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Research article

Can an aversive, extinction-resistant memory trigger impairments in walking adaptability? An experimental study using adult rats

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ABSTRACT

Cognitive demands can influence the adaptation of walking, a crucial skill to maintain body stability and prevent falls. Whilst previous research has shown emotional load tunes goal-directed movements, little attention has been given to this finding. This study sought to assess the effects of suffering an extinction-resistant memory on skilled walking performance in adult rats, as an indicator of walking adaptability. Thus, 36 Wistar rats were divided in a two-part experiment. In the first part (n = 16), the aversive, extinction-resistance memory paradigm was established using a fear-conditioning chamber. In the second, rats (n = 20) were assessed in a neutral room using the ladder rung walking test before and tree days after inducing an extinction-resistance memory. In addition, the elevated plus-maze test was used to control the influence of the anxiety-like status on gait adaptability. Our results revealed the shock group exhibited worse walking adaptability (lower skilled walking score), when compared to the sham group. Moreover, the immobility time in the ladder rung walking test was similar to the controls, suggesting that gait adaptability performance was not a consequence of the fear generalization. No anxiety-like behavior was observed in the plus maze test. Finally, correlation coefficients also showed the skilled walking performance score was positively correlated with the number of gait cycles and trial time in the ladder rung walking test and the total crossings in the plus maze. Overall, these preliminary findings provide evidence to hypothesize an aversive, extinction-resistant experience might change the emotional load, affecting the ability to adapt walking.

1. Introduction

The ability to adjust the feet to environmental context is a crucial aspect of walking adaptability and is an important skill to maintain the stability of the body and prevent falls [1,2]. Moreover, the task of adapting walking (e.g. turning, stepping to safe footfall locations etc.) is thought to be more demanding of cognitive resources, as seen in stroke survivors and older adults [2]. In addition, previous studies in humans have also suggested the activation of primary sensorimotor cortex, prefrontal area and hippocampus are associated with an adequate walking adaptability [3,4]. In the context of the cognitive demands of altering walking in response to the environment [1–4], the stress/emotional state of an individual may modulate the performance of skilled/adaptable walking to a different extent than motor acts such as reaching or walking over flat level surfaces.

Whilst the connections between stress, fear and movement have been studied in terms of freezing and fight-or-flight behaviors in different animal models, recent findings have suggested stressful/aversive experiences can also influence skilled sensorimotor functions in the short and long-term [5–8]. For example, acute and chronic stress and corticosterone infusion impair skilled movements [6,8] as well as the normal kinematic pattern during forelimb reaching in rodents [7]. Indeed, several reports support the hypothesis that aversive experiences may affect the locomotor system in both the short and long-term, although the mechanisms are not fully understood [6–10].

Consolidation and evocation of aversive memories require a complex organized neuroanatomical pathway, involving structures such as the amygdala, hippocampus [5] and, more recently discovered, the

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cerebellum [11], an important structure involved in the learning and performance of visually-cued movements (such as adjusting steps in response to what we see in the environment) [12]. Despite evidence of overlap of cognitive and emotional networks while adapting walking [4], little attention has been given to the effects of suffering an aversive experience, which generates an extinction-resistant memory, on the walking adaptability/skilled walking performance. Thus, the proposed study aims to assess, preliminarily, whether suffering an aversive experience that generates an extinction-resistant memory [5,13] can trigger impairments in walking adaptability of adult rats [6,8,14].

2. Methods

Male Wistar rats (3 months old, ± 300 g) were obtained from the *Centro de Modelos Biológicos Experimentais (CeMBE)* of the Pontifícia Universidade Católica do Rio Grande do Sul (our regular provider). The animals were housed four to a cage and kept with free access to food and water, under a 12-h light/dark cycle (lights on at 7:00 a.m.) with room temperature maintained at 22–24 °C. All procedures were in accordance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals and with the Brazilian Council for Animal Experiments Control (Concea). The Animal Bioethics Committee of the Pontifical Catholic University of Rio Grande do Sul approved the study protocol (number 15/00442).

Inescapable footshocks (IF) are an aversive and stressful event for rodents that is widely used to model fear conditioning and aversive memory in rats [5,10,15,16]. IF has been shown to elicit dysregulation of the hypothalamus–pituitary–adrenal (HPA) axis and the neurotransmitter systems as well as functional and/or structural changes in the hippocampus, amygdala, prefrontal cortex, locus coeruleus, and ventral tegmental area [5,13,16].

