wistar rats. Genetic differences between Ribeirão Pretos's and UFMG's WAR breeding stocks may account for such behavioral differences. Besides reducing burying behavior [57], it has been shown that hippocampal lesions increase open arm and central area permanence in the EPM and OFT, respectively [58]. Thus, despite not significantly staying longer or approaching the inner circle of the OFT or the open arms of the EPM test more often, the results of these tasks show a tendency of WARs being less anxious than resistant animals when presented with situations of mild aversiveness, which is congruent with the marble burying results.

The prompt interpretation of the much better performance of WARs in the IAT (Fig. 4A), i.e., having better cognitive/memory performance, must be considered with caution in light of the previous findings regarding the enhanced despair behavioral traits of WARs. In fact, that was the purpose of complementing memory evaluation testing using the TWAA protocol (Fig. 4B). The step-down inhibitory avoidance test is an ambiguous test in the sense that performance, i.e., expression of inhibitory behavior, does not necessarily result only from learning and memory of the instrumental contingency, being also affected by Pavlovian fear memory expression dependent on subcortical circuits [59–61]. Therefore, the inhibitory avoidance test may suffer interference from mood disorders associated with potentiated fear to inescapable situations. The learning and memory deficits of WARs in the two-way active avoidance, which is an instrumental paradigm highly dependent on cortical and temporal processing [62–64], are more congruent with the mnemonic impairments observed in patients with TLE. Thus, by contrasting the paradoxical performance results in both learning and memory tests (i.e., IAT and TWAA), the inherited low threshold for evaluating helplessness situations (as depicted in the FST) might be interfering with the experimental memory evaluation of WARs. In summary, although the IAT and TWAA tests analyzed together may suggest that WARs are unable to properly evaluate degrees of aversiveness within environmental or experimental contingencies, results regarding cognitive impairment are most likely compromised. The WAR's abnormal capacity to evaluate aversive contingencies is further corroborated by the results from the MBT (Fig. 2G). The marble burying test was designed to evaluate obsessive–compulsive behavior, anxiety (as observed by the referee), and neophobia (fear of new things). It has been suggested that this kind of behavior is probably associated with the defensive burying typically found in rodents. The fact that marbles modified to have specific aversive characteristics (e.g., coated with aversive substances or electrified) are buried more than innocuous ones (i.e., defensive behavior) reinforces the hypothesis that WARs do not properly evaluate "mild" aversive stimuli.

In summary, nonkindled WARs present a complex mosaic of abnormal interictal behaviors apparently related to a compromised ability to evaluate emotional contingencies with different degrees of aversiveness, especially inescapable situations, either not properly recognizing or over interpreting current aversiveness. These interictal behavioral alterations, given that rats were not submitted to any seizure inducing chronic protocol, are related to WARs' proneness to seizure and compensatory mechanisms, shedding light on the relation between epilepsy and psychiatric and cognitive comorbidities.

Acknowledgments

This work was supported by Fundação de Amparo à Pesquisa do Estado de Minas Gerais (CBB-APQ-02290-13 and TEC-APQ-01084-13), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (MINCYT 0951/2013 and PNPD 02875/09-8), and Conselho Nacional de Desenvolvimento Científico e Tecnológico (454458/2014-2, 476681/2012-0, 470532/2012-2, and 306767/2013-9).

Conflict of interest

We confirm that we have read the Journal's position on issues involving ethical publication and affirm that this report is consistent with its guidelines. In addition, the authors declare that there is no conflict of interest in this work and that no secondary interest (e.g., financial gain) has in any way interfered or influenced the authors' professional judgment regarding the information being divulged.

