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Effect of exercise on learning and memory in a rat model of developmental stress

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Abstract

Introduction—Adverse life events occurring in early development can result in long-term effects on behavioural, physiological and cognitive processes. In particular, perinatal stressors impair neurogenesis in the hippocampus which consequently impairs memory formation. Exercise has previously been shown to have antidepressant effects and to increase cognitive functioning by increasing neurogenesis and neurotrophins in the hippocampus. The current study examined the effects of maternal separation, which has been shown to model anxiety in animals, and the effects of exercise on learning and memory.

Methods—Forty-five male Sprague-Dawley rats were divided into 4 groups, maternally separated / non-runners, maternally separated / runners, non-separated / runners and non-separated / non-runners. Maternal separation occurred from postnatal day 2 (P2) to 14 (P14) for 3 hours per day. Exercised rats were given voluntary access to individual running wheels attached to their cages from P29 to P49. Behavioural testing (Morris water maze (MWM) and object recognition tests) took place from P49 to P63.

Results—Maternally separated rats showed no significant difference in anxiety levels in the elevated plus maze and the open field compared to the normally reared controls. However, rats that were allowed voluntary access to running wheels showed increased levels of anxiety in the elevated plus maze and in the open field. Maternal separation did not have any effect on memory performance in the MWM or the object recognition tasks. Exercise increased spatial learning and memory in the MWM with the exercised rats displaying a decreased latency in locating the hidden platform than the non-exercised rats. The exercised rats spent significantly less time exploring the most recently encountered object in the temporal order task in comparison to the non-exercised controls, therefore showing improved temporal recognition memory. All groups performed the same on the other recognition tasks, with all rats showing intact memory performance.

Conclusion—Results indicate that maternal separation had little effect on the rats whereas exercise enhanced both spatial and recognition memory.

Keywords

Maternal separation; stress; exercise; learning and memory

1. INTRODUCTION

The neonatal period in both humans and rodents is a fundamental phase in the normal developmental process. Adverse life events during this critical period can result in long term

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effects on behavioural, physiological and cognitive processes (Gareau *et al*. 2007; Horvath *et al.* 2004). One of the characteristic biochemical features of the pathological outcome of these early adverse life-events is dysregulation of the hypothalamic-pituitary-adrenal axis (HPA axis) (Mesquita *et al*. 2007; Tang *et al*. 2003). The HPA axis is a neuroendocrine system that responds to environmental challenges (Kandel *et al.* 2006). Stress stimulates the release of corticotrophin-releasing hormone (CRH) which in turn causes the release of adrenocorticotropic hormone (ACTH). ACTH stimulates the release of glucocorticoids from the adrenal glands (Kandel *et al.* 2006). The interplay between the elements of the HPA axis act to maintain a balanced homeostatic environment. In humans alterations to the HPA axis have accompanied certain psychiatric diseases such as depression and anxiety (Kathol *et al*. 1989).

Disturbances in mother-infant interaction have been shown to be a natural stressor which may lead to maladaptive development (Daniels *et al*. 2004). Maternal separation (MS) has widely been used to model the effects of adverse early life-experiences because it has been shown to produce the clinical features of depression and anxiety such as the abnormal HPA axis which is accompanied by the associated behavioural effects (Marais *et al*. 2008). Other neurochemical changes that occur in response to stress include decreased levels of brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) (Griesbach *et al*. 2004, Roceri *et al*. 2004).

Down-regulation of hippocampal BDNF was observed in maternally deprived rats in accordance with impaired memory formation and studies showing increased anxiety-like behaviour even in adult rats (Knuth & Etgen 2007; Roceri *et al*. 2002). The hippocampus is a brain region that is especially vulnerable to stress in early development due to the high density of glucocorticoid receptors (Pickering *et al*. 2006). Stress may reduce the proliferation of synapses in the hippocampus seen in the first 3 weeks of a pup's life and contribute to impaired cognition in adulthood (Andersen & Teicher 2004).

Voluntary exercise has been shown to reduce anxiety levels and counteract the cognitive and mood effects of stress and depression (Clark *et al*. 2008). Running has also been shown to enhance neurogenesis and improve cognitive performance (Duman *et al*. 2008; Stranahan *et al*. 2007). One reason may be due to the increase in hippocampal BDNF levels seen in exercised rats (Vaynman *et al*. 2004). BDNF has previously been shown to be positively correlated with nerve terminal proteins (Ying *et al*. 2008).

The purpose of this study was firstly to determine whether maternal separation impairs certain aspects of memory formation (spatial memory in the Morris Water Maze and object recognition memory). Secondly, the effects of voluntary exercise were examined to determine if rats given voluntary access to running wheels displayed lower levels of anxiety (caused by maternal separation) and better memory performance than non-exercised rats.

