Delayed voluntary physical exercise restores "when" and "where" object recognition memory after traumatic brain injury

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Running title: Exercise effects on "when" and "where" memory after TBI

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ABSTRACT

Physical exercise has been associated with improved cognition and may even reduce memory deficits after brain injuries. The aims of this work were to: 1) assess whether voluntary physical exercise can reduce the deficits associated with traumatic brain injury (TBI) in two different components of episodic-like memory based on object recognition, temporal order memory ("when"), and object location memory ("where"); and 2) determine whether changes in levels of brain-derived neurotrophic factor (BDNF) in the hippocampus and prefrontal cortex, as well as alterations in hippocampal cytokines, insulin-like growth factor-1 (IGF-1) and vascular endothelial growth factor (VEGF), may influence the effects exercise has on either or both tasks. The rats were distributed into a sham group, a TBI group that remained sedentary (TBI-sed), and a TBI group that had access to a running wheel for a 25-day period from post-injury day 11 (TBI-exe). The rats were sacrificed after the "where" memory task, at post-injury day 37. Physical exercise restored the "when" and "where" memories, which had been impaired by the TBI, and increased the concentration of BDNF in the hippocampus, but not the prefrontal cortex. Neither TBI nor exercise were found to significantly affect hippocampal cytokines, IGF-1 or VEGF at this time post-injury. BDNF levels showed significant positive correlations with exercise, and with "when" (but not "where") memory. These results indicate that post-injury physical exercise restores "when" and "where" object recognition memory tasks after TBI, and that increased BDNF seems to be involved in this effect, particularly with regard to "when" memory.

Key words: Traumatic brain injury; physical exercise; temporal order memory; object location memory; cytokines; neurotrophins

1. Introduction

Traumatic brain injury (TBI) is a critical health and socioeconomic problem affecting millions of people in both low- and high-income countries across the globe, with an estimated worldwide incidence rate of 939 cases per 100,000 people [1]. Despite afflicting people of all ages, TBI is the major source of acquired brain damage in children and young adults, with a peak incidence in late adolescence. Epidemiological studies also demonstrate that males in this age range are a higher risk population compared to females [2].

In a substantial proportion of patients, TBI ultimately leads to chronic functional deficits in multiple domains, including cognitive impairment. The cognitive functions most frequently affected include attention, working memory, executive functions and long-term memory, particularly episodic memory [3,4].

Some prospective studies suggest that aerobic physical exercise may play a role in cognitive recovery after TBI in humans. Thus, positive relationships have been found between the amount of physical exercise and/or physical activity and cognitive status in individuals with a prior history of TBI [5,6]. Positive correlations between the increased fitness induced by exercising and the amount of cognitive improvement have also been reported in TBI patients [7]. Despite this, the number of studies testing the effectiveness of exercise interventions on patients with moderate to severe TBI is still scarce and the findings are inconclusive [8,9].

In contrast, animal research has made an important contribution to our understanding of the pathophysiological and cognitive effects of TBI, in addition to testing the efficacy of exercise interventions. Indeed, there is a great deal of experimental evidence that physical exercise can reduce TBI-related deficits in several learning and memory tasks [10], particularly spatial and non-spatial hippocampal-dependent tasks such as the

Morris water maze [11–13], Barnes maze [14], radial-arm maze [15], and novel object recognition memory (ORM) tasks [16–18]. ORM tasks involve components of episodic-like memory, since memory of the objects may rely on the conjunction of several components, such as their physical features (object identity or "what"), their position (object location or "where"), the time of event occurrence ("when"), and so on [19]. However, the standard version of the task [20] does not allow the different episodic-like components that are guiding the performance of the animals to be discriminated. This is particularly important, as impaired temporal order memory (the "when" component), which has been associated with prefrontal dysfunction, is highly prevalent in TBI patients [21]. In addition, and with a few exceptions [22], the effects of exercise on tasks closely linked to the prefrontal cortex have seldom been tested after experimental TBI, despite the prefrontal cortex, jointly with the hippocampus, being the brain regions most affected by physical exercise in various conditions [23].

Several variations have been introduced to the standard ORM task to address specific components of episodic-like memory and the underlying neural circuits predominantly involved in these. The variations include, but are not limited to, temporal order ("when") and object-location ("where") memory tasks [24–27]. The "when" task tests the animal's ability to discriminate between familiar objects previously presented at different times, while object location examines a subject's capacity to detect whether a familiar object has been moved to a novel location, emphasising the requirement of spatial features. The various ORM procedures also differ in the specific neural substrate involved. For example, the perirhinal cortex is involved in object recognition and plays a crucial role in the standard form of the task [24], while different behavioural and electrophysiological studies suggest that both the perirhinal cortex and the medial prefrontal cortex functionally interact in temporal order memory [25,28–31]. The

hippocampus has also been implicated in several ORM tasks, especially when spatial features are emphasised (as in the object location task), or when the delay between acquisition and retention is increased [32]. Connections between the hippocampus and the medial prefrontal cortex seem to be involved in "when" and "where" ORM tasks, but there are regional differences [31,33].

A few studies have shown impairment in either "when" or "where" ORM after experimental TBI. For example, diffuse TBI (medial fluid-percussion injury) sustained at different ages (from postnatal day 17 to 6 months of age) led to deficits in a working memory version of object location (with only a 3 min delay between sample and memory tests), tested at 8 and 9 months post-injury [34]. Paterno et al. [35] also showed deficits in an object location task after mild controlled cortical impact (CCI) injury. Object location impairment was also found after repeated concussion (by means of closed head injury) in juvenile rats [36]. Darwish et al. [37] found temporal order memory deficits in a Y-shaped maze at different times after mild-to-moderate CCI in adult rats, and impaired temporal order memory of odours in a working-memory version of this task was also reported after lateral fluid-percussion injury [38]. However, to our knowledge, the benefits of physical exercise on these specific memory tasks after TBI have not been studied.

The benefits of exercise on memory recovery after TBI are mediated by a variety of neural mechanisms. For example, exercise enhances neurogenesis in the hippocampus, and these novel adult-born neurons may contribute to cognitive recovery [16–18]. TBI induces complex alterations in the levels of several neurotrophins, including brain-derived neurotrophic factor (BDNF) [39], insulin-like growth factor 1 (IGF-1) [40], and vascular endothelial growth factor (VEGF) [41], usually characterised by increased levels soon after the injury (as part of the endogenous repair response), followed by a

subsequent decrease. There is evidence that increased levels of hippocampal BDNF [11,12,15,18,42–44] and IGF-1 [18] contribute to the benefits of physical exercise on memory after TBI, through mechanisms such as increased plasticity and neurogenesis. Less is known, however, about the role of exercise-induced increases of BDNF in the prefrontal cortex after TBI. It has also been reported that increased levels of VEGF in the cortex and the hippocampus are involved in the neuroprotective effects of pre-injury exercise [45].

Exercise can also reduce secondary neuronal death and myelin loss, and attenuate the neuroinflammatory response associated with TBI, by reducing chronic microgliosis and modifying the cytokine profile [16–18]. While there is ample evidence that cytokines are elevated in the acute phase of TBI, less is known about their profile in the brain in the chronic stage [46]. The effects of exercise on late post-injury cytokine levels are also little known and could vary depending on when the exercise was initiated. Indeed, Piao et al. [18] found that exercise starting at 1 week post-injury increased the levels of pro-inflammatory interleukin-1b (IL-1β) at 5 weeks post-injury, while it had no effect on the expression of another pro-inflammatory cytokine, interleukin-6 (IL-6), or anti-inflammatory interleukin-10 (IL-10). In contrast, exercise initiated 5 weeks after injury reduced the expression of IL-1β and increased the expression of IL-6 and IL-10 at 9 weeks post-injury.

It seems that the time that exercise is initiated after the injury may also influence other neural mechanisms involved in its memory benefits. A previous study on rats found that voluntary exercise could reduce memory deficits both when started 4 days and 4 weeks after injury, but the earlier start to exercise was associated with reduced neuron loss and attenuated microglial reactivity, while the latter seemed mainly mediated by increased neurogenesis [17]. This suggests that the neuroprotective effects of exercise are higher

shortly after the events leading to the secondary injury have been initiated. In line with this, better outcomes were found in an experimental model of concussion when exercise was started within the first 3 days after injury [47]. However, with higher severity injuries, very early initiation of exercise can result in adverse effects, because the increased energy consumption induced by exercise can prevent coping with the increased energy demands associated to injury and exacerbate the major alterations in brain metabolism produced early after TBI [10]. In addition, from the translational point of view, testing the effectiveness of delayed exercise seems highly relevant, as patients with moderate-severe TBI cannot exercise at the acute stage, when disrupted consciousness and other neurological and non-neurological symptoms requiring intensive care are common. In those studies with moderate/severe patients highly variable post-injury delays of exercise initiation have been used, but most commonly this delay ranged from several weeks to several years [8,9].

Considering all these findings, the aim of this study was to examine whether delayed voluntary physical exercise (starting 11 days after injury) can reduce the deficits induced by the CCI model of TBI in "when" (32 days post-injury) and "where" (36 days post-injury) memory tasks. According to some estimations 11 days of rat's life during late adolescence/early adulthood correspond to around 1 year of human life [48]. The intervention was thus initiated at the chronic stage of TBI.

