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Original Article

The Effect of Voluntary Exercise and Prenatal Exposure to Sodium Valproate on Learning, Memory, and Anxiety of Rats' Offspring

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Abstract

Background: Antiepileptic drugs, such as sodium valproate (SV), are teratogenic as their usage by the pregnant mother has been associated with an increased risk of major congenital abnormalities in the fetus. In this study, the effects of voluntary exercise and prenatal exposure to SV on learning, memory, and anxiety in rats' offspring are investigated.

Methods: In the present study, 70 female albino Wistar rats (200-240g) were used. The rats were categorized in seven groups: 1 and 2, pregnant rats with exposure to SV (10 mg/kg/day i.p) 3 and 4, pregnant rats with exposure to SV (20 mg/kg/day i.p) 5 and 6, pregnant rats with exposure to normal saline (0.4 ml/day i.p) and 7, pregnant rats with exposure to lamotrigine (20 mg/kg/day i.p). The even and odd groups were sedentary and voluntary exercise groups, respectively. Learning and memory were tested in male offspring using shuttle-box; anxiety was tested by elevated plus-maze (each group n=12). Statistical analyses were performed using the one-way ANOVA (the Tukey test) and/or two-way ANOVA on rank.

Results: The results showed that voluntary exercise in male rats caused improvement of latency and duration time in the dark box compared to sedentary groups (P=0.004). Moreover, the group administrated with 10 mg/kg SV showed better learning capability than the group administrated with 20 mg/kg SV. Voluntary exercise could also improve anxiety (P=0.001).

Conclusion: This study indicated that exercise could increase learning capacity and improve memories in rats' offspring whose mothers were exposed to SV. Voluntary exercise could improve anxiety too, and the effect was dose-dependent.

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Keywords ● Exercise ● Sodium valproate ● Epilepsy ● Pregnancy

What's Known

- Antiepileptic drugs, such as sodium valproate (SV), are used to control different types of convulsive disorders or stabilize mood.
- The pharmacologic treatment of epilepsy and bipolar disorder is seldom more challenging during pregnancy. Most of these women need to continue using antiepileptic drugs during pregnancy.

What's New

- Maternal exercise on running wheels was shown to positively affect the cognitive impairments in the offspring by the administration of SV to the mother during pregnancy.
- Voluntary exercise during pregnancy could reduce the adverse effects of prenatal exposure to 10 and 20 mg/kg of SV in the male rats' offspring such as memory-related disturbances in performance.

Introduction

The prevalence of epilepsy among pregnant women is 0.3-0.7%; most of these women require continuous treatment with anti-epileptic drugs (AED) throughout their pregnancies. Similarly, there is growing evidence pointing to the importance of treatment during pregnancy for women with bipolar disorders, because several adverse pregnancy outcomes as well as the risk of relapse are associated with untreated psychiatric conditions in

pregnant women.[1, 2]

The pharmacologic treatment of bipolar disorders and epilepsy is especially challenging during pregnancy. Given the fact that uncontrollable seizures can be possibly harmful to both the mother and the fetus and exposes them to risk, most epileptic women need continuous treatment during pregnancy.[3] Continued treatment, however, has its own concerns, as antiepileptic medications are associated with major congenital malformations and are linked to certain adverse effects on postnatal cognitive development.[4]

Lamotrigine is known to be one of the most frequently used AEDs in North America as well as in Europe. However, valproic acid (VPA), as one of the four first-line approved medications for the treatment of epilepsy, is also used vastly and increasingly to treat bipolar disorders. However, some types of seizure could not be controlled by lamotrigine.[5]

The majority of studies about the teratogenic effects of VPA in humans exclusively focus on the effects of exposure during the first trimester. Exposure to VPA during this period has been associated with several facial abnormalities (e.g. cleft lips and palates, broad nasal base, shallow philtrum, etc.), a number of more severe malformations (e.g. cardiovascular abnormalities, genitourinary defects, limb defects, etc.), and neural tube defects.[6] This collection of physical defects and malformations associated with VPA has been referred to as "fetal valproate syndrome".[7, 8] Moreover, cognitive issues, such as low verbal intelligence quotient, memory and learning deficits, and autistic spectrum disorder, have also been reported in children previously diagnosed with valproate syndrome.[9, 10] Whereas the adverse effects of exposure to VPA during the first half of gestation have been well understood for a long time now, recent studies have brought to light that exposure in the late stages of gestation may equally contribute to fetal anticonvulsant syndrome phenotype.[11-13]