2. MATERIALS AND METHODS

2.1 Animals

Forty-five male Sprague-Dawley rats were obtained from the University of Cape Town Animal Unit. On post-natal day two (P2) all litters were sexed (selecting males first) and then culled to 8 in order to standardize litter size. Rats were divided into 4 groups: the maternally separated/exercised (MSR) group (n=10), the maternally separated/non-exercised (MSnR) group (n=11), the non-separated/exercised (nMSR) group (n=11) and the non-separated/non-exercised (nMSnR) group (n=13). Dams and pups were housed in a 12-hour light/dark cycle, lights on at 06h00. On P21 rats were transferred to a new 12-hour light/dark cycle with the lights off at 11h00. Temperatures were maintained at 23-25°C. Food and

water was available *ad libitum*. Rats were weighed on P49, P52, P55, P63 and P65. All experiments were approved by the Research Ethics Committee of the University of Cape Town.

2.2 Maternal Separation Paradigm

Starting on P2 the dams of the maternally separated pups were physically removed from the home cage and the pups were transferred in their home cage to a different room in order to prevent communication via ultra-sound vocalizations. Pups were separated for 3 hours per day, until P14 after which normal interaction with the dam was resumed. The control pups (non-maternally separated runners, nMSR, and non-runners, nMSnR) were not separated from the dams. On P21 all rats were weaned. Males were housed communally until P29.

2.3 Voluntary Exercise

On P29 rats were given free access to individual running wheels attached to their cages. The number of revolutions was mechanically recorded on a counter and the distance run was calculated from the revolution count and circumference of the wheel where one revolution was equal to 1 m. Recordings were taken daily 1 hour before the dark cycle began (when the rats became active) in order to get an accurate reading of daily running distances. Non-exercised rats (maternally separated non-runners, MSnR, and nMSnR) were housed singly in standard plastic cages from P29 – P49.

On P49 rats were removed from running wheels and all groups were then housed in standard plastic cages in two's or three's, until termination of the experiment.

2.4 Behavioural Testing

Rats were placed in separate cages, taken to the behavioural room and were allowed to habituate for 1 hour prior to behavioural testing. The behavioural rooms used were ventilated and maintained at a temperature of 21-23°C with a light intensity of 58 lux. Behavioural testing occurred during the dark phase of the rats light/dark cycle.

All arena and objects (except for the Morris Water Maze) used in the behavioural experiments were cleaned using 70% alcohol after each trial. The apparatus was allowed to dry to allow alcohol vapor to dissipate before further testing resumed. Faeces and other debris were removed from the Morris Water Maze between trials. All tests were recorded using a video camera and videos were analyzed using Noldus Ethovision version 5.

2.4.1 Open Field—At P49, rats were tested in the open field for anxiety-like behaviour and locomotor activity. Rats were placed in the corner of a wooden box measuring 100 cm × 100 cm × 50cm and allowed to freely explore for 5 minutes. The box was divided into an inner and outer zone demarcated by white tape on the floor 10 cm from the outer wall of the box. Time spent and distance covered in the inner and outer zones of the box as well as the frequency of transition from the outer to inner zones were analyzed. Significantly more time spent in the inner zone indicates increased exploration and therefore reduced anxiety (Prut & Belzung 2003). Distance covered is an indication of the locomotor activity; an increase indicates a desire to explore and therefore reduced anxiety (Prut & Belzung 2003).

2.4.2 Elevated Plus Maze—At P49, immediately after being tested in the open field, the rats were placed in the elevated plus maze, facing the open arm, for 5 minutes. The protocol of Walf and Frye (2007) was followed. The elevated plus maze is used to test for anxiety-like behaviour. Time spent in each arm, time spent in the centre zone and the frequency of entry into each arm were analyzed. Significantly more time spent in the open arm compared

to the closed arm indicates reduced anxiety levels, as the closed arm provides shelter for the rat (Walf & Frye 2007).

2.4.3 Morris Water Maze (MWM)—The Morris Water Maze (MWM) protocol of Vorhees and Willams (2006) was used. The MWM consisted of a white circular tank with a diameter of 175 cm and height of 63 cm. The tank was filled with water maintained between 21°C – 24°C and made opaque with non-toxic tempera paint. The pool was divided into four quadrants and arbitrarily divided into southeast, northeast, northwest and southwest quadrants. A circular Perspex escape platform of 15cm diameter was submerged 30cm from the wall in the southeast quadrant of the pool (the target quadrant). The position of the platform was kept constant for the duration of the 5-day acquisition period. Four A3 sized pictures (black, white and blue circles, triangles, stripes and squares) were permanently fixed on the surrounding walls and served as distal navigation cues to enable location of the platform.