References

[1] Loscher W, Honack D. Intravenous carbamazepine: comparison of different parenteral formulations in a mouse model of convulsive status epilepticus. Epilepsia 1997;38:106–13.
[2] Fisher RS. Redefining epilepsy. Curr Opin Neurol 2015;28:130–5.
[3] Garcia-Cairasco N, Terra VC, Doretto MC. Midbrain substrates of audiogenic seizures in rats. Behav Brain Res 1993;58:57–67.
[4] Moraes MF, Del Vecchio F, Terra VC, Garcia-Cairasco N. Time evolution of acoustic 'information' processing in the mesencephalon of Wistar rats. Neurosci Lett 2000; 284:13–6.
[5] Doretto MC, Oliveira-e-Silva M, Ferreira-Alves DL, Pires SG, Garcia-Cairasco N, Reis AM. Effect of lactation on the expression of audiogenic seizures: association with plasma prolactin profiles. Epilepsy Res 2003;54:109–21.
[6] Doretto MC, Garcia-Cairasco N. Differential audiogenic seizure sensitization by selective unilateral substantia nigra lesions in resistant Wistar rats. Physiol Behav 1995; 58:273–82.
[7] Garcia-Cairasco N, Sabbatini RM. Possible interaction between the inferior colliculus and the substantia nigra in audiogenic seizures in Wistar rats. Physiol Behav 1991; 50:421–7.
[8] Garcia-Cairasco N, Sabbatini RM. Neuroethological evaluation of audiogenic seizures in hemidetelencephalated rats. Behav Brain Res 1989;33:65–77.
[9] Garcia-Cairasco N, Rossetti F, Oliveira JA, Furtado Mde A. Neuroethological study of status epilepticus induced by systemic pilocarpine in Wistar audiogenic rats (WAR strain). Epilepsy Behav 2004;5:455–63.
[10] Galvis-Alonso OY, Cortes De Oliveira JA, Garcia-Cairasco N. Limbic epileptogenicity, cell loss and axonal reorganization induced by audiogenic and amygdala kindling in Wistar audiogenic rats (WAR strain). Neuroscience 2004;125:787–802.
[11] Chakravarty DN, Faingold CL. Differential roles in the neuronal network for audiogenic seizures are observed among the inferior colliculus subnuclei and the amygdala. Exp Neurol 1999;157:135–41.
[12] Gerdes JS, Keller SS, Schwindt W, Evers S, Mohammadi S, Deppe M. Progression of microstructural putamen alterations in a case of symptomatic recurrent seizures using diffusion tensor imaging. Seizure 2012;21:478–81.