Maternal exercise during pregnancy has been known to promote the offspring's brain functions. More specifically, cell proliferation and/or neurogenesis in the dentate gyrus have been reported to improve as a result of physical exercise.[14, 15] It is also worth mentioning that studies in this regard have established that regular exercise has no harmful effect on either the mother or the fetus.[16] In fact, many studies have strongly suggested that maternal exercise exerts beneficial effects on the brain functions of the developing fetus. As for the pregnant mother, maternal exercise improves endurance and muscle strength, mitigates excessive weight gain, relieves back pain as well as anxiety, depression, and a number of other discomforts commonly associated with pregnancy.[17, 18]

The present study aimed to find whether voluntary exercise could significantly improve learning and memory and alleviate anxiety in male rats' offspring with prenatal exposure to sodium valproate (SV). The step-through passive avoidance task was used to examine the hypothesized association between learning and memory enhancements (on the one hand) and voluntary exercise (on the other hand). To test the effects of voluntary exercise on anxiety, the elevated plus-maze was employed.

Materials and Methods

Animal Study

In this experimental study, 70 male Wistar rats with body weights between 200 and 240 g were allowed to mate with 70 female virgin Wistar rats with body weights between 200 and 240 g over a 24-hour period. The presence of vaginal plugs in female rats was checked twice, at midnight and then again at 5 a.m. in the following morning. The presence of a vaginal plug was taken to indicate pregnancy.[3] 70 pregnant rats were then randomly divided into sedentary groups (S. group) and voluntary groups (V. exercise group) with and without the administration of SV (details will follow). The animals were housed in individual cages with cycles of 12 hours light and 12 hours dark at 22-24 °C, receiving food and water ad libitum.[3] Cages were checked 21 days after mating for the presence of offspring. Postnatal Day 0 (PND0) was defined for each pup as the day it was first observed. Moreover, each mother and its offspring were taken as a colony.[16] Procedures involving animals and their care were conducted in accordance with the recommendations of the European Council Directive (86/609/EEC) of November 24, 1986, regarding the protection of animals used for experimental purposes (http://data.europa.eu/eli/dir/1986/609/oj).[19] All procedures were approved by the Institutional Committee for Care and Use of Laboratory Animals in Iran University for Medical Sciences (No. 95-07-63199).

Animal Treatment

This study used SV prepared by dissolving in normal saline. The rats were randomized into seven groups (10 rats in each group):

1) Sedentary pregnant rats with exposure to SV (10 mg/kg/day i.p);

2) Pregnant rats with exposure to SV (10 mg/kg/day i.p) with SV exercise;

3) Sedentary pregnant rats with exposure to SV (20 mg/kg/day i.p);

4) Pregnant rats with exposure to SV (20 mg/kg/day i.p) with voluntary exercise;

5) Sedentary pregnant rats with exposure to normal saline (0.5 ml/kg/day i.p);

6) Pregnant rats with exposure to normal saline (0.5 ml/kg/day i.p) with voluntary exercise;

7) Sedentary pregnant rats with exposure to lamotrigine (20 mg/kg/day i.p) as positive control.

The animals were injected with 10 mg/kg and 20 mg/kg SV as well as saline solution and lamotrigine (20 mg/kg intraperitoneal [IP]) from day 7 through day 18. The step-through passive avoidance task and the elevated plus-maze were evaluated on Postnatal Day (PND) 30.[16]

Exercise Paradigm

The study used a detailed exercise model.[20] In sum, the V. exercise group animals had access, throughout their pregnancies, to a running wheel (diameter=34.5 cm, width=9.5 cm) freely rotating at a resistance of 100 g with each wheel equipped with a counter to report the number of rotations. Exercise data were taken once daily at 6 a.m. up to day 21 after mating and twice daily at 9 a.m. and 6 p.m. thereafter. Postnatal Day 0 (PND0) was defined as the first day pups were observed. Mothers and their respective pups were moved to individual cages after delivery. 72 male pups, 30 days of age, were selected randomly from 477 mice (206 male and 271 female mice), 12 mice in each group. The aforementioned procedure was repeated for animals in the sedentary group with no running wheels.[3, 17, 18]