The MWM spatial learning test took place over a period of six days, from P50 to P55. The first five days (P50 – P54) were the acquisition or training days and on the sixth day (P55) the probe or retention trial was performed. The training period consisted of four trials per day, starting the rat at four different positions in the water maze, to avoid left and right navigation to the platform. This is a response strategy, described by Clements *et al.* (2007) where the rat remembers which direction their body turned instead of using visuospatial cues to guide them. Instead of hippocampal memory used in a place strategy where the rat uses spatial cues to guide them, the response strategy relies on striatal memory. Each trial began with the rat in the pool facing the sidewalls and ended when the rat found the platform or after 120 seconds; in both cases the rat was allowed to stay on the platform for 10 seconds.

On the 6^{th} day a probe trial of 60 seconds was performed after the escape platform had been removed. The release position (northeast) for the probe was different from the three positions used for the spatial trials on the previous five days of testing. For the training trials, time taken to reach the platform; time spent in the target quadrant, mean swimming velocity and distance to platform were analyzed. For the probe trial, time spent in the peripheral and target quadrants (southeast quadrant), frequency of entry into the target and peripheral quadrant (northeast, northwest and southwest quadrants), mean velocity and distance covered were analyzed.

2.4.4 Object recognition tasks—No behavioural testing took place between P55 and P62. Memory testing (for object recognition/location) resumed at P63. All object recognition/location memory tasks were performed sequentially at the same time of day. All memory testing protocols were according to Barker *et al.* (2007) using a box (65 cm × 65 cm × 60 cm box in dimension) described by Bertsina-Anglade *et al.* (2006). Objects used were wooden shapes ranging in size from 5 cm × 5 cm to 5 cm × 10 cm in diameter and height. The objects were placed in the corners of the box, 3 cm from each adjacent wall and taped down to prevent the object from shifting. If an object was used in the following days, it was not placed in the same position. Positions of the novel and familiar objects were changed around from one rat to the next to rule out left/right preference.

On P62 all rats were allowed to explore the empty box for 2 minutes in order to familiarize the rat with the testing environment (Bevins & Besheer 2006). On P63, the novel object recognition task was performed, followed, approximately 2 hours later, by the object location task (Barker *et al.* 2007). On P64, the temporal order task was performed followed 2 hours later by the object-in-place task. The rats' behaviour was recorded using a video camera. Time spent exploring each object was determined manually, using a stopwatch to record time spent with each object.

Exploratory behaviour for all rats was defined as the nose pointing toward the object at a distance of < 2 cm for > 2 seconds. Using the object to rear upwards was not counted as exploration.

2.4.4.1 Novel Object Recognition Task (NORT): The Novel Object Recognition Task (NORT) is based on differential exploration of new objects. The task consisted of a trial phase and a test phase. Rats were placed at the mid-point of the wall opposite to the objects, with its nose pointing away from the objects. In the trial phase, rats were allowed to freely explore two identical objects for 5 minutes. Rats were removed from the box and the test phase took place 5 minutes later where one object was replaced by a different, novel object.

During the test, the rats were allowed to explore the novel object and previously experienced object for 3 minutes. During the test phase the exploratory behaviour towards each object was recorded using a stopwatch. More time spent investigating the novel object was considered to be indicative of working memory being intact (Barker *et al.* 2007).

2.4.4.2 Object Location task (OL): The object location (OL) task measures recognition memory of object location. The task consisted of 2 phases the trial and test phase. Rats were placed at the mid-point of the wall opposite to the objects, with its nose pointing away from the objects. During the trial phase the rats were allowed to explore two identical objects for 3 minutes, after which the rats were removed from the box. In the test phase, performed 5 minutes after the sample phase, one object was placed in a different location to its original position and rats were then allowed to explore the objects for 3 minutes. Rats that showed superior recognition memory were expected to spend more time exploring the object that had been moved (Barker *et al.* 2007).

2.4.4.3 Temporal order task (TO): The temporal order (TO) task consists of 3 phases; 2 trial phases and a test phase. Rats were placed at the mid-point of the wall opposite to the objects, with their nose pointing away from the objects. During phase 1, the rat was allowed to explore 2 identical objects for 4 minutes. The second phase took place 1 hour later when the rat was allowed to explore 2 new identical objects for another 4 minutes. A 3-minutes test trial was performed 3 hours later where 1 object from phase 1 and 2 were used. Between trial phases the rats remained in the behavioural room in their home cages. In the TO task rats with intact temporal memory were expected to spend more time exploring the objects used in the first phase relative to the object used in the second phase (Barker *et al.* 2007).