We also examined whether the effects of exercise on these memory tasks are related to late changes (37 days post-injury) in the levels of BDNF in the hippocampus and prefrontal cortex, and in hippocampal levels of IGF-1 and VEGF, as well as in a panel of cytokines.

2. Materials and Methods

2.1. Ethics

All the procedures were conducted in compliance with European Community legislation for the protection of animals used for scientific purposes (2010/63/EU, September 22nd, 2010), and with Spanish national legislation (Royal Decree 53/2013, February 1st, 2013) regulating the care and ethical issues related to animal experimentation. The experimental protocols were approved by the Ethics Committee for Animal and Human Experimentation of Universitat Autònoma de Barcelona and the Autonomous Government of Catalonia.

2.2. Animals

The initial sample comprised 30 male Sprague-Dawley albino rats obtained from Charles River Laboratories (Abresle, France) and supplied by Prolabor (Barcelona, Spain) at 6 weeks of age. On their arrival, the rats were kept in the quarantine room for 1 week. After this, they were individually housed in 28 cm wide x 52 cm long x 18 cm high cages. Over the following 2 consecutive days, the animals were handled for approximately 5 min in order to familiarise them with the experimenters. During the entire procedure, they were kept on a 12 h light/dark cycle (light phase starting at 8.00 am) under a controlled temperature (20-22 ºC) and humidity (40-70%) regime. They were provided with approximately 28-30 g of food pellets (2014 Teklad global 14% protein rodent maintenance diet: Envigo, Valencia, Spain) on a daily basis, which is above the daily food requirements for adult rats, and water was available *ad libitum*.

2.3. Experimental groups

The rats were randomly allocated to the following three groups: TBI-sed group (n=10), TBI-exe group (n=10), and sham group (n=10). The sham and TBI-sed groups remained sedentary and were kept in their standard cages throughout the experiment, while the animals in the TBI-exe group had access to a running wheel from the 11th day post injury until the end of the experiment.

2.4. Surgery

Between 2 and 3 days after the 2nd handling session, at 7 weeks of age (considered to correspond to late adolescence) [48] the rats were subjected to stereotaxic surgery to induce CCI. Anaesthesia was induced with 5% isoflurane (Forane, Abbot Laboratories, SA, Madrid, Spain) in oxygen (2 l/min) inside a Plexiglas chamber (13 cm wide x 20 cm long x 13 cm high) for 7 min. The rats were then placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA), and the anaesthesia was continued by delivering 2% isoflurane in oxygen (1 l/min) through a nose mask. After exposing the skull through an incision on the scalp midline, a 5 mm diameter craniotomy was performed over the parietal cortex on the right hemisphere.

TBI was induced using a CCI device (Pittsburgh Precision Instruments, Pittsburgh, PA). An impactor rod with a 3 mm diameter tip, that was pneumatically operated, was positioned over the dura mater at coordinates 4.5 posterior to Bregma and 3 mm lateral to the midline. The rod impacted the brain at a velocity of 6 m/s reaching a depth of 2 mm below the dura mater layer, where it remained for 150 ms. It was angled 15° to the vertical to maintain a perpendicular position in reference to the tangential plane of the brain curvature at the impact surface. A transducer connected to the impactor measured both the velocity and the duration of the impact. These parameters have been shown to result in TBI of moderate severity [49]. Animals in the sham group were exposed to the

same procedures, but no impact was applied. Following these procedures, the scalp was sutured, and a single 0.2 ml dose of buprenorphine (Buprex, Schering-Plough, SA, Madrid, Spain) was injected subcutaneously to control postoperative pain.

2.5. Physical exercise

The rats in the TBI-exe group remained sedentary until the 11th day after injury. At this time, they were moved to other cages (26 cm wide x 48 cm long x 20 cm high) attached to a 37 cm diameter running wheel (Rat Wheel W/Brake, ENV-042: Med Associates Inc, St. Albans, VT, USA). The time and distance spent running were recorded each day using a bicycle computer (Sigma BC 506: Sigma Elektro GmbH, Neustadt, Germany). The exercise condition was maintained until the end of the experiment, for a total of 25 days.

2.6. "When" and "where" object recognition memory procedures

Training in the "when" task took place on days 30 to 32 post surgery, while the "where" procedures were carried out on days 35 and 36 after surgery (See Fig. 1). An open box (65.5 cm wide x 65.5 cm long x 35 cm high) made of a conglomerate covered with dark brown melamine was used for both tasks. The box was enclosed in a sound-attenuating cage of white melamine (72 cm wide x 72 cm long x 157 cm high) and ventilated using an extractor fan. The illumination in the centre of the training box was 30 lux.

The objects used for the procedure varied in shape, colour and size. An object composed of Lego pieces and a blue hanger were chosen for the "when" memory task. Two identical orange drink cans were used for the "where" task. These objects were not known to have any ethological significance for the rats and had never been encountered by the animals prior to the procedure. The three different objects were available in

duplicate copies. They were fixed on the floor with double-sided adhesive tape that was strong enough to prevent the rats from moving them.

All the behavioural sessions were recorded using a video camera mounted above the experimental apparatus and controlled with the video tracking software ANY-Maze (Stoelting Europe, Dublin, Ireland). The locomotion data (distances travelled in m) from each session was acquired through the ANY-Maze software, while object exploration was measured off-line by two trained experimenters unaware of the treatment condition and the object order and position.

To avoid olfactory cues, the apparatus and objects were carefully cleaned with a 70% ethanol solution and dried before each animal attempted the task, as well as after the last one had finished. Between the different daily trials, the animals were returned to their home cages in the vivarium.

2.6.1. Temporal order memory task ("when" task).

The animals were habituated to the experimental apparatus (4 habituation sessions, 2 per day at a 2 h interval, on 2 consecutive days) by being placed in the training box without any objects or stimuli being present. Each habituation session lasted 12 min. The sequence of acquisition and retention sessions is depicted in Fig. 2A. The 1st acquisition session took place 24 h after the final habituation session and was followed, 1 h later, by the 2nd acquisition trial. In both acquisition trials, the animals were allowed to freely explore two identical copies of an object for 4 min. The positions of the specific objects used in each sample trial were counterbalanced between the animals. The objects were located near adjacent corners of the apparatus, 10 cm from the surrounding walls. The subjects were placed into the box facing the centre of the opposite wall of the box.

The retention trial was performed 3 h after the 2nd acquisition trial and lasted 3 min. In this test, one copy of the objects from acquisition trial 1 ("old" object) and one copy of the objects from acquisition trial 2 ("recent" object) were used. The objects were placed in the two corners of the experimental apparatus that had contained the objects in the acquisition trials. Again, the animals were introduced into the apparatus facing the centre of the opposite wall, and the position of the old and the recent objects was also counterbalanced: the old object was presented in the right corner for half the animals and in the left corner for the other half. Thus, all the possible combinations were randomly achieved in the three groups to avoid potential bias due to the preferences of the animals for particular locations or objects.

2.6.2. Object location task ("where" task).

The ORM task based on object location began 3 days after completion of the "when" task (i.e., on post-injury day 35; see Fig. 1). For this behavioural procedure, a 12 cm diameter white disk was fixed, by means of double-sided adhesive tape, onto the upper central part of the wall nearest the position of the objects in the acquisition trial, in order to serve as a spatial cue for the relative location of the objects.

The animals received two habituation sessions separated by an interval of 1.5 h. In each session the rats were placed in the experimental apparatus with the white disk but without any of the objects being present. These sessions lasted 12 min. The acquisition session took place 24 h after the 2nd habituation session. Two identical objects (drink cans), which were different to the objects used in the *"when"* task, were fixed at an approximate distance of 10 cm from the surrounding walls in each trial. The retention session was performed 1.5 h after the acquisition trial. The objects presented to the animals were identical to those presented during acquisition, but one of them was

moved to the opposite corner ("displaced" object). The positions of the "displaced" and "stationary" objects were counterbalanced in each group. In both the acquisition and the retention trials, the animals were introduced into the training box facing the centre of the wall opposite the objects, and were allowed to freely explore them for 4 min (see Fig. 2B).

In both tasks, the variables recorded during the acquisition and retention trials were total distance travelled, latency of first exploration, and total time spent exploring each object. The time exploring the objects was measured by two experimenters blinded to the group assigned to each subject and the nature of each object (old/recent for "when" task, displaced/stationary for "where" task). Exploration was only considered when the animal directed its nose towards the object at a distance of less than 2 cm. Any other behaviour, such as looking around while sitting on or resting against the object, was not considered exploration. Since ORM tasks are based on object exploration, any animal that explored for less than 10 seconds during the acquisition trial of the "where" task or during one or both acquisition trials of the "when" task was excluded from the analysis of the corresponding memory task, since less exploration may not result in reliable encoding levels [50].

Healthy rodents show a preference for novelty. In temporal order memory tasks, greater exploration of a less-recently presented object (old object) is interpreted as recency discrimination and, thus, as memory of the temporal order in which the stimuli were presented [26]. For object location, rodents tend to spend more time exploring the displaced object rather than the stationary one, which is considered to indicate good recall of object positioning [27]. Therefore, to analyse memory, we calculated a discrimination index based on the differential exploration of old/recent ("when" task) or displaced/stationary ("where" task) objects for the retention trials, as follows.