The Passive Avoidance Task Apparatus

The passive avoidance task apparatus in this study was modeled after the apparatus used by a previous study.[21] It consisted of a shuttle-box divided by a wall into two compartments of equal size (20×20×30 cm). The wall was equipped with a guillotine-like door (7×3×9 cm) to be lifted manually at will. The walls and the floor were both opaque resin in one compartment (i.e. the light compartment), and, respectively, dark and comprised stainless steel grids (2.5 mm in diameter at 1 cm-distances from each other) in the other (i.e. the dark compartment). A stimulator was connected to the apparatus as the source of electric shocks, used in the dark compartment. Electric shocks (50- Hz, 3-s, 1- mA) were delivered to the grid floor of the dark compartment during training days in the trial.[15]

Training and Testing

The training model used in this study was employed earlier.[22, 23] All animals were allowed 30 minutes to get used to the experiment room before experiments began. Each animal was then gently moved to the light compartment with 5 seconds to roam before the guillotine door was opened and the animal was allowed entry to the dark compartment. The latency with which each animal moved from the light compartment into the dark one was recorded. Animals with latencies above 100 seconds were eliminated from the experiment. The guillotine door would be closed once the animal fully entered the dark compartment. Then, the animal would be moved to its cage. This trial was then repeated after 30 min where, this time, a shock (50 Hz, 1 mA, and 3 seconds) would be delivered to the grid floor immediately after the animal enters the dark compartment; the animal was then moved back to its cage after 20 seconds. Next, the animal was moved back to the apparatus after 2 min; a successful acquisition of passive avoidance response was recorded given the animal had not entered the dark compartment in under 120 seconds upon which the trial would be terminated. In cases where the animal entered the dark compartment in under 120 seconds, a second shock of the same intensity and duration was delivered to the grid floor, and the animal would be listed for a re-test. This would go on until the acquisition of passive avoidance.

The memory retrieval of the acquired passive avoidance was tested on day 2 (24 hours after training). Animals were individually moved to the light compartment and given 20 seconds before the door was opened; whereby the step-through latency for each animal would be recorded. Tests were terminated when the animal (a) entered the dark compartment or (b) stayed in the light compartment for 300 seconds (the defined retrieval criterion). No electric shocks were delivered during testing sessions on day 2.

Elevated Plus-Maze

The detection of any significant effects from the drugs was validated behaviorally, physiologically, and pharmacologically. The number of animals that entered into open arms was significantly lower than those that entered into closed arms; the time they spent in open arms was also significantly lower. Significantly heightened anxiety-related behavior was associated with confinement to open arms as well as a significant increase in corticosterone concentrations. Elevated plus-maze, it may be worth mentioning, is a widely-employed method for identifying drug effects on anxiety. The

method uses two open and two closed arms and measures the effects of drug on anxiety as the extent to which a certain drug affects the open-arms-to-closed-arms ratio.[19]

The drug-exposed rats were placed at the center of the elevated plus-maze immediately after the pretest facing one of the two open arms. The following measurements were taken during the 5-min period of the test: The number of entries into open arms; the number of entries into closed arms; the time spent in open arms; and, the time spent in closed arms. Two indices of anxiety were thus defined based on these measurements: Time spent in open arms expressed as the percentage of the total time spent in both open and closed arms; and, entries into open arms expressed as a percentage of the total number of entries into both open and closed arms. An entry was taken to happen when the animal entered into an arm with all four paws. The maze was cleaned after each trial.[19, 20]

Statistical Analysis

Data are expressed as mean±SEM. Statistical analyses were performed using one- and two-way analyses of variance (ANOVA). The post-hoc comparison of the obtained means was performed using the Tukey test for multiple comparisons when necessary. Previously, all variables were tested for normal and homogeneous variances by Kolmogorov–Smirnov test statistic test. All data had $P>0.05$, which indicated the normal distribution of data. The homogeneity of variances was determined using the Levene's test. Statistical significance was set at $P<0.05$. The calculations were performed using the Graph Pad Prism (7.0) software.

Results

Running Distance during Pregnancy

As figure 1 shows, the pregnant rats from the voluntary exercise control group not only ran during their pregnancies but also adapted their running distance with their new physiological states as they moved further into their pregnancies. A significant difference ($P=0.76$) was observed in the running distance of the 20 mg/kg SV group in comparison to the other groups in that they ran significantly less distance than their peers.