2.4.4.4 Object-in-place task (OIP): The object-in-place task consisted of a 3 minutes sample phase and a 3 minutes test phase. In the sample phase the rats were allowed to explore 4 different objects (A, B, C, D), two of these objects were swapped around for the test phase which took place 5 minutes after completion of the sample phase. For both the trial and test phase, the rat was placed in the centre of the box facing the same wall from trial to test phase. Time spent exploring the 2 objects that had changed position and those that remained in the same position was recorded. If object-in-place memory was intact, the rats were expected to spend more time exploring the objects that had changed position (Barker *et al.* 2007).

2.6 Statistical Analysis

All statistical analyses were performed using Statistica version 8. Graphs were plotted using Graph Pad Prism 5. Data are presented as the means ± SEM. Statistically significant differences between groups and factorial effects were tested by 2-way ANOVA. When the interaction was shown to be significant, post-hoc analysis was performed using Tukey's

HSD test. Differences between two groups were assessed using the Students t-test for independent groups. Two-sided $P < 0.05$ was considered significant.

3. RESULTS

3.1 Voluntary exercise and weight

No significant difference was found between the maternally separated rats and the non-separated rats when the mean distance run daily, as well as the mean distance run over the 21 days of exercise, were compared.

Factorial ANOVA revealed that the non-separated rats (nMSnR and nMSR) had significantly lower body weights in comparison to the maternally separated rats (MSR and MSnR). This was noted on P21 ($F_{(1,41)} =7$, $p < 0.01$), P50 ($F_{(1,11)} =8.7$, $p < 0.01$), P55 ($F_{(1,35)} = 5.6$, $p < 0.05$), P63 ($F_{(1,41)} = 11$, $p < 0.001$), and P65 ($F_{(1,41)} = 6$, $p < 0.05$).

3.2 The Open Field

No significant difference ($F_{(1,41)} = 0.02$, $p > 0.05$) was observed between the groups when comparing time spent in the inner and outer zones of the open field. Although the maternally separated rats seemed to spend more time in the outer zone, it did not reach a significant level. However, rats that were exercised ($n = 21$, 10 MSR and 11 nMSR) showed a significant reduction in locomotor activity when compared to the non-exercised ($n = 24$, 11 MSnR and 13 nMSnR) rats ($F_{(1,41)} = 16.03$, $p < 0.001$, Figure 1).

3.3 The Elevated Plus Maze

Rats that were exercised (MSR and nMSR) spent significantly less time in the open arms of the elevated plus maze and made significantly fewer entries into the open arms compared to the non-exercised rats ($F_{(1,41)} =10.8$, $p < 0.01$, $F_{(1,41)} = 7.1$, $p < 0.01$, Figure 2).

3.4 The Morris Water Maze

Six rats were excluded from the MWM data (4 from nMSnR group and 2 from the nMSR group). The exclusion criteria were as follows: If by day three the rats did not remain on the platform when guided to it, or placed on the platform, it was assumed that no learning had taken place and therefore the rats were excluded from the study.

Performance in the MWM for each of the 5 trial days was determined by the average latency to locate the platform. Rats quickly learned the task, showing asymptotic levels of performance (Figure 3). Analysis of the day-to-day recordings showed a significant improvement from day 1 through to day 4 ($n = 39$; $p < 0.01$). From day 3 the exercised rats displayed a lower latency to locate the platform, this decrease reached a significant level on day 5 when the exercised rats showed a significant decrease in the time taken to locate the platform compared to the non-exercised rats ($F_{(1,35)} = 8.5$, $p < 0.01$). The MSnR group took longer to locate the platform than other rats during the 5 day trial period, however, this difference did not achieve significance.

No correlation was found between the total distance run over the 21 days of exercise by each of the exercising rats and the latency to locate the platform on day 5.

The mean velocity was analyzed using Noldus Ethovision version 5 in order to determine whether the differences in latency to locate the platform were due to differences in learning or due to differences in physical abilities. The mean velocity for the 4 trials of each day was calculated for each rat; thereafter the group means were calculated. Factorial ANOVA revealed a significant difference in velocity on day 1 between the exercised (MSR and

nMSR) and the non-exercised rats (MSnR and nMSnR), where the exercised rats (runners) swam more slowly than the non-exercised rats (non-runners, $F_{(1,35)} = 5$, $p < 0.01$, Figure 4). This was replicated on day 4 ($F_{(1,35)} = 7.2$, $p < 0.01$) and day 5 ($F_{(1,28)} = 5$, $p < 0.05$), where exercised rats swam more slowly than non-exercised rats.