[13] Luo C, Li Q, Lai Y, Xia Y, Qin Y, Liao W, et al. Altered functional connectivity in default mode network in absence epilepsy: a resting-state fMRI study. Hum Brain Mapp 2011;32:438–49.
[14] Labate A, Ventura P, Gambardella A, Le Piane E, Colosimo E, Leggio U, et al. MRI evidence of mesial temporal sclerosis in sporadic "benign" temporal lobe epilepsy. Neurology 2006;66:562–5.
[15] Detour J, Schroeder H, Desor D, Nehlig A. A 5-month period of epilepsy impairs spatial memory, decreases anxiety, but spares object recognition in the lithium–pilocarpine model in adult rats. Epilepsia 2005;46:499–508.
[16] Bernasconi N. Mesial temporal damage in temporal lobe epilepsy: a volumetric MRI study of the hippocampus, amygdala and parahippocampal region. Brain 2003;126:462–9.
[17] Magalhaes LH, Garcia-Cairasco N, Massensini AR, Doretto MC, Moraes MF. Evidence for augmented brainstem activated forebrain seizures in Wistar Audiogenic rats subjected to transauricular electroshock. Neurosci Lett 2004;369:19–23.
[18] Scarlatelli-Lima AV, Magalhaes LH, Doretto MC, Moraes MF. Assessment of the seizure susceptibility of Wistar audiogenic rat to electroshock, pentylenetetrazole and pilocarpine. Brain Res 2003;960:184–9.
[19] Drumond LE, Kushmerick C, Guidine PAM, Doretto MC, Moraes MFD, Massensini AR. Reduced hippocampal GABAergic function in Wistar audiogenic rats. Braz J Med Biol Res 2011;44:1054–9.
[20] Mesquita MBS, Medeiros DD, Cota VR, Richardson MP, Williams S, Moraes MFD. Distinct temporal patterns of electrical stimulation influence neural recruitment during PTZ infusion: an fMRI study. Progr Biophys Mol Biol 2011;105:109–18.
[21] Fonseca AG, Santos RA, Moraes MF, Leite MF, Doretto MC. Vasopressinergic hypothalamic neurons are recruited during the audiogenic seizure of WARs. Brain Res 2005;1038:32–40.
[22] Umeoka EH, Garcia SB, Antunes-Rodrigues J, Elias LL, Garcia-Cairasco N. Functional characterization of the hypothalamic–pituitary–adrenal axis of the Wistar audiogenic rat (WAR) strain. Brain Res 2011;1381:141–7.
[23] Zohar I, Weinstock M. Differential effect of prenatal stress on the expression of corticotrophin-releasing hormone and its receptors in the hypothalamus and amygdala in male and female rats. J Neuroendocrinol 2011;23:320–8.
[24] Ribak CE, Baram TZ. Selective death of hippocampal CA3 pyramidal cells with mossy fiber afferents after CRH-induced status epilepticus in infant rats. Brain Res Dev Brain Res 1996;91:245–51.
[25] Baram TZ, Schultz L. Corticotropin-releasing hormone is a rapid and potent convulsant in the infant rat. Brain Res Dev Brain Res 1991;61:97–101.
[26] Navarria A, Tamburella A, Iannotti FA, Micale V, Camillieri G, Gozzo L, et al. The dual blocker of FAAH/TRPV1 N-arachidonoyl serotonin reverses the behavioral despair induced by stress in rats and modulates the HPA-axis. Pharmacol Res 2014;87:151–9.
[27] Cho KO, Lybrand ZR, Ito N, Brulet R, Tafacory F, Zhang L, et al. Aberrant hippocampal neurogenesis contributes to epilepsy and associated cognitive decline. Nat Commun 2015;6:6606.
[28] Witt JA, Helmstaedter C. Cognition in the early stages of adult epilepsy. Seizure 2015;26:65–8.
[29] Brooks-Kayal AR, Bath KG, Berg AT, Galanopoulou AS, Holmes GL, Jensen FE, et al. Issues related to symptomatic and disease-modifying treatments affecting cognitive and neuropsychiatric comorbidities of epilepsy. Epilepsia 2013;54(Suppl. 4):44–60.
[30] Pearson JN, Schulz KM, Patel M. Specific alterations in the performance of learning and memory tasks in models of chemoconvulsant-induced status epilepticus. Epilepsy Res 2014;108:1032–40.
[31] Rocha L, Alonso-Vanegas M, Martinez-Juarez IE, Orozco-Suarez S, Escalante-Santiago D, Feria-Romero IA, et al. GABAergic alterations in neocortex of patients with pharmacoresistant temporal lobe epilepsy can explain the comorbidity of anxiety and depression: the potential impact of clinical factors. Front Cell Neurosci 2014;8:442.
[32] Kanner AM. Depression and epilepsy: a review of multiple facets of their close relation. Neurol Clin 2009;27:865–80.
[33] Kandratavicius L, Hallak JE, Carlotti Jr CG, Assirati Jr JA, Leite JP. Hippocampal expression of heat shock proteins in mesial temporal lobe epilepsy with psychiatric comorbidities and their relation to seizure outcome. Epilepsia 2014;55:1834–43.
[34] Munger Clary HM. Anxiety and epilepsy: what neurologists and epileptologists should know. Curr Neurol Neurosci Rep 2014;14:445.
[35] Kanner AM, Schachter SC, Barry JJ, Hesdorffer DC, Mula M, Trimble M, et al. Depression and epilepsy: epidemiologic and neurobiologic perspectives that may explain their high comorbid occurrence. Epilepsy Behav 2012;24:156–68.
[36] Kandratavicius L, Ruggiero RN, Hallak JE, Garcia-Cairasco N, Leite JP. Pathophysiology of mood disorders in temporal lobe epilepsy. Rev Bras Psiquiatr 2012;34(Suppl. 2):S233–45.
[37] Rossetti F, Rodrigues MC, de Oliveira JA, Garcia-Cairasco N. EEG wavelet analyses of the striatum-substantia nigra pars reticulata-superior colliculus circuitry: audiogenic seizures and anticonvulsant drug administration in Wistar audiogenic rats (War strain). Epilepsy Res 2006;72:192–208.