Effects of Treatment on the Passive Avoidance Learning Test[21]

The Number of Trials to Acquire Passive Avoidance

The results from the passive avoidance experiments showed that the animals from the 20 mg/kg SV sedentary group learned the avoidance task significantly more slowly than the animals from the control, 10 mg/kg SV, and 20 mg/kg lamotrigine groups. No significant difference was observed among other groups in this regard.

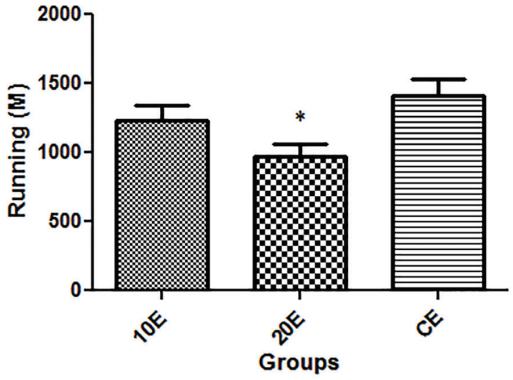

Figure 1: The figure shows the average running distance (expressed in meter per day) in the pregnant rat in voluntary exercising groups. 10E: a group 10 mg/kg sodium valproate receiving voluntary exercise (n=10); 20E: a group 20 mg/kg sodium valproate receiving voluntary exercise (n=10); CE: control group receiving voluntary exercise (n=10); *P=0.0076 is significant between 20E and CE group. Each value represents mean±SEM

A significant difference was also observed in the number of trials to acquire passive avoidance of the exercise group, on the one hand, and that of the sedentary group, on the other hand. (figure 2).

Effects of Treatment on the (Step-through Apparatus) Passive Avoidance Learning test retention

The step-through latencies and the amounts of time spent in the dark compartment were also compared for the exercise and the sedentary groups. The results showed that exercise had a significantly positive effect on the memory deficit in both the 10 and the 20 mg/kg SV groups with regard to the passive avoidance task (figure 3).

Elevated Plus-Maze Behavior

Effects of valproate treatment and exercise on elevated plus-maze behavior

Figure 4 shows the effects of injecting SV (10 and 20 mg/kg) to pregnant rats on the behavior shown by their pups in elevated plus-maze.

Male rat pups whose mothers were exposed to 20 mg/kg SV in pregnancy displayed an increase in the percentage of entries and time on the open arms compared to other groups, suggesting that this group was significantly less anxious on the open arms (A and B). However, there were no significant differences between the groups in the

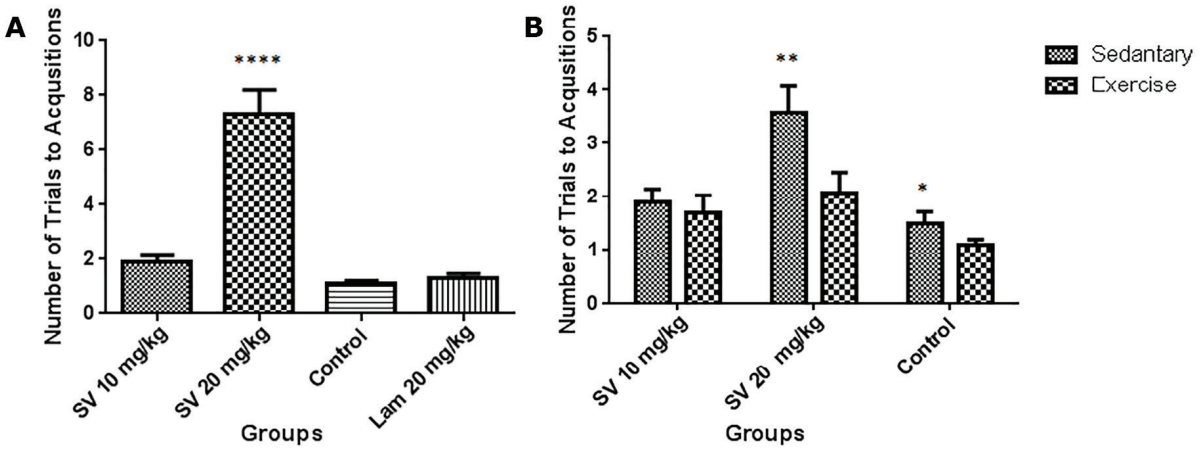

Figure 2: The figure illustrates the effect of voluntary exercise on the comparison of passive avoidance learning in 1st day on 30-day male rat pups between control, sodium valproate (SV), and lamotrigine (Lam) sedentary groups. Part A shows that the number of trials to acquisitions of SV 20 mg/kg group was significantly higher than the other groups, means at least learning compared to other groups (****P<0.0001); Part B shows that the number of trials to acquisitions of SV 20 mg/kg group was significantly higher than its exercise group, means at least learning compared to exercise group (**P=0.01); Compared with the SV 20 mg/kg exercise group (*P=0.02); Compared with the control exercise; Each value represents mean±SEM