No significant ($F_{(1,41)} = 0.1$ $p > 0.05$) difference was observed in time spent in the target quadrant between any of the groups during the probe trial. This result was replicated with no significant difference ($F_{(1,41)} = 1.7$ $p > 0.05$) between groups with regard to frequency of entry into the target quadrant.

3.5. Object Recognition Tasks

Factorial ANOVA was performed and results showed that all rats displayed intact memory. They spent significantly more time with the novel object, less with the recent object and the object that had changed position. There were no significant group differences in the object-in-place test, novel object recognition task or object location task. This was also true when discrimination ratios were calculated (time spent exploring novel or original object/total time spent exploring). In the temporal order task, factorial ANOVA revealed that the exercised rats (MSR and nMSR) had spent significantly less time exploring the object from the second phase in comparison to the non-exercised rats (MSnR and nMSnR) ($F_{(1,41)} = 5$, $p < 0.05$, Figure 5).

4. DISCUSSION

Animals were tested in the open field and elevated plus maze to examine the effects of maternal separation and exercise on locomotor activity and anxiety respectively. Although maternally separated rats spent less time in the open arms of the elevated plus maze and inner zone of the open field, the difference failed to reach significant levels. Using the same MS paradigm as the current study, Kalinichev *et al.* (2006) also found that the maternally separated group did not spend significantly less time in the open arms of the elevated plus maze in comparison to the normally reared group. Faure *et al.* (2007) showed that even with an additional triple stressor, maternally separated rats displayed no significant difference in behaviour in the elevated plus maze and open field when compared to controls.

The failure of the maternally separated rats to display anxiety-like behaviour in various studies, including the current study, may be due to the dam-pup interaction when the pup is returned to the cage (Marci *et al*. 2008). Dams have previously been shown to care more actively for maternally separated pups after separation in comparison to handled and normally reared controls (Marci *et al*. 2008, Marmendal *et al*. 2006). It has been suggested that dams counteract the previously deprived environment through compensatory up-regulation of maternal care (Marci *et al*. 2008). One critique of the study of Macri *et al*. (2008) however is that pups were not placed in a separate room from the dam during the separation period therefore potentially allowing communication between the dam and the pups, thereby influencing the subsequent dam-pup interaction when reunited. Up-regulation in maternal care like high levels of licking, grooming and arched-back nursing have been shown to be positively correlated with levels of synaptophysin and increased learning in the MWM (Liu *et al*. 2000) which would counteract the expected decrease in neurogenesis seen with stress (Roceri *et al*. 2002). On the contrary, many other studies also show that MS dams are slower to initiate pup retrieval when compared to dams of the normally reared litters (Hunsaker *et al*. 2008). Madruga *et al.* (2006) suggest that the smell of the dam on the saw-dust may influence the stressful nature of the separation. When Wigger *et al*. (1999) removed the pups from the home cage and placed the pups in a different cage during the separation period they found that the MS pups displayed less exploration of the open arms of the elevated plus maze, this however may have been due to the stress of handling.

The exercised rats displayed a significant reduction in locomotor activity in the open field in comparison to the non-exercised rats. This result of increased levels of anxiety was reinforced in the elevated plus maze where exercised rats spent significantly less time in the open arms as well as a decreased frequency of entry into the open arm of the elevated plus maze. Similar findings were shown by Binder *et al*. (2004) where non-exercised controls explored the centre zone more frequently and had a decreased latency to enter the centre of the open field in comparison to the exercised rats. The unexpected result of increased anxiety in the exercised rats in the current study may be due to the stress of being deprived of habitual running (Howells *et al*. 2005). Rats were removed from their cages with attached running wheels on the day of the open field and elevated plus maze testing; the testing time corresponded with the dark cycle when the rats would be physically active in the running wheel. In support of this suggestion, Howells *et al.* (2005) used immobilized running wheels for one hour per day as a stressor in their study. In addition, Widenfalk *et al.* (1999), showed that interruption of exercise decreased BDNF and TrkB levels in rats that had been allowed to run for 5 weeks, demonstrating that deprivation of habitual running caused changes in central brain function (Widenfolk *et al*. 1999).

Running has also been shown to activate the HPA axis (Coleman *et al*. 1998). Stranahan *et al*. (2006) found that running increased the levels of glucocorticoids, which were significantly higher in the rats running in isolation compared to the socially grouped rats. It was therefore suggested by Stranahan *et al*. (2006) that social interaction buffers the exercised rats from negative actions of high glucocorticoid levels. The increase in glucocorticoid levels seen in the isolated exercised rats could account for the increased anxiety shown in this study.