Discrimination index of the temporal order memory task: [(time exploring the old object – time exploring the recent object) / total exploration time] *100. Discrimination index for the object location task: [(time exploring the displaced object – time exploring the stationary object)/ total exploration time] * 100. Thus, the values of the discrimination indices can range from +100 (only the old/displaced object is explored) to -100 (only the recent/stationary object is explored), with 0 being chance level. A value around 0 therefore indicates a lack of recall, while indices that are significantly higher than 0 are interpreted as indicating good recall.

Given that previous reports indicate that maximal memory in ORM tasks is expressed during the 1st minute of the test [27], the time spent exploring each object was recorded for the 1st minute of the retention tests, as well as for the total test.

2.7. Brain processing

One day after completing the object location task, on post-injury day 37, the rats were sacrificed by decapitation, and multiple brain areas were dissected on ice. The areas of interest (ipsilateral and contralateral hippocampi and prefrontal cortices) were weighed, placed in Eppendorf tubes on dry ice and immediately frozen at -70ºC. The tissue was homogenised and centrifuged, and the supernatant was collected and aliquoted for subsequent biochemical analyses.

2.7.1. BDNF protein quantification

The levels of BDNF protein in the prefrontal cortex and hippocampus were determined by enzyme-linked immunosorbent assay (ELISA) using a commercially available rat BDNF kit (RAB1138, Merck-Millipore, Darmstadt, Germany) and following the manufacturer's instructions. The assays were performed in 96-well microtitre plates pre-

coated with a specific capture antibody. 100 µl of standards or samples were introduced into the corresponding wells and incubated at 4°C overnight. The plates were then washed four times, and 100 µl of the biotinylated detection antibody was added to each well and left to incubate for 1 h at room temperature (RT). The plates were washed again, and a diluted horseradish peroxidase-streptavidin solution was added to each well and left to incubate for 45 min at RT. After washing, an ELISA colorimetric reagent was added to the wells for 30 min at RT, followed by a stop solution. Each plate was immediately read at 450 nm. A standard curve was plotted for each plate. The BDNF concentrations were assayed from the regression line of the BDNF standards.
The prefrontal cortex samples were assayed in duplicate and averaged over the two measurements. Duplicates were not possible for the hippocampal BDNF due to the large amount of sample required by the BDNF ELISA kit and the fact that the samples were also required for two other assays (IGF-1 ELISA and multiplex).

2.7.2. IGF-1 protein quantification

The levels of hippocampal IGF-1 protein were determined using the commercially available rat IGF-1 ELISA kit (R&D systems, Inc.; Minneapolis, USA) following the manufacturer's instructions. All the samples were assayed in duplicate and averaged over the two measurements.

2.7.3. Quantification of cytokines and VEGF

Multiplex bead-based immunoassay (Luminex), following the instructions of the commercially available kit (RECYTMAG-65K; Merck-Millipore; Darmstadt, Germany), was used to determine the hippocampal levels of VEGF and the following cytokines: IL-1β, IL-6, IL-10, interleukin 12p40 (IL-12), tumour necrosis factor α

(TNF-α), interleukin 4 (IL-4), and interleukin 13 (IL-13). The immunoassay was performed with beads coated with antibodies for each of the proteins under study, which were added after the addition of 25 µl of the standards and samples into the respective wells of the microtitre filter plates. This was followed by a 2 h incubation at RT under agitation at 450 rpm. The plate was then washed and incubated for 1 h at RT with detection antibodies, followed by another 30 min of incubation with streptavidin-phycoerythrin. The plate was then washed twice and sheath fluids were added to all the wells. Subsequently, the plate was read using a magnetic bead-based Luminex™ MAGPIX™ Instrument System plate reader (Invitrogen-Thermo Fisher Scientific; Waltham, MA; USA), and standard VEGF and cytokine curves were constructed automatically. The VEGF and cytokine concentrations in the experimental samples were calculated based on the standard curves generated. All the samples were assayed in duplicate and the resulting values were averaged.

All the biochemical data was expressed as pg/mg of total protein. The values were calculated for the ipsilateral and contralateral hemispheres, as well as for the two hemispheres pooled.

2.8. Statistical analyses

The statistical analyses were carried out using the Stata 16 software package (Stata Corp. Texas, USA). Statistical outliers were identified using boxplots, quartiles (Q) and interquartile ranges (IR), and those animals with values lower than Q1 – 3 x IR or higher than Q3 + 3 x IR (extreme outliers) were excluded from the corresponding analysis. One-way analysis of variance (ANOVA) was used to test for possible differences between the groups. *Post-hoc* comparisons between pairs of groups were made using the Tukey method whenever the ANOVA indicated the existence of

between-group differences. In the event of a lack of variance homogeneity, we replaced the ANOVA with the non-parametric Kruskal-Wallis test, and, if the group effect was significant, Mann-Whitney tests were applied to compare pairs of groups. The p values were only considered to be significant if they were lower than the p values resulting from Holm-Bonferroni corrections. General linear models were used to study the evolution of running behaviour in the TBI-exe group (with subsequent polynomial contrasts), while mixed analyses of variance were applied to analyse the ambulatory behaviour of the animals in the 3 groups during their habituation to the "when" and "where" memory tasks. One-sample t-tests were used to determine whether the discrimination index differed from 0 in any group. Finally, Pearson correlations were calculated for several measurements. Statistical significance was set as $p < 0.05$.

3. Results

One animal died 2 days after surgery. The final sample therefore comprised 29 rats, distributed as follows: sham (n=10), TBI-sed (n=10), and TBI-exe (n=9).

3.1. Evolution of exercise

We first examined the evolution of running distances (Fig. 3A) and times (Fig 3B) in the TBI-exe group for five blocks of five days each over the total duration of the exercise period (25 days). We found a high degree of interindividual variability in the amount of running undertaken. A general linear model for repeated measurements indicated significant differences across running blocks for both running distance [$F(4) = 4.88$; $p = .003$] and time [$F(4) = 5.69$; $p = .027$]. Polynomial contrasts indicated a linear ascending evolution of running distance across the blocks ($p = .036$), in addition to a quadratic evolution of running times ($p = .042$). Specifically, we observed a linear

increase in the running distance and time up to the 4th block, with decreases from the 4th to 5th blocks, although this inflection was only significant for running time.

3.2. Temporal order memory task ("When" task)

Mixed analysis of variance revealed a group [$F(2, 26) = 4.72$; $p = .018$] and session [$F(3, 78) = 29.17$; $p < .001$] effect on the distances travelled in the training box during the 4 habituation sessions, but no interaction between these. Simple contrasts between the experimental groups indicated that the TBI-exe group presented significantly lower distances than the TBI-sed group ($t = 3.066$; $p = .005$), while no significant differences were found between any other pairs of groups. Polynomial contrasts indicated that the evolution of distances over the 4 habituation sessions fitted a quadratic function ($t = 3.16$; $p = .003$), with a substantial decrease from the 1st to the 2nd session, and a less marked decrease thereafter.

No differences were found between groups for locomotion (travelled distances), total object exploration, and latency of first exploration in the two acquisition trials and the retention test. There were, however, 6 animals (3 in the sham group, 1 in the TBI-sed group and 2 in the TBI-exe group) that explored below the criterion. The data from these animals was therefore excluded from the discrimination index analyses.

We then examined the discrimination indices obtained for the 1st minute (Fig. 4A) and the entire test (Fig. 4B) in each group, as a measure of temporal order memory.

The one-sample t-tests indicated that the discrimination index of the 1st minute of the retention test was only significantly higher than 0 in the TBI-exe group [$t(6) = 2.61$; $p = .040$], indicating good recall only in this group. The discrimination index for the entire retention test was significantly higher than 0 (chance level) in the sham [$t(6) = 2.83$; $p = .029$], and TBI-exe [$t(6) = 2.73$; $p = .034$] groups, indicating greater exploration of the

old object. The discrimination index for the TBI-sed group approached significance ($p = .056$), but in this case the mean was below 0, indicating a tendency to explore the recent object more. Given the lack of variance homogeneity, between-group comparisons were analysed using the Kruskal-Wallis tests, which revealed the lack of significant differences between the groups for the discrimination index in the first minute of the test. In contrast, there were significant differences between the groups for the entire retention session [$\chi^2(2) = 10.96$; $p = .004$]. Comparing pairs of groups using the Mann-Whitney test and subsequently applying Holm-Bonferroni corrections indicated that the TBI-sed group had a significantly lower discrimination index than the TBI-exe ($p < .028$) and sham ($p < .007$) groups. No differences were found between the sham and TBI-exe groups.

Thus, exercise induced good recall in both the 1st minute and entire test, and restored the preference for the first object encountered after TBI when the entire retention test was taken into account.

Positive correlations were found between the amount of running in the last exercise block (which coincided with memory training and testing) and the discrimination index for the entire session (see Table 1).

3.3. Object location task ("where" task)

For the distances travelled by the animals during the two training box habituation sessions, mixed analyses of variance indicated a lack of significance for the main factors (group and session), as well as their interaction.

There were no significant differences between the groups for latency in the first object exploration episode, total exploration time, and locomotion, in either the acquisition or retention trials of the "where" task.