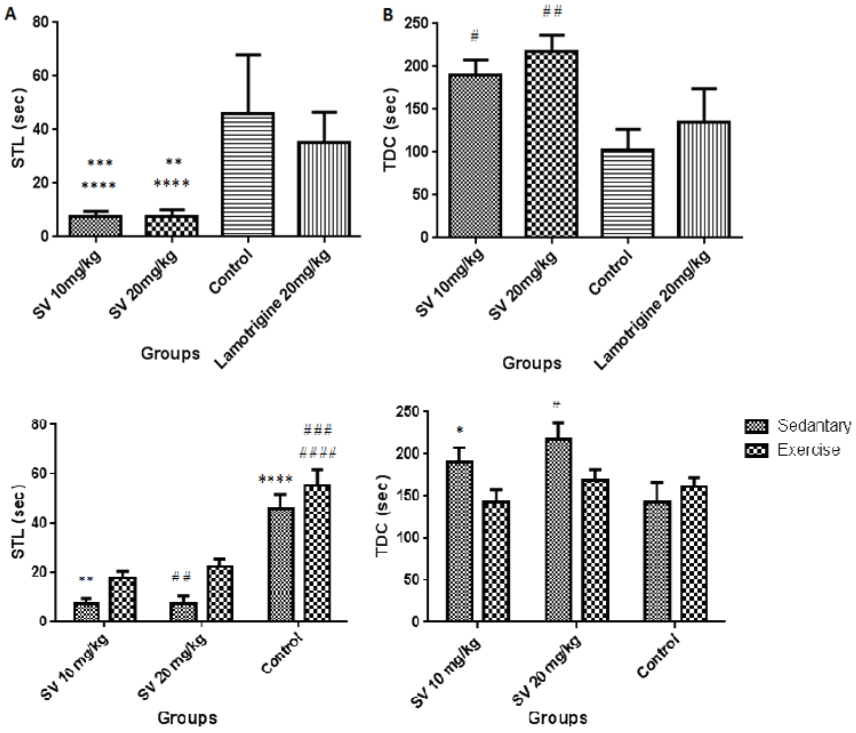

Figure 3: The figure indicates the effect of sodium valproate (SV) intraperitoneal on the step-through latency (STLr) dark/light avoidance test. Part A shows the time spent in the dark compartment (TDC); part B shows the time spent in the retention trial of passive avoidance task which was carried out 24 h after acquisition trial; part C shows the time spent in the dark compartment (TDC); and part D shows the retention trial of passive avoidance task compared with sedentary groups; Part A shows that **P=0.004 compared between SV 20 mg/kg with lamotrigine group, ***P=0.0002 compared between SV 10 mg/kg with lamotrigine group, and ****P<0.0001 compared between SV 10 and 20mg/kg with control group; Part B shows that #P=0.01 compared between SV 10 mg/kg with control group and ##P=0.002 compared between SV 20 mg/kg with control group; Part C shows that **P=0.006 as compared between sedentary and exercise groups in 10 mg/kg SV, ****P<0.0001 as compared between sedentary and exercise groups in control groups, ##P=0.007 as compared between sedentary and exercise groups in 20 mg/kg SV, ###P=0.0005 as compared with 20 mg/kg SV sedentary group, and ####P<0.0001 as compared with 10 mg/kg SV sedentary group; Part D shows that #P=0.02 and ##P=0.004 compared with control group. Each value represents mean±SEM (n=12)

number of closed arm entries (panel C) (figure 4).

Figure 5 indicates elevated plus-maze behavior. The evaluation of prenatal exposure to maternal voluntary exercise during pregnancy was compared to the sedentary groups protected against the adverse effects of prenatal SV in male rat pups (figure 5). The results indicated that there were no significant differences between voluntary exercise groups and the sedentary groups. Plane B indicates that

voluntary exercise in 20 mg/kg SV led to a decrease in the percentage of time spent on the open arm; exercise in this group could improve abnormally low anxiety, and in the control group exercise indicated less anxiety compared to the sedentary group.