The MWM is a test of spatial learning and reference memory. Maternal separation had no effect on spatial learning and memory. This was in accordance with previous literature (Vallee *et al*. 1997, Lee 2005, Aisa *et al*. 2007, Hunsaker *et al*. 2008). The exercised rats showed enhanced spatial learning and memory in the MWM behavioural testing. The exercised rats had a lower latency to reach the hidden platform from day 3 onwards; the decrease reached a significant level on day 5. This was in accordance with previous studies (Vaynman *et al*. 2004 and 2007). The latency to locate the platform on day 5 was not correlated with the total distance run by each of the exercising rats. This suggests that the amount of exercise was not related to spatial memory. The probe test showed no significant difference in memory retention, with all groups spending similar amounts of time in the target quadrant. This was unexpected as it has previously been shown that exercised rats spend more time in the target quadrant when compared to controls (Vaynman *et al*. 2004 and 2007).

A negative correlation was found between latency to locate the platform and weight. Nutritional factors have been suggested to influence the BDNF system and therefore affect hippocampal development (Gomez-Pinilla *et al*. 2005). The effects of MS-induced stress may have been masked by the significantly lower weight of the non-separated rats in comparison to the maternally separated rats.

Other memory tasks investigated the effects of exercise and maternal separation on different components of recognition memory which included the discrimination of individual objects, object-location and the order of presented objects (Barker *et al*. 2007). Exercise had an effect on memory of the temporal order of objects placed in the arena, with exercised rats spending less time exploring the more recently encountered object from the second phase compared to non-exercised rats. All groups however did not show impaired temporal order memory as time spent exploring the second phase object was significantly lower than the time spent exploring the object from the first phase; exercise therefore improved temporal

order memory. Exercise and maternal separation had no effect on novel object recognition, object location and object-in-place memory. Vallee *et al*. (1997) reported similar results using handling as a form of neonatal stress. They showed that the number of visits into the previously visited arms in a two-trial memory test on the Y-Maze were similar for developmentally stressed and non-stressed animals therefore showing intact responses to novelty. Binder *et al*. (2004) showed that exercise had no effect on object recognition memory, which agreed with results found by Mello *et al*. (2008), where chronic exercise (8 weeks of treadmill running) did not affect long or short term memory in object recognition tasks. However, Aisa *et al*. (2007) found that maternally separated rats had a significantly lower discrimination index in the novel object recognition tests compared to normally reared controls. Maternal separation has also been shown to prevent long-term potentiation in rat hippocampal slices using electrophysiological recordings (Gruss *et al*. 2008).

Conclusion

No effect of MS was seen on behaviour whereas running enhanced learning and memory in the MWM task and improved object recognition memory in the temporal order task.

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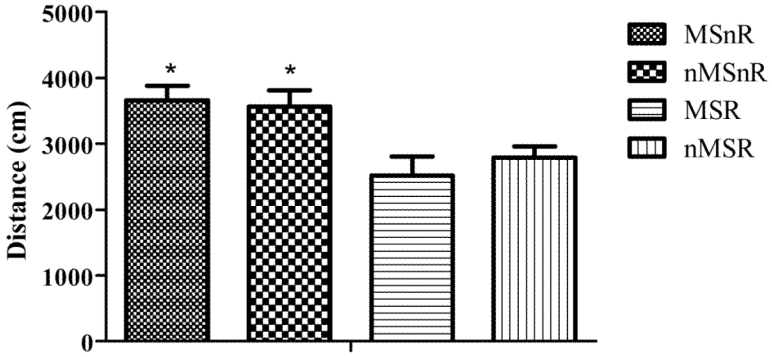

Figure 1.
Graph showing the mean distance covered ±SEM in the open field as an indication of locomotor activity. Factorial ANOVA revealed that the non-exercised rats covered a greater distance in the open field (* $p < 0.01$) than exercised rats. MSnR = maternally separated non-runners, n = 11; nMSnR = non-separated non-runners, n = 13; MSR = maternally separated runners, n = 10, nMSR = non-separated runners, n = 11.