One of the animals in the TBI-exe group did not fulfil the exploration criterion in the acquisition trial of the "where" memory task and was, therefore, excluded from the discrimination index analyses of this task. We then investigated the effect of TBI and physical exercise on the spatial component of the ORM ("where" task) using the discrimination indices obtained for the 1st minute (Fig. 5A) and entire test (Fig. 5B) in each group. The one-sample t-tests indicated that the discrimination index differed significantly from 0 (indicating good recall) in the sham [1st minute: $t(9) = 8.20$; $p < 0.001$; entire session: $t(9) = 6.55$; $p < 0.001$], and TBI-exe [1st minute: $t(7) = 3.58$; $p = 0.009$; entire session: $t(7) = 2.64$; $p = 0.03$] groups. In contrast, the discrimination index for the TBI-sed animals did not differ significantly from 0, indicating a lack of recall. The ANOVA revealed the existence of differences between the groups in the discrimination index for the 1st minute [$F(2, 25) = 8.85$; $p = .001$], but not for the entire session. *Post-hoc* comparison between pairs of groups for the 1st minute discrimination index indicated significantly lower values in TBI-sed animals compared to sham rats ($p = .001$) and TBI-exe rats ($p = .045$), while no significant differences were found between the sham and TBI-exe groups.

Thus, both the sham and TBI-exe rats demonstrated a good "where" memory for both the entire session and the 1st minute, while the memory of the TBI-sed rats was impaired. The benefits of exercise were particularly evident in the 1st minute of the test, when the performance of the exercising rats was significantly higher compared to that presented by the TBI-sed rats, and similar to the sham group.

In contrast to the findings in the temporal order memory task, there were no significant correlations between the discrimination index in the object location task and the amount of running behaviour.

3.4. BDNF protein levels

An outlier (as defined in 2.8) was detected in the sham group with regard to BDNF in the hippocampus. This animal's data was removed from these analyses.

Figure 6A depicts the mean (+SEM) concentrations (pg/mg total protein) of BDNF in each experimental group in the ipsilateral (A) and contralateral (B) hippocampi, as well as in both hippocampi pooled (C). The ANOVA revealed no significant differences between the groups for either the ipsilateral ($p = .09$) or contralateral ($p = .09$) sides, but did indicate significant differences between them for the levels of BDNF pooled for the two hemispheres [$F(2,19) = 4.06$; $p = .033$). *Post-hoc* analyses (Tukey correction) indicated that the TBI-exe rats had significantly higher BDNF levels than the sham group ($p = .049$), while the comparison between the TBI-exe and TBI-sed groups was not significant ($p = .067$). Neither were there any differences between the sham and TBI-sed groups.

Similar analyses were performed to determine BDNF concentrations in the prefrontal cortex (Table 2). No significant differences were detected between the groups for either separate hemispheres or the two hemispheres pooled.

As indicated in Table 1, positive correlations were found between the amount of running behaviour and BDNF concentrations in the hippocampus and prefrontal cortex, mainly accounted for by the levels in the contralateral hemisphere.

In addition, positive significant correlations were also found between the discrimination index in the temporal order memory task and BDNF levels in the contralateral hippocampus ($r = 0.49$; $p = .038$), and in the pooled hippocampi ($r = 0.49$; $p = .038$), as well as in the ipsilateral prefrontal cortex ($r = 0.50$; $p = .047$). In contrast, BDNF levels showed no significant correlation with the discrimination index in the object location task.

3.5. IGF-1 and VEGF protein levels in the hippocampus

An outlier (as defined in 2.8) was found for IGF-1 in the TBI-exe group, and excluded from the analyses.

The mean values (± SD) of IGF-1 and VEGF for each experimental group are indicated in Table 2. No significant differences between the experimental groups were found in hippocampal concentrations of IGF-1 and VEGF in either hemisphere.

3.6. Cytokines

Finally, we quantified the hippocampal protein levels of a battery of pro- and anti-inflammatory cytokines for each experimental group (Table 2). The ANOVA revealed no significant differences between the three groups in the hippocampal levels of either of the cytokines.

There were, however, some significant correlations between memory performance and several cytokines. For the "when" task discrimination index (entire session), positive correlations were found with the levels of the anti-inflammatory cytokine IL-13 in the ipsilateral hippocampus for the entire sample ($r = 0.524$; $p = .025$). Additionally, positive correlations were found, again for the whole sample, between the "where" task discrimination index (entire session) and hippocampal levels of the anti-inflammatory cytokine IL-10 in the contralateral hemisphere ($r = 0.493$; $p = .016$), as well as in the two hemispheres pooled ($r= 0.417$; $p = .047$).

4. Discussion

The present data show that 25 days of voluntary physical exercise initiated with a post-injury delay of 11 days (which would correspond to the chronic stage of injury) can reverse the deficits associated with TBI in specific components of episodic memory. Specifically, exercise reduced the deficits in temporal order ("when" component) and object location ("where" component) memories, which rely on partially separated neural circuits. To our knowledge, this is the first report of exercise improving memory for these two separate components after a TBI.

4.1. Effects of TBI and exercise on temporal order memory

Novelty preference is short-lived and fades progressively with exposure to the object. For this reason, maximal exploration of the novel object is expected to take place at the beginning of the retention test [27]. Despite this, sham-operated rats only showed a significant preference for the old object when the entire test time was considered, and not in the 1st minute. It should be noted that in this group the discrimination indices, particularly in the 1st minute, presented a high degree of inter-individual variability, suggesting that the training conditions used in this task may have been associated with a high level of difficulty. This was unexpected, as good temporal order memory has been described, in healthy rats, with delays of up to 24 h between the 2nd acquisition session and the retention trial [26]. Using a temporal order memory task in a Y maze, Darwish et al. [37] found sham-operated rats displayed good memory after a 1-h delay (no longer delays were applied). Multiple variables influence task difficulty (the features of the specific objects used, the amount of habituation to the training cage, etc.), and we cannot rule out the possibility that, under the study conditions in this case, a delay of 3 h may have been too demanding for this specific kind of ORM task in operated animals.

Another unexpected finding was that a total of 6 animals (including 3 in the sham group) did not explore enough during either, or both, of the acquisition trials for their discrimination index to be considered reliable. The animals had 4 training cage habituation sessions, and all the groups seemed to be well habituated, as indicated by the significant reduction in locomotor activity between the 1st and 2nd sessions. Similarly to the procedures used in other laboratories for temporal order memory training [26], the rats were habituated to the empty training cage, but not to the presence of a novel object. This is in contrast to the usual procedures for the standard version of ORM used in our laboratory, which include a session where rats are habituated to encountering a novel object inside the training cage [16,17]. Under these conditions the number of animals that fail to explore during acquisition is very low. There is, however, the possibility that some rats might fail to explore in the acquisition sessions as a consequence of neophobia.

TBI was associated with a lack of temporal order memory in the 1st minute and to a tendency (approaching significance; $p=0.056$) to explore the most recently encountered object more, rather than the older one, in the entire session. This is very unusual in normal rats, but Darwish et al. [37] used a Y-maze to examine temporal order memory in animals with mild-to-moderate CCI and found that at several post-injury times lesioned rats also showed a preference for the recent object. The tendency to prefer the most recent object could be a consequence of proactive interference, whereby the memory of the old object could interfere in the subsequent codification of the recent one. This interference would lead to greater exploration of the recent object in the retention test, with the recent object being treated as novel [51]. Familiarity preference can also be found when subjects have only a very residual memory of the familiar (recent) object [52]. Another possible explanation of recency preference in the TBI-sed

group may be a change in the normal tendency to prefer novel (or less recent) objects. However, this seems unlikely considering that this changed preference pattern is not seen in the standard version of the ORM task (where TBI rats explore familiar and novel objects to a similar extent) [16,17].

Physical exercise resulted in good temporal order memory, evidenced both in the 1st minute and the total duration of the memory test. Moreover, exercise normalised the performance of TBI animals when the entire test was considered, since the discrimination indices of TBI-exe rats were not different from those of sham group animals and were significantly higher than those of TBI-sed rats. The results of this study therefore indicate that the benefits of physical exercise on temporal order memory do not only affect animals with no brain injury [53], or those with memory deficits induced, for example, by interpheron α [54], but also those that have suffered a TBI. In addition, the positive correlations between the discrimination index and the mean daily running distance and time shortly before and during training, suggests that the amount of running undertaken may influence the performance level in this task. These positive correlations are also in line with previous findings involving standard ORM tests on rats starting voluntary wheel running 4 weeks after CCI, but not in those starting exercise 4 days post-injury [17]. Unfortunately, most studies on voluntary physical exercise fail to report the amount of running the animals actually perform.

4.2. Effects of TBI and exercise on object location

As expected, sham rats showed a marked preference for the displaced object, indicating good "where" memory, while TBI impaired performance in this task. However, the rats who had been able to exercise performed similarly to the sham animals. Moreover,

physical exercise reversed the memory deficits associated with TBI in the 1st minute of the test.