Discussion

The findings of this study suggested that exposure to 20 mg/kg of SV during pregnancy could significantly impair learning in the rats' 30-day-old male offspring. Moreover, it was found that maternal exercise on running wheels during pregnancy could significantly improve learning in offspring with prenatal exposure to 20 mg/kg of SV. In addition, voluntary exercise may significantly improve the cognitive impairments resulted from exposure to SV. In fact, exercise may entirely eliminate SV-related adverse effects. An association is suggested between the use of antiepileptic drugs during gestation or in infancy and behavioral impairments.[4, 24, 25] Considering the need for further research on the side effects of antiepileptic drugs with regard to cognitive impairments and congenital abnormalities in fetuses, this study aimed to assess exactly such cognitive disorders.

The administration of SV in newborns, while the brain is still developing, was shown to cause impaired learning in young rats. The study researchers investigated the semi-daily administration of 200 mg/kg SV to rats between days 4 and 10 after birth and collecting data using the Morris water maze twice on days 23 and 30 after the rats were born. The findings from day 23 suggested significant dysfunction and impairment in both memory and learning as well as affected swimming pace. Analyses of the data from day 30, however, revealed no significant differences between the control and the experimental groups. SV, it was concluded, caused transient neural defects during the development of the brain.[26] The present study examined the effects of the administration of 10 and 20 mg/kg SV for 12 days on rats starting from day 7 after conception. The offspring were observed for 30 days for cognitive deficits. Other similar studies have been previously carried out on the effects of SV during the first

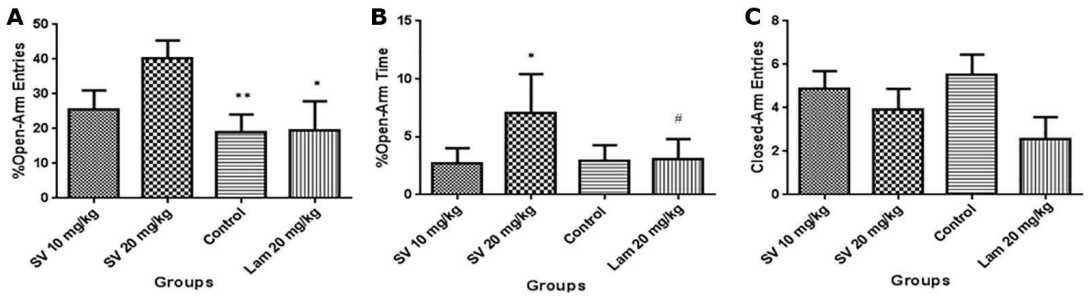

Figure 4: The figure illustrates the effects of sodium valproate (SV) (10 and 20 mg/kg) on pregnant rats and the behavior of their pups in elevated Plus-Maze. Part A shows *P=0.04, **P=0.009 as compared with 20 mg/kg SV; Part B shows *P=0.01 as compared with 10 mg/kg SV and control groups; #P=0.04 as compared with 20 mg/kg SV. Each value represents mean±SEM (n=12)