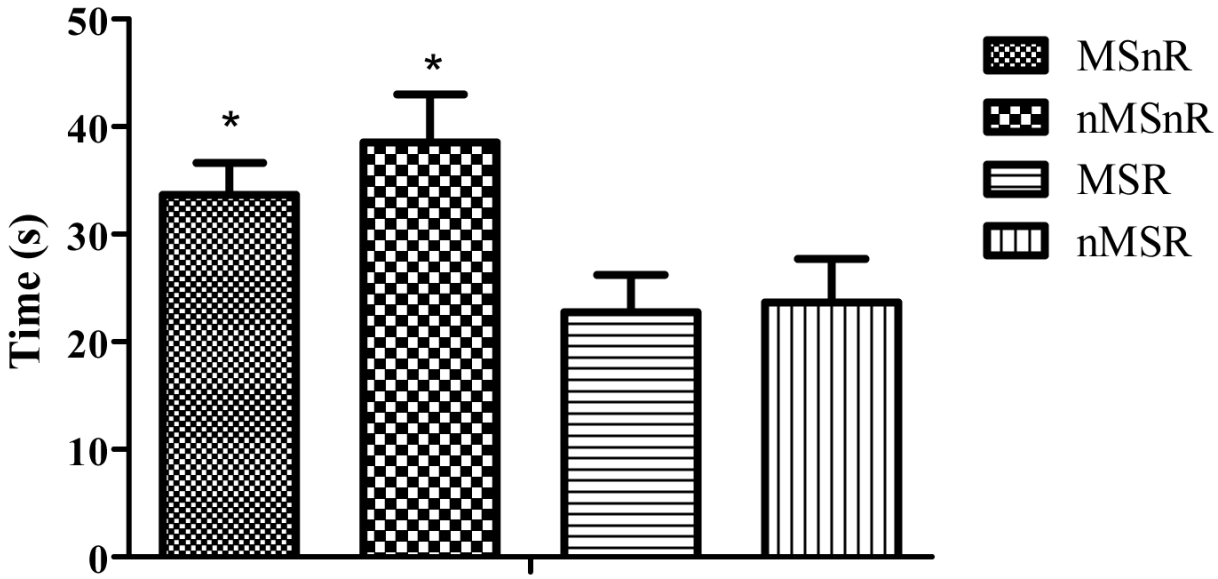

Figure 2.
Graph showing mean time spent ±SEM in the open arms of the elevated plus maze. Non-exercised rats spent a significantly greater amount of time (* $p < 0.01$) in the open arms in comparison to exercised rats. MSnR = maternally separated non-runners, n = 11; nMSnR = non-separated non-runners, n = 13; MSR = maternally separated runners, n = 10, nMSR = non-separated runners, n = 11.