The fear-conditioning chamber (Panlab, Barcelona, Spain) is made of aluminum walls (35 × 35 × 35 cm) and a clear front access panel. The floor of the chamber consists of parallel grid bars made from stainless-steel, spaced 0.8 mm apart. The grid is connected to a device that delivers the footshocks and the apparatus was placed inside a sound-attenuating box (Panlab, Barcelona, Spain) with a ventilating fan. Rats were placed, one at a time, into the fear-conditioning chamber, and left to explore it for two minutes, then two intense electrical footshocks (1 mA, 60 Hz, 20 s) were delivered at a 30 s interval. Thirty seconds after the last footshock, the animal was removed from the conditioning chamber and returned to its home cage. The animals in the sham group (control) underwent the described procedure, but did not receive the footshocks. The chamber was cleaned with 70% ethanol before and after each use. This protocol was adapted from previous studies [5,13,17].

In the first part of the experiment, to assess extinction-resistance of the aversive memory [5,13,17], we randomly assigned rats into two groups: shock (N = 7) and sham (N = 9). Twenty-four (S1) and 48 h (S2) after the exposure to IF, the rats were placed back in the same apparatus for ten minutes of extinction training (no footshocks were given). The following day (72 h after the traumatic protocol), the animals were placed back in the apparatus for a three minute retention test (also without footshocks) (Fig. 1). The time (sec) of freezing behavior (no visible movement except for respiration) was assessed during the first and last three minutes of the extinction training, and throughout the three minutes the retention test. Freezing behavior was scored by two independent researchers and converted into a time percentage [13,17].

After confirming IF induced an extinction-resistant memory (first part of the experiment), we evaluated its effects on walking adaptability using the ladder rung walking test [8,14]. This test provides an evaluation of skilled walking by measuring forelimb and hindlimb placement to ladder rungs (which demand accurate foot placement); stepping, and inter-limb co-ordination, reflecting skilled walking performance, and providing an indicator of walking adaptability in rats [14]. The ladder rung walking apparatus consists of a 1 m long horizontal ladder placed 30 cm above the table with stainless-steel rungs that can be repositioned (3.0 mm diameter/1–3 cm apart); the arrangement varied between the trials to prevent the animals learning the rung positions and reinforce the need of walking adaptation, as previously described [14].

In the second part of the experiment, the animals from the shock (N = 12) and sham (N = 8) groups were tested in the ladder rung walking test at baseline (before the footshocks) and post-shock (72 h after the shocks). We assumed the time-window between delivering the footshocks and the walking adaptability assessment (~72 h post-shock) was large enough to reduce the acute effects of stress hormones, i.e., corticosterone, as suggested by previous studies [18,19]. Thus, walking adaptability would have been assessed while classical stress-related hormones were at normal levels, especially because the animals were familiarized with the ladder waking apparatus before the footshocks, preventing a novelty-related stress [20]. Two days prior to baseline evaluation, the rats were trained (four trials a day) to cross the apparatus, as an acclimation procedure [14]. During the testing sessions, the rats were filmed crossing the apparatus. We assessed the following endpoints: a) skilled walking performance score (SWPS) (% baseline performance); b) number of valid gait cycles; c) immobility time (the time spent immobile during the trial); d) number of stops during the trial; e) trial time (time spent to cross the apparatus); and f) walking speed during the trial (cm/sec).

Additionally, we performed the elevated plus maze test (≈3 h prior to the post-shock ladder rung walking test) to evaluate the animals' anxiety-like behavior [15]. After the last ladder rung walking test evaluation, animals received a lethal injection of sodic pentobarbital associated with lidocaine (10 mg/mL) (i.p.) [16].

2.1. Statistical analysis

The statistical analyses were performed using SPSS 17.0 (Statistical Package for the Social Sciences, USA). One-way ANOVA or repeated measures ANOVA were used to detect group differences, as appropriate. When nonparametric, logarithm transformation was applied before performing the tests. Pearson's or Spearman's correlation coefficient

Day - 3	Day - 2	Day - 1	Day 0	Day 1	Day 2	Day 3
Experiment, part one: Establishment of an aversive, resistant-extinction memory model						
Home cage	Home cage	Home cage	Footshocks protocol	Extinction training (S1)	Extinction training (S2)	Retention test (RT)
Experiment, part two: Effects of the aversive memory model on walking adaptation/performance.						
Ladder walking acclimation	Ladder walking acclimation	Ladder walking Baseline assessment	Footshocks protocol	Home cage	Home cage	Elevated Plus Maze Ladder Waking Post-shock assessment Euthanasia

Fig. 1. Experimental design of experiments 1 and 2. S1: first session of extinction training; S2: second session of extinction training; RT: retention test.