[38] Papp M, Willner P, Muscat R. An animal model of anhedonia: attenuation of sucrose consumption and place preference conditioning by chronic unpredictable mild stress. Psychopharmacology (Berl) 1991;104:255–9.
[39] Sampson D, Muscat R, Phillips G, Willner P. Decreased reactivity to sweetness following chronic exposure to mild unpredictable stress or acute administration of pimozide. Neurosci Biobehav Rev 1992;16:519–24.
[40] Crawley JN, Chen T, Puri A, Washburn R, Sullivan TL, Hill JM, et al. Social approach behaviors in oxytocin knockout mice: comparison of two independent lines tested in different laboratory environments. Neuropeptides 2007;41:145–63.
[41] Novaes GF, Amado D, Scorza FA, Cysneiros RM. Social behavior impairment in offspring exposed to maternal seizures in utero. J Neural Transm 2012;119:639–44.
[42] Garcia AMB, Cardenas FP, Morato S. Effect of different illumination levels on rat behavior in the elevated plus-maze. Physiol Behav 2005;85:265–70.
[43] Pellow S, Chopin P, File SE, Briley M. Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods 1985;14:149–67.
[44] Treit D, Pinel JP, Fibiger HC. Conditioned defensive burying: a new paradigm for the study of anxiolytic agents. Pharmacol Biochem Behav 1981;15:619–26.
[45] Nicolas LB, Kolb Y, Prinssen EP. A combined marble burying-locomotor activity test in mice: a practical screening test with sensitivity to different classes of anxiolytics and antidepressants. Eur J Pharmacol 2006;547:106–15.
[46] Barros-Loscertales A, Ventura-Campos N, Sanjuan-Tomas A, Belloch V, Parcet MA, Avila C. Behavioral activation system modulation on brain activation during appetitive and aversive stimulus processing. Soc Cogn Affect Neurosci 2010;5:18–28.
[47] Castagne V, Moser P, Roux S, Porsolt RD. Rodent models of depression: forced swim and tail suspension behavioral despair tests in rats and mice. Curr Protoc Neurosci 2011. http://dx.doi.org/10.1002/0471141755.ph0508s49 [Chapter 8: Unit 8 10A].
[48] Lim BK, Huang KW, Grueter BA, Rothwell PE, Malenka RC. Anhedonia requires MC4R-mediated synaptic adaptations in nucleus accumbens. Nature 2012;487:183–9.
[49] Epps SA, Weinshenker D. Rhythm and blues: animal models of epilepsy and depression comorbidity. Biochem Pharmacol 2012;85:135–46.
[50] Brenes Sáenz JC, Villagra OR, Fornaguera Trias J. Factor analysis of Forced Swimming test, Sucrose Preference test and Open Field test on enriched, social and isolated reared rats. Behav Brain Res 2006;169:57–65.
[51] Garcia-Cairasco N, Doretto MC, Prado RP, Jorge BP, Terra VC, Oliveira JA. New insights into behavioral evaluation of audiogenic seizures. A comparison of two ethological methods. Behav Brain Res 1992;48:49–56.
[52] Racine RJ. Modification of seizure activity by electrical stimulation. II. Motor seizure. Electroencephalogr Clin Neurophysiol 1972;32:281–94.
[53] Garcia-Cairasco N, Wakamatsu H, Oliveira JA, Gomes EL, Del Bel EA, Mello LE. Neuroethological and morphological (Neo-Timm staining) correlates of limbic recruitment during the development of audiogenic kindling in seizure susceptible Wistar rats. Epilepsy Res 1996;26:177–92.
[54] Deacon RM, Rawlins JN. Hippocampal lesions, species-typical behaviours and anxiety in mice. Behav Brain Res 2005;156:241–9.
[55] Deacon RM. The successive alleys test of anxiety in mice and rats. J Vis Exp 2013;76:2705.
[56] Garcia-Cairasco N, Oliveira JA, Wakamatsu H, Bueno ST, Guimaraes FS. Reduced exploratory activity of audiogenic seizures susceptible Wistar rats. Physiol Behav 1998;64:671–4.
[57] Degroot A, Treit D. Anxiety is functionally segregated within the septo-hippocampal system. Brain Res 2004;1001:60–71.
[58] Weeden CS, Roberts JM, Kamm AM, Kesner RP. The role of the ventral dentate gyrus in anxiety-based behaviors. Neurobiol Learn Mem 2015;118:143–9.
[59] Maren S. What the amygdala does and doesn't do in aversive learning. Learn Mem 2003;10:306–8.
[60] Wilensky AE, Schafe GE, LeDoux JE. The amygdala modulates memory consolidation of fear-motivated inhibitory avoidance learning but not classical fear conditioning. J Neurosci 2000;20:7059–66.
[61] Maren S, Aharonov G, Fanselow MS. Neurotoxic lesions of the dorsal hippocampus and Pavlovian fear conditioning in rats. Behav Brain Res 1997;88:261–74.
[62] Moscarello JM, LeDoux JE. Active avoidance learning requires prefrontal suppression of amygdala-mediated defensive reactions. J Neurosci 2013;33:3815–23.
[63] Alvarez EO, Ruarte MB. Glutamic acid and histamine-sensitive neurons in the ventral hippocampus and the basolateral amygdala of the rat: functional interaction on memory and learning processes. Behav Brain Res 2004;152:209–19.
[64] Alvarez EO, Ruarte MB. Histaminergic neurons of the ventral hippocampus and the baso-lateral amygdala of the rat: functional interaction on memory and learning mechanisms. Behav Brain Res 2002;128:81–90.