Encoding deficits have been reported to play a crucial role in impaired performance in an object location task 6 days after lateral fluid percussion injury in mice, as indicated by the differences between lesioned and control animals in object exploration tasks in acquisition sessions [35]. While the amount of exploration during acquisition of our lesioned rats, whether exercising or sedentary, did not differ from that of the sham rats, we cannot disregard the contribution of encoding deficits to the impaired performance of lesioned rats, since both acquisition and retention trials were performed after the brain injury. However, in our opinion, memory deficits also seem rather likely, in line with the memory impairment found using the standard form of the ORM task [16,17]. Physical exercise reversed the deficits in the "where" memory task associated with TBI. Exercise has been reported to improve object location in an animal model of vascular dementia [55], but, to our knowledge no other studies had previously tested the influence of physical exercise on object location after TBI.

In contrast to that observed in the temporal order memory task, there were no significant correlations between the running distances and times and the discrimination index in the object location task, suggesting that the effects on this task are less dependent on the amount of the exercise.

4.3. Mechanisms mediating the positive effects of exercise on "when" and "where" memory

4.3.1. BDNF

BDNF exerts both neuroprotective and neuroreparative effects through various mechanisms, including the promotion of synaptic plasticity, neuronal survival, and

neurogenesis, thus contributing to reducing some of the negative consequences of TBI [39].

BDNF upregulation is one of the typical effects that mediate the influence of physical exercise on memory recovery after experimental TBI [11,42,44,56–58], although the reverse effect has also been reported. For example, Ko et al. [59] found that eight weeks of forced treadmill exercise, initiated 3 weeks post-injury, normalised hippocampal BDNF levels that were upregulated by TBI.

In line with previous reports, in this study, physical exercise increased BDNF levels in the hippocampus (but not the prefrontal cortex), and this increase was statistically significant, compared to sham animals, when the values from the two hemispheres were pooled. In addition, there were very high positive correlations between the amount of exercise and BDNF levels in both the hippocampus and the prefrontal cortex, which also agrees with previous reports [11]. Moreover, there were positive correlations between BDNF levels in both the hippocampus and the prefrontal cortex, and the discrimination index in the temporal order memory task. Since the prefrontal cortex and certain connections between this cortical region and the hippocampus are known to be involved in temporal order memory [25,31,33], the data suggests that BDNF upregulation in the hippocampus may have played a crucial role in reversing the memory deficits in the "when" memory, and BDNF in the prefrontal cortex may also have contributed to memory improvement in some way. In rats with no brain damage, one report states that increased BDNF upregulation in the prefrontal cortex is only found in high exercise intensity conditions on a treadmill, while BDNF in the hippocampus is elevated after both high and low intensity exercise [60]. In this study, therefore, the variability in the amount of running behaviour might have prevented us detecting a significant increase in prefrontal BDNF.

Increased hippocampal BDNF may also have contributed to reversing the memory deficits in exercising animals related to the object location task, since the role of the hippocampus in ORM seems particularly relevant when spatial components are emphasised. However, there were no significant correlations between the discrimination index for the "where" task and BDNF concentrations. It must be noted that the training conditions used here did not require the use of allocentric strategies, and that the single proximal spatial cue (a white disk that was always placed on the same wall of the training cage) may have alternatively facilitated the use of an egocentric strategy, or strategies based on metric and topological information (distance between the disk and any of the two objects, and between the objects themselves). It is known that the posterior parietal cortex is particularly involved in spatial tasks when they are based on proximal cues and egocentric strategies [61]. Based on previous work from our laboratory, in which the volume and location of the lesion cavity were examined, we also know that the posterior parietal cortex is damaged, on the side ipsilateral to the impact (right hemisphere), when CCI is applied over the parietal cortex. For this reason, damage to the posterior parietal cortex may be involved in the deficits found in the TBI-sed rats in the "where" task. A study on rats submitted to lateral fluid percussion injury over the right parietal cortex showed that, 14 days after the injury, the animals had impaired working memory in a metric task (where the distance between two objects was changed), but not in a topological task (where the positions of two of the four explored objects was flipped) [38]. In our work only one of the objects was moved in the retention test, while the other remained stationary. Under these conditions, both the distance between the displaced object and the proximal cue (the disk), as well as the position of the displaced object in relation to the stationary one and to the disk, were changed. On the other hand, memory was tested 1.5 h after training (not in a working

memory paradigm). Therefore the "where" task included both metric and topological components, relatively long retention times, and it may have also involved greater participation of the posterior parietal cortex (mainly with regard to the topological component), as well as other regions, including the dorsal hippocampus and the prefrontal cortex.

In summary, the mechanisms mediating the deficits found in the "where" task in the TBI-sed group, as well as the benefits of exercise on this task, may involve other critical neural structures and biochemical changes, besides those examined here.

4.3.2. IGF-1

Dysregulation of IGF-1 pathways is common after a TBI, and the benefits of several therapies seem to be related to increased levels of this neurotrophin [40]. Increased IGF-1 levels in the brain contribute to the beneficial action of physical exercise in healthy animals [62], and after brain insults, such as focal ischaemia [63]. However, no evidence of this relationship was found in this work.

4.3.3. VEGF

VEGF plays an important role in angiogenesis, vasculogenesis, and neurogenesis. Altered VEGF levels contribute to the physiopathology of TBI, particularly to cerebral hypoperfusion, ischaemia, hypoxia, haemorrhage, blood-brain barrier disruption, and oedema [41]. In healthy subjects it is known that physical exercise increases VEGF and angiogenesis, and this increase contributes to the cognitive benefits of exercise [64]. Pre-injury exercise has been found to exert neuroprotective effects against subsequent TBI, and part of this benefit is related to increased cortical and hippocampal VEGF levels [45]. Less is known about the involvement of VEGF in the effects of post-TBI

exercise. In this study, TBI did not induce significant changes in the hippocampal levels of VEGF, and the benefits of post-injury exercise were not associated with changes in VEGF levels.

4.3.4. Cytokines

Upregulation of multiple cytokines is common shortly after CCI and in other animal models of TBI [65,66]. Increased cytokines at the acute post-injury stage contribute to long-term microgliosis and other chronic inflammatory responses that may exacerbate the consequences of injury [46]. In line with this, there are reports that CCI leads to increased microglial reactivity that lasts for weeks [17,18] after the injury, even as long as a year [67]. A few reports also indicate certain changes in brain cytokine levels several weeks after CCI. Thus, increased levels of the pro-inflammatory cytokines IL-1β and IL-12 have been found in the pericontusional area (bilateral medial frontal cortex) several weeks after CCI in rats [68,69]. Increased hippocampal expression of IL-1β at 1, 5, and 9 weeks post-injury were reported after CCI in mice, while the expression of the pro-inflammatory IL-6 and the anti-inflammatory IL-10 were elevated 1 week post-injury, gradually returning to control levels up to 9 weeks post-injury [18]. In contrast, another work failed to find measurable levels of TNF-α in either the cortex or the hippocampus 3 months after lateral fluid percussion injury in rats [70].

In this work, no changes were found in either of the analysed cytokines as a consequence of either TBI or exercise. Several factors may have contributed to this disparity with other studies. Firstly, this could be related to whether the cytokine levels are measured in the perilesional area [68, 69], or in underlying regions, such as the hippocampus. Secondly, it could be affected by whether the uninjured control group is made up of sham-operated animals submitted to craniotomy (as here), operated animals

with no craniotomy [68, 69], or naïve animals [18]. Indeed, craniotomy is known to activate a neuroinflammatory response [71,72], although this response seems to last no longer than 21 days, at least in mice [72], which is well before the time at which the rats were sacrificed in this study. Finally, other factors such as lesion severity or specific post-injury time may be crucial in determining whether certain cytokine levels are altered or not.

In spite of having no effects on the concentrations of the cytokines analysed, both TBI and exercise influence other responses related to neuroinflammation, such as microgliosis. Voluntary physical exercise initiated 4 days after CCI (but not at 4 weeks) attenuated the enhancement of microglial reactivity associated with TBI at 7 weeks after injury [17]. In contrast, delayed, but not early, exercise was found to reduce the hippocampal levels of the proinflammatory cytokine IL-1β, as well as markers of the microglial M1 phenotype (galectin-3 and C1qB), while increasing the levels of the anti-inflammatory cytokine IL-10 [18]. In summary, the data on the possible effects of TBI and exercise on brain cytokine levels, as well as on other neuroinflammatory reactions, including microglial reactivity, at the chronic stage of injury are unclear and warrant further investigation.

Although positive correlations were found, for the pooled data of the 3 groups, between the discrimination indices and the levels of Il-10 or IL-13, which correspond with the regulatory role played by several cytokines in memory processes [73], these correlations do not explain the benefits of the exercise intervention in TBI rats.

5. Conclusions

Voluntary physical exercise, initiated 11 days after CCI, reversed the deficits associated with TBI in two components of episodic-like memory ("when" and "where"). The

increased BDNF levels induced by exercise (particularly in the hippocampus) seem involved in the benefits of exercise on memory performance, at least with regard to temporal order memory.

These results could be important in the clinical setting, as they indicate that delayed exercise could result in cognitive benefits in patients afflicted by moderate and severe TBI, who may be unable to begin an exercise intervention very quickly after the injury. In addition, they show that tasks related to the prefrontal cortex are also sensitive to exercise interventions after experimental TBI.