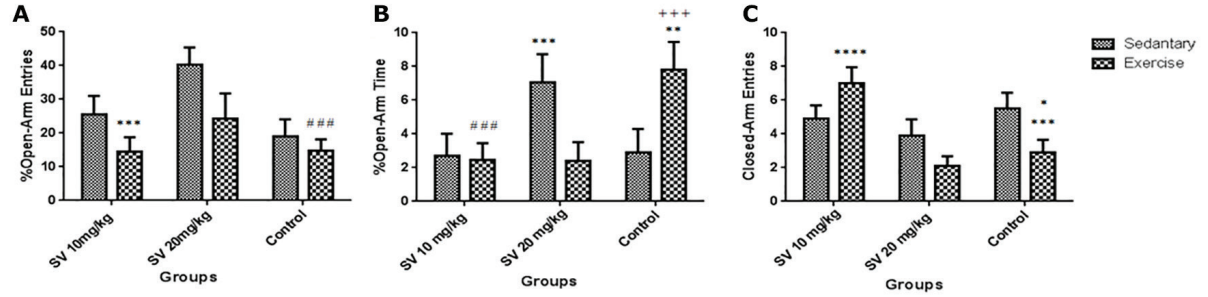

Figure 5: The figure captures the evaluation of prenatal exposure to maternal voluntary exercise during pregnancy as compared to sedentary groups protected against the adverse effects of sodium valproate (SV) prenatal in male rat pups. Part A shows ***P=0.001, ###P=0.005 as compared with 20 mg/kg SV sedentary group. In this panel, no significant differences were observed between voluntary exercise groups and the sedentary groups; Part B shows **P=0.01 as compared with sedentary control and 10 mg/kg SV sedentary groups; ***P=0.003 as compared with 20 mg/kg SV exercise group; ###P=0.001 as compared with 20 mg/kg SV sedentary group; +++P=0.002 as compared with 10 and 20 mg/kg SV exercise groups. In this panel indicating that voluntary exercise in 20 mg/kg SV the observed decrease in the percentage of time spent on the open arm, exercise in this group could improve an abnormally low anxiety; In control group, exercise indicated less anxiety compared to the sedentary group; Part C shows *P= 0.04 as compared with sedentary control group; ***P=0.002 as compared with 10 mg/kg SV exercise group. ****P=0.0002 as compared with 20 mg/kg; Each value represents mean±SEM (n=12)

trimester. Findings suggested that exposure to the drug during the first trimester could be associated with spina bifida in children; more specifically, cases where daily doses of over 1,000 mg were involved revealed significantly higher occurrence rates.[9] Exposure to SV in rats during what is considered equivalent to the third trimester of human pregnancy has been shown to impair spatial learning in offspring. Findings suggest that valproic acid may result in malformations as well as brain and nervous system disorders, sometimes accompanied with long-term complications.[27] Lowered intelligent quotients (IQs) in children with prenatal exposure to valproate have also been reported,[9, 27] but no such adverse effects have been observed for carbamazepine and phenytoin.[9] Additionally, valproic acid can reportedly cause learning impairments and dysfunction in retentive memory.[28]

The present study findings are in agreement with those reporting that exposure to SV during the third trimester of pregnancy impairs learning and memory.

Good exercise during pregnancy can positively affect both the mother and the fetus. The present study suggests that voluntary maternal exercise is effective in improving certain cognitive problems such as memory and learning impairments in male rat offspring. Voluntary exercise, further, seems to have an advantage over other types of exercise in this regard, probably due to a lack of stress on the mother's side, which, for instance, may accompany forced exercise. The findings also corroborate those of an earlier study by the same researchers indicating the efficacy of running wheels in enhancing cognition and memory.[29] Prenatal voluntary exercise was also shown to play a protective role in rat offspring against postnatal hypoxia.[30] Not-so-intense exercise on treadmills was also associated with the expression of certain genes such as those linked with a brain-derived factor, vascular endothelial growth factor, and enhanced neurogenesis in the hippocampus.[31] The result of this study indicated that exposure to 20 mg/kg of SV was associated with a significant drop in the amount of exercise on running wheels. Studies have also suggested the efficacy of exercise during pregnancy on spatial learning in rats.[32] Moreover, access to running wheels has been reported to increase hippocampal cell proliferation and cell survival in rats.[15] Importantly, the present findings in conjunction with those of a study done in 2007 suggest that maternal exercise on a treadmill may also enhance offspring's brain functions.[33] Interestingly, a study by Hydari and colleagues reported that maternal exercise on wheels led to significantly lowered anxiety, both in the control group and in the group receiving morphine.[34] The present findings, in contrast, revealed that exercise reduced anxiety in the control group but led to increased anxiety in the experimental groups (where the animals received SV). The sedative properties of SV, evidently, is more efficient in relieving anxiety.[34]

This study investigated the effects of voluntary exercise on behavioral variation in rats' offspring. The limitation of the study was lack of surveying molecular mechanisms such as the correlation between hippocampal brain-derived neurotrophic factor (BDNF) mRNA expression and memory performance.

Conclusion

The results of the present study showed what degree of prenatal exposure (expressed in mg/kg) to SV caused impaired cognitive behavior. In addition, maternal exercise on running wheels was shown to affect positively the cognitive impairments in the male offspring caused by the administration of SV to the mother during pregnancy. This study provides some insights about the possible effects of voluntary exercise on learning and anxiety in offspring rats. Moreover, the findings of the present study provide insights into future research into the effects of mandatory exercise and the investigations of molecular mechanisms (role of BDNF) of exercise on memory and anxiety in animal models.

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Conflict of Interest: None declared.

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