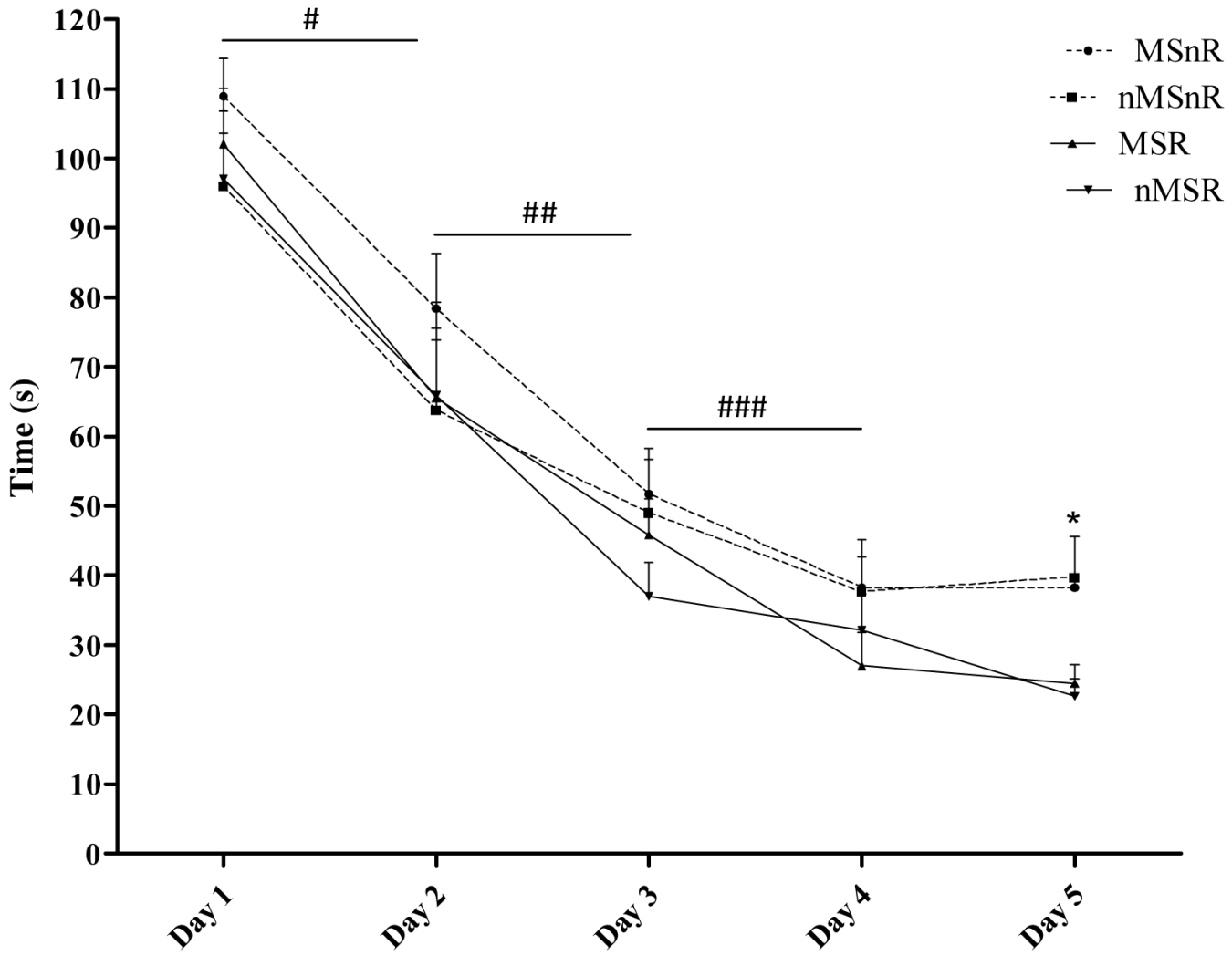

Figure 3.

Graph showing the mean time ±SEM taken to reach the platform for the 5 trial days. A significant decrease in the latency to reach the platform was found on the following days: day 1 compared to day 2, # $p < 0.01$; day 2 compared to day 3, ## $p < 0.01$; day 3 compared to day 4, ### $p < 0.01$. Factorial ANOVA showed that exercised rats had a significantly lower latency to locate the platform on day 5 than non-exercised rats, *$p < 0.01$ MSnR and nMSnR compared to MSR and nMSR.

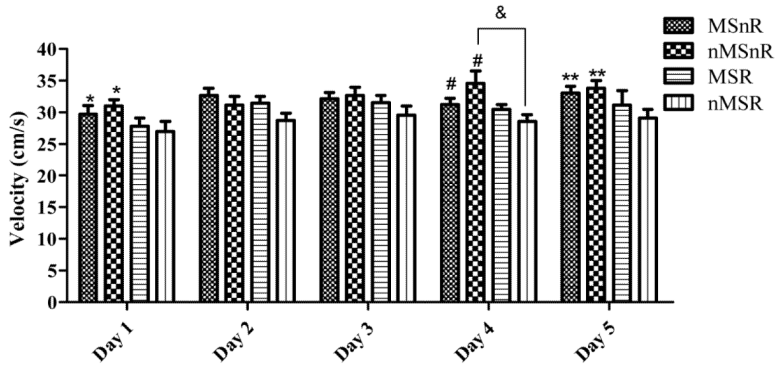

Figure 4.

Graph showing the mean velocity ±SEM for each day. Exercised rats had a lower velocity than non- exercised rats (MSnR and nMSnR compared to MSR and nMSR) on day 1, * $p < 0.01$; day 4, # $p < 0.01$ and day 5, ** $p < 0.05$. On day 4 there was a significant interaction between maternal separation and exercise with nMSnR swimming faster than nMSR, & $p < 0.05$.

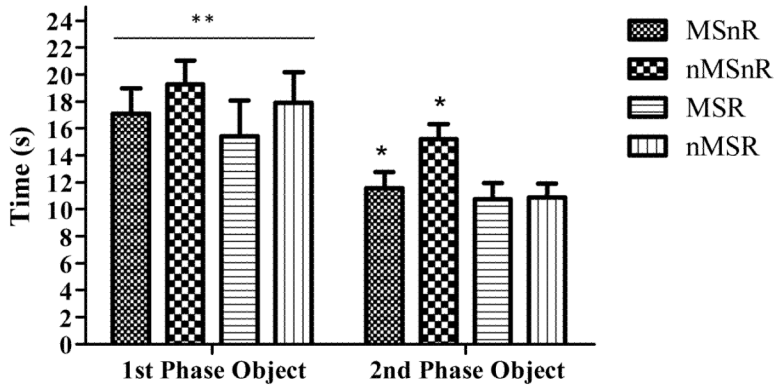

Figure 5.

Graph showing mean time spent ±SEM with the object from the 1st phase and the object from the 2nd phase of the temporal order task. Factorial ANOVA revealed that non-exercised rats spent a greater amount of time with the object from the 2nd phase in comparison to the exercised rats, * $p < 0.05$ (MSnR and nMSnR compared to MSR and nMSR). All rats spent significantly more time with the object from the 1st phase in comparison to the object from the 2nd phase, ** $p < 0.01$.