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Declaration of competing interest

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Author contributions

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Table 1. Full correlation matrix between amount of exercise (mean daily running distance and time in each running block, and mean cumulated running distance and time) and BDNF concentrations in the hippocampus and the prefrontal cortex, and between amount of exercise and discrimination index in the temporal order memory task.

			BDNF in the hippocampus			BDNF in the prefrontal cortex		
	Running amount	**Discrimination index in the temporal order memory task**	**Ipsilateral**	**Contralateral**	**Both hemispheres**	**Ipsilateral**	**Contralateral**	**Both hemispheres**
Mean daily running distance	**Days 1-5**	NS	NS	NS	NS	NS	NS	NS
	Days 6-10	NS	NS	r =0.867 p=.0115	NS	NS	NS	NS
	Days 11-15	NS	NS	r=0.956 p<.001	r=0.858 p=.0133	NS	r=0.907 p=.0048	r=0.887 p=.007
	Days 16-20	NS	NS	r=0.946 p=.0013	r=0.854 p=.0144	NS	r=0.916 p=.0037	r=0.894 p=.0066
	Days 21-25	r=0.792 p=.0336	NS	r=0.949 p=.0011	r=0.817 p=.0249	NS	r=0.818 p=.024	r=0.817 p=.0248
Cumulated running distance		NS	NS	r=0.957 p<.001	r=0.837 p=.0186	NS	r=0.841 p=.0177	r=0.838 p=.0185
Mean daily running time	**Days 1-5**	NS	NS	NS	NS	NS	NS	NS
	Days 6-10	NS	NS	r=0.812 p=.0263	NS	NS	NS	NS
	Days 11-15	NS	NS	r=0.925 p=.0024	r=0.788 p=.0353	NS	r=0.791 p=.0342	r=0.804 p=.029
	Days 16-20	NS	NS	r=0.943 p=.0014	r=0.817 p=.0248	NS	r=0.846 p=.0162	r=0.850 p=.0152
	Days 21-25	r=0.80 p=.0304	NS	r=0.905 p=.0051	r=0.755 p=.0497	NS	NS	NS
Cumulated running time		NS	NS	r=0.906 p=.0048	r=0.761 p=.0131	NS	NS	NS

T**able 2.** Mean (Std dev) concentration (pg/mg total protein) of BDNF in the prefrontal cortex, as well as IGF-1, VEGF and a set of cytokines in the hippocampus, for each experimental group.

		Ipsilateral Mean (Std Dev)	**Contralateral** Mean (Std Dev)	**Overall** Mean (Std Dev)
BDNF (prefrontal cortex)	Sham	11.33 (3.26)	14.08 (2.70)	12.74 (2.32)
	TBI-sed	9.51 (1.10)	13.8 (2.01)	12.06 (1.4)
	TBI-exe	10.6 (2.21)	15.43 (5.13)	13.53 (3.59)
IGF-1	Sham	122.12 (21.69)	117 (24.06)	119.06 (16.96)
	TBI-sed	155.96 (59.04)	131.42 (34.45)	143.05 (38.48)
	TBI-exe	114.96 (26.21)	112.82 (32.52)	109.79 (27.26)
VEGF	Sham	3.40 (0.63)	2.71 (0.51)	3.01 (0.48)
	TBI-sed	3.14 (0.59)	2.74 (0.46)	2.91 (0.49)
	TBI-exe	3.76 (1.57)	2.75 (0.66)	3.06 (0.78)
IL-1β	Sham	12.74 (3.00)	10 (2.46)	11.20 (2.05)
	TBI-sed	11.93 (2.30)	9.36 (2.03)	10.64 (1.69)
	TBI-exe	13.22 (2.80)	9.28 (2.84)	10.81 (2.29)
IL-4	Sham	1.33 (0.58)	1.51 (0.69)	1.42 (0.50)
	TBI-sed	1.31 (0.67)	1.07 (0.41)	1.16 (0.27)
	TBI-exe	2.32 (1.66)	1.39 (0.95)	1.72 (1.07)
IL-6	Sham	180.38 (23.21)	150.68 (37.65)	165.06 (23.45)
	TBI-sed	197.51 (47.61)	151.52 (49.51)	172.63 (33.36)
	TBI-exe	198.54 (101.75)	129.68 (52.77)	163.74 (67.51)
IL-10	Sham	3.75 (1.01)	3.24 (1.00)	3.43 (0.46)
	TBI-sed	3.37 (0.81)	2.92 (1.3)	3.09 (0.79)
	TBI-exe	4.37 (1.99)	2.72 (0.71)	3.31 (0.85)
IL-12	Sham	17.47 (3.26)	15.09 (3.86)	16.19 (3.48)
	TBI-sed	16.18 (3.31)	13.13 (3.07)	14.45 (2.29)
	TBI-exe	17.17 (6.8)	16.17 (5.5)	16.93 (5.04)
IL-13	Sham	1.09 (0.35)	0.75 (0.19)	0.86 (0.16)
	TBI-sed	0.81 (0.36)	0.57 (0.17)	0.68 (0.19)
	TBI-exe	1.09 (0.64)	0.56 (0.37)	0.81 (0.35)
TNF-α	Sham	0.67 (0.14)	0.56 (0.28)	0.59 (0.16)
	TBI-sed	0.81 (0.24)	0.52 (0.18)	0.65 (0.16)
	TBI-exe	0.99 (0.47)	0.49 (0.25)	0.67 (0.20)

Captions for figures

Fig. 1. Timeline of the experimental procedures.

Fig. 2. Procedures for "when" (A) and "where" (B) object recognition memory.

Fig. 3. Evolution of exercise behavior: mean daily distance (A) and time (B) run across the exercise period (distributed into blocks composed of 5 days each) in TBI-exe group.

Fig. 4. Discrimination index for the temporal order memory task. Mean (+ SEM) values in each of the 3 experimental groups during the 1st minute of the retention test (A) and during the whole test (B). *: Significant differences compared to 0, indicative of good recall. +: Significant differences compared to TBI-sed group.

Fig. 5. Discrimination index for the object location task. Mean values (+SEM) in each of the 3 experimental groups during the 1st minute of the retention test (A) and during the whole test (B). *: Significant differences compared to 0, indicative of good recall. +: Significant differences compared to TBI-sed group.

Fig. 6. Hippocampal BDNF**.** Mean (+SEM) concentration (pg/mg of total protein) of BDNF protein in the ipsilateral (A) and contralateral (B) hippocampus, as well as in both hippocampi pooled (C). *: Significant differences compared to Sham group.

Figure 1

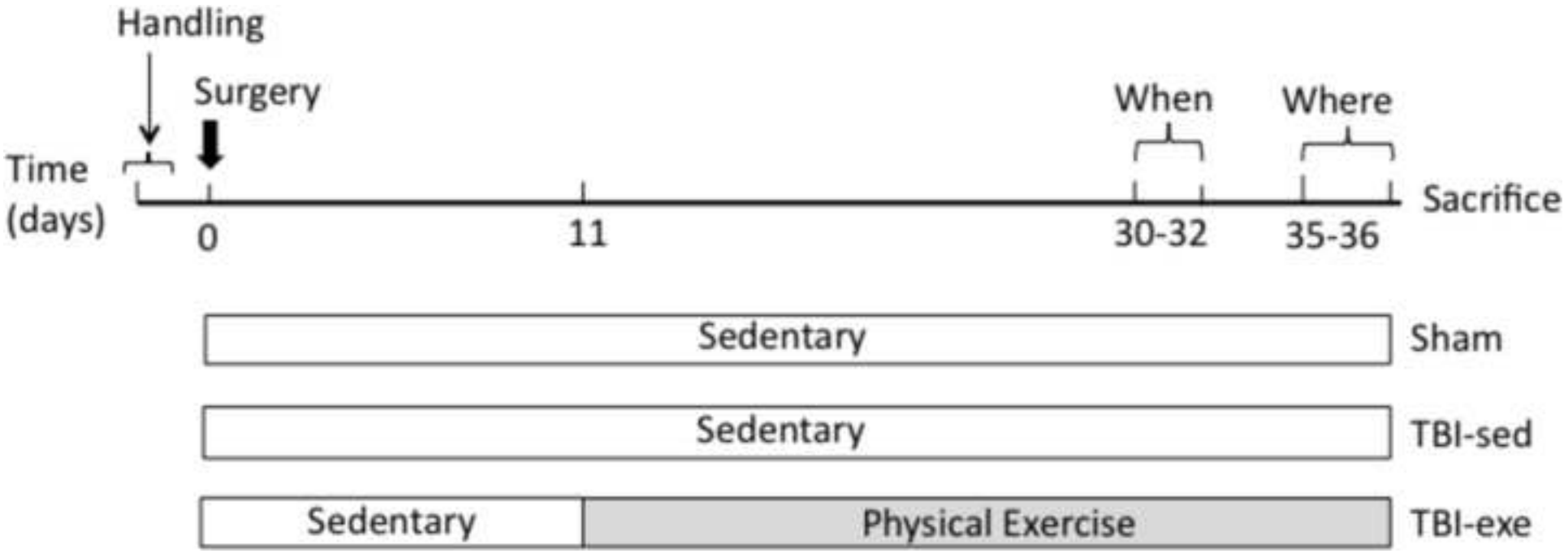

Figure 2

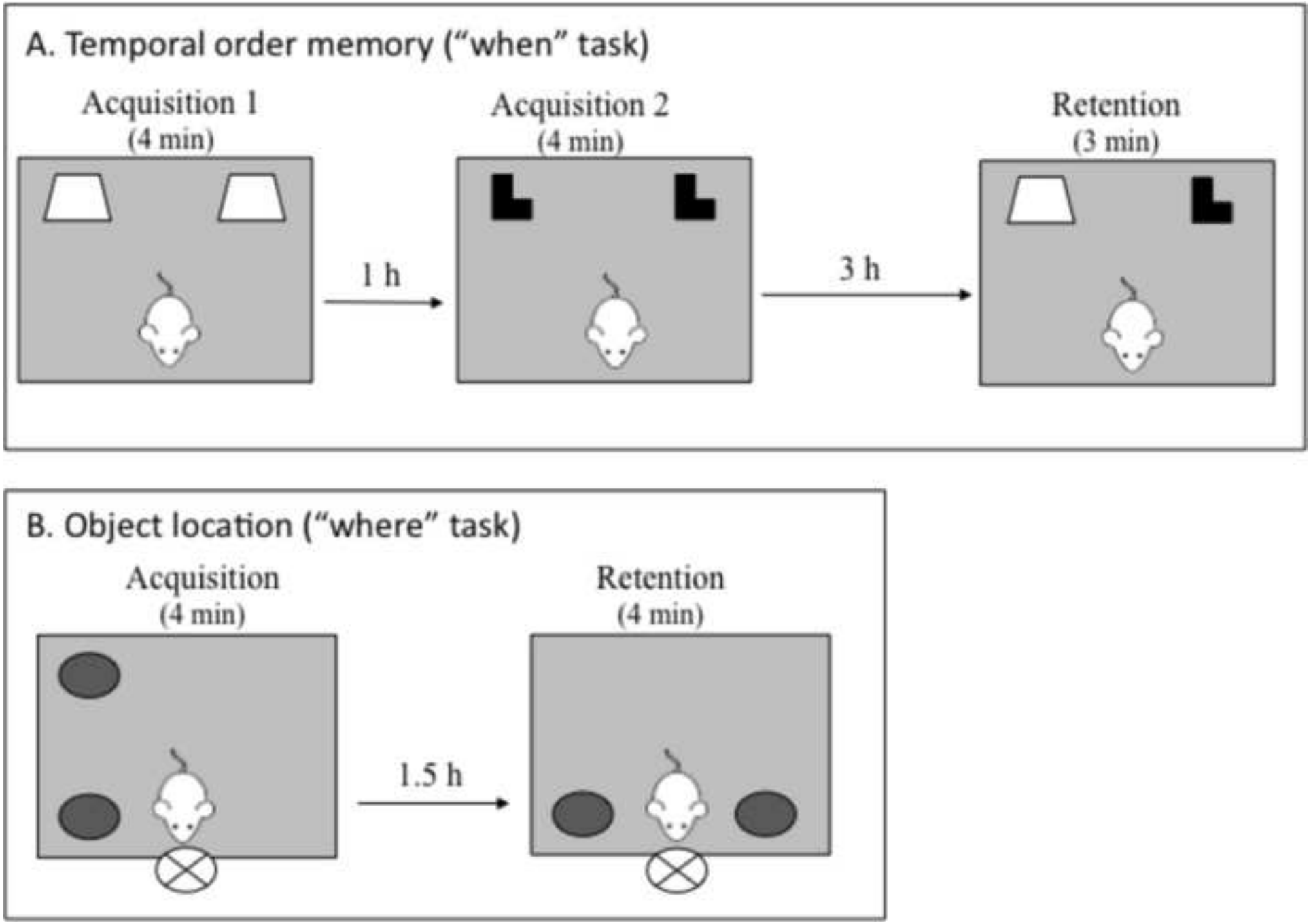

Figure 3

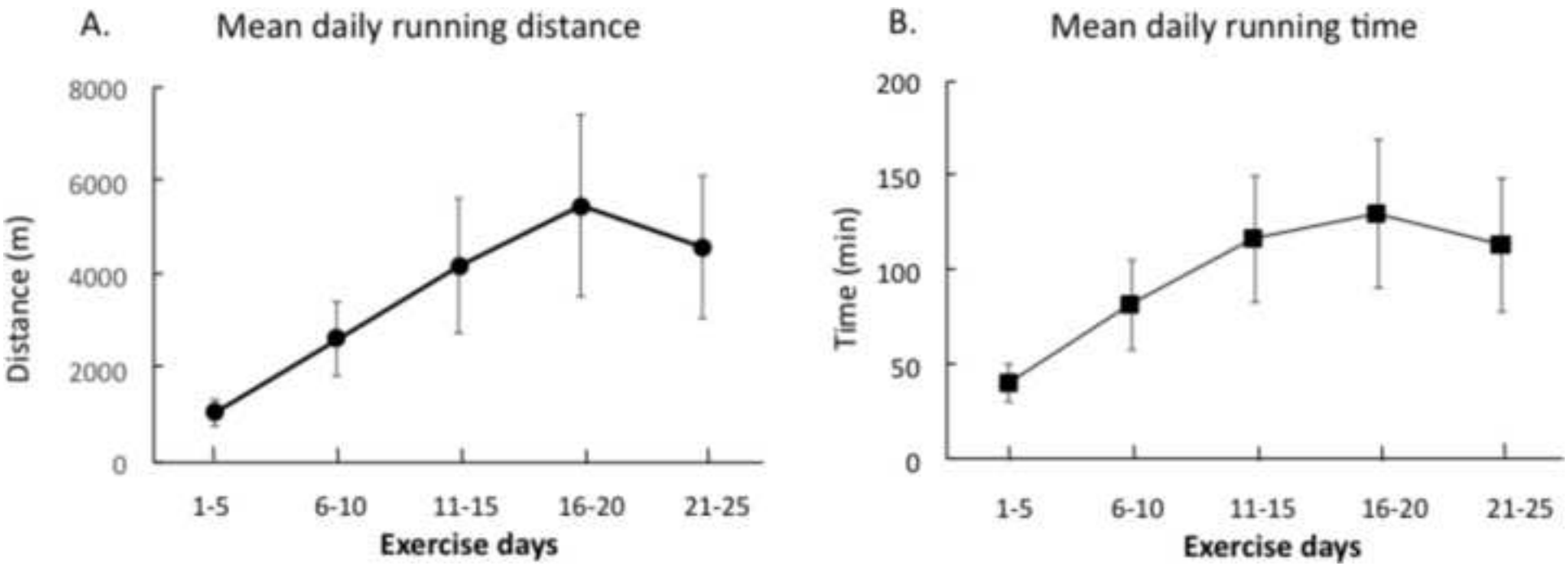

Figure 4

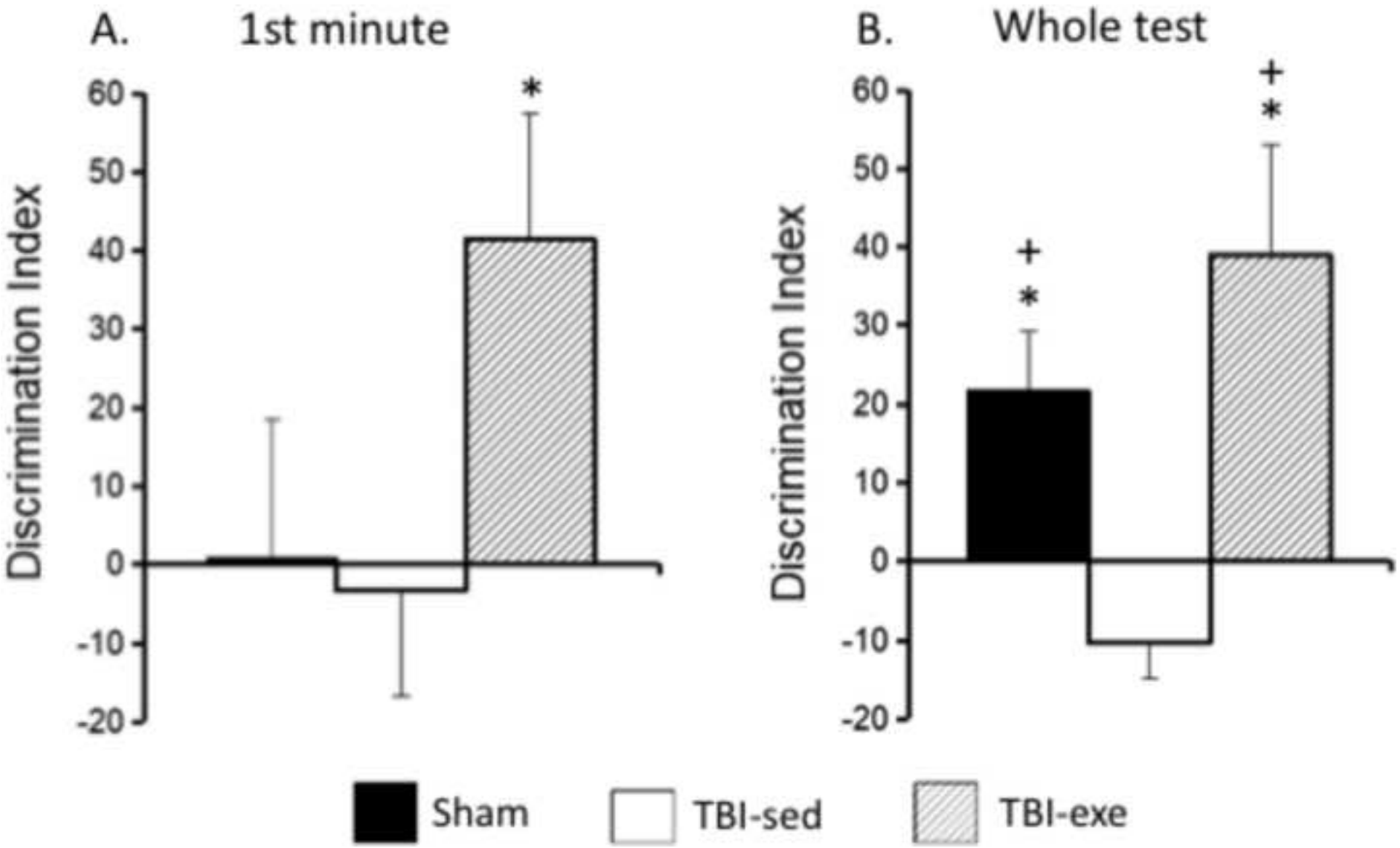

Figure 5

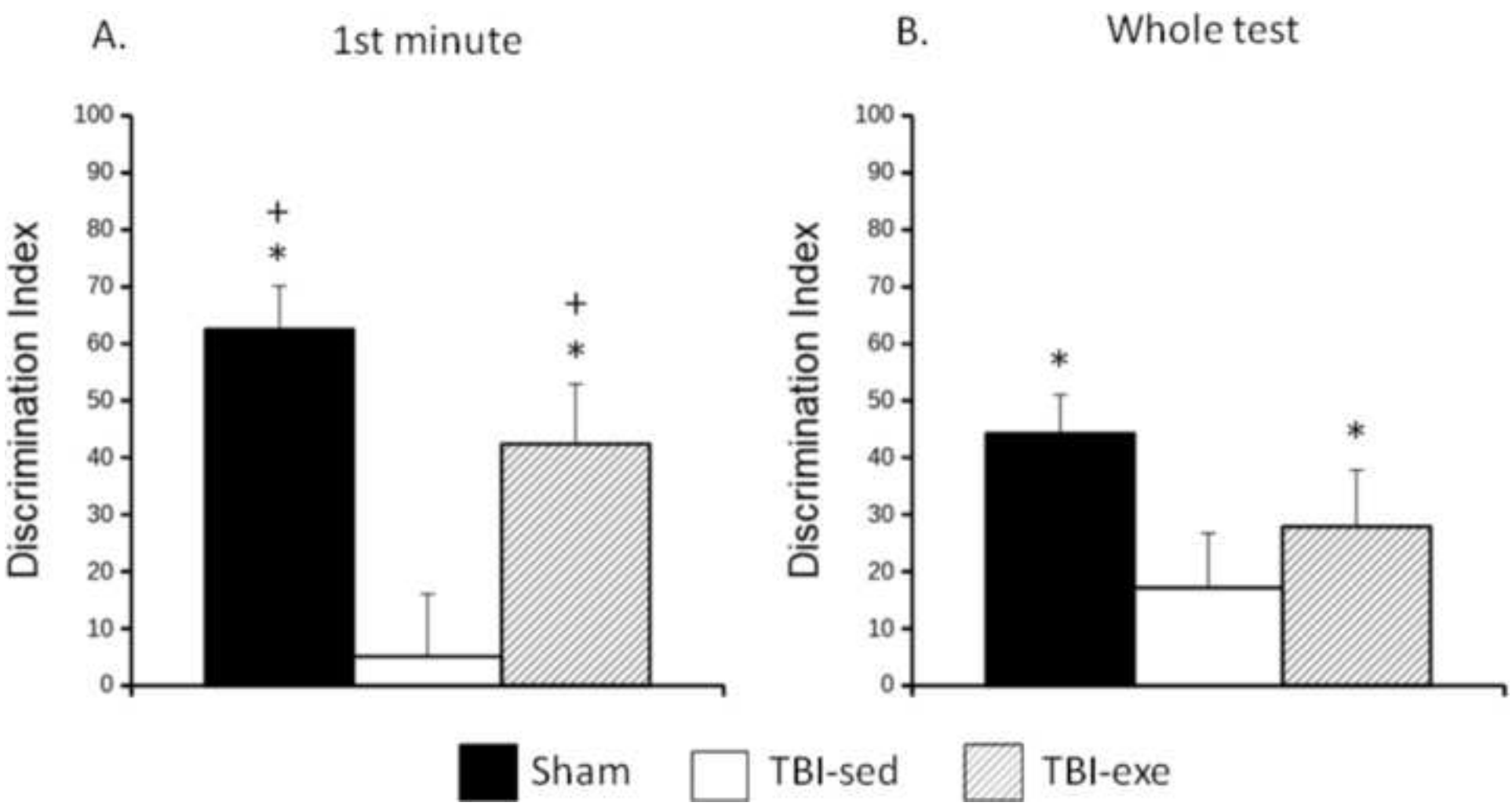

Figure 6

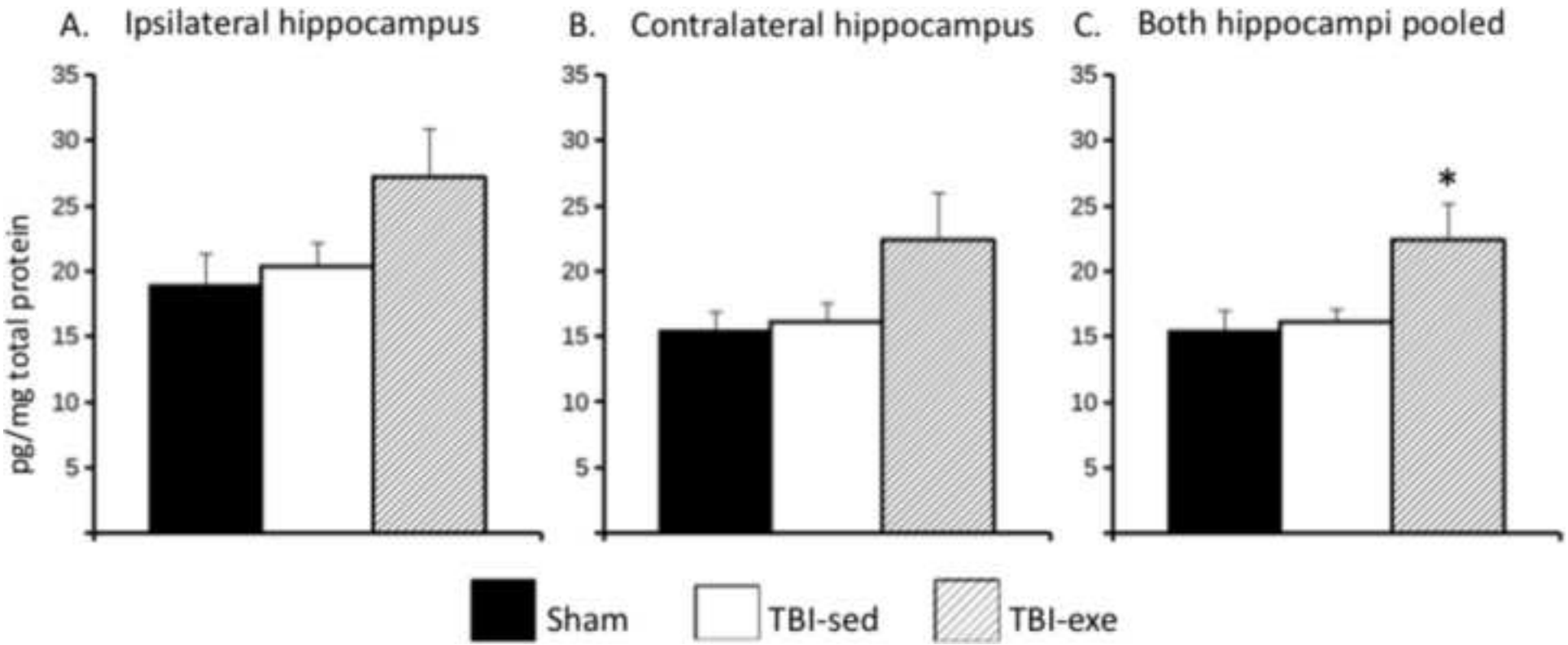

Table 1

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Table

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Table 2

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Author contributions

L. Martinez-Drudis; Investigation; Formal analysis; Writing, review & editing; L. Amorós-Aguilar: Investigation; Formal analysis; Writing, review & editing; B. Serra-Elias: investigation; Formal analysis; M. Torras-Garcia: Conceptualization; Methodology; Investigation; Supervision; Writing, review & editing; D. Costa-Miserachs: Software; Conceptualization; Writing, review & editing; I. Portell-Cortés: Funding acquisition; Conceptualization; Investigation; Writing, review & editing; M. Coll-Andreu: Conceptualization; Investigation; Formal analysis; Supervision; Writing original draft.