Brief Communication

Post pubertal behavioral changes in rats with neonatal lesions of the ventral hippocampus

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The neuropathology and psychopathology of schizophrenia are still poorly understood. This is attributed to the paucity of adequate animal models. The potency of animal models in this disorder research is limited to certain aspects of the disease.[1] It has been shown previously that rats with a neonatal excitotoxic lesion of the ventral hippocampus display in adulthood a variety of abnormalities reminiscent of schizophrenia, and can be used as an animal model of this disorder. The excitotoxic lesion involves regions of the hippocampus that directly project to the prefrontal cortex, in the ventral hippocampus, and ventral subiculum.[2] These regions correspond to the anterior hippocampus in humans, an area that shows anatomic abnormalities in schizophrenia.[3] Evidence suggests that the neonatal lesion of the ventral hippocampus in the rat comprises the architectural integrity of medial prefrontal cortex (mPFC) (a region that receives extensive projections from the ventral hippocampus) for example, this lesion decreases GAD67 mRNA expression, N acetyllaspartate (NAA) concentration and spine density, and dendrites length of mPFC pyramidal neurons. Complex behavior thought to be dependent on the integrity of the prefrontal cortex, such as social interactions are also disrupted by the neonatal but not adult ventral hippocampal lesions.[4] It seems that the social interaction test is a relevant tool to study animal models of schizophrenia.[1] In this study, we use the neonatal ventral hippocampal lesion (NVHL) model that was developed in 1993 by Lipska, et al[2] and there are several reports on this model, specially related to social behaviors and stereotypic movements. Therefore our objective was to evaluate the post pubertal behavioral changes in rats with neonatal lesions of the ventral hippocampus.

In this study, we used 3 groups, 7 rats in each group: control group, sham control group, and test group. Pregnant Wistar rats were obtained at the last days of gestation and were individually housed in a humidity and temperature controlled environment on a 12/12 hours light/dark cycle with free access to standard chow and tap water. On the day of the birth, litters of 6-10 male pups were formed. Wistar pups were born in house on postnatal day (PD=0) and maintained in litters under standard conditions. All efforts were made to minimize animal suffering, and to reduce the number of animals used. All of the surgical procedures were approved by the Tehran University of Medical Sciences, Animal Care Committee (Guide for the care and use of laboratory animals, national research council, 1996), Tehran, Iran.

Following delivery of the pups, the rats were left undisturbed for 7 days. On PD=7, body weight was 11-15g, the male pups of each litter were randomized to sham, control, and test animals. Surgery was performed according to the method described earlier.[2] First, anesthesia by hypothermia was obtained by placing the male pups on wet ice for 10-15 minutes, and anesthesia was verified by non responsiveness to tactile stimulation. An incision was made midline, longitudinal over the skull, and 0.3 microliter of biogenic acid (10 microgram/micro liter) or an equal volume of sterile physiological serum was injected through a 30 gauge track needle. The rate of injection was 0.15 microliter/minutes. Surgery was carried out by a Stereotaxic apparatus, and it was modified by a platform. It was performed through the coordinates: anteroposterior=-3.0mm, Lateral-medial=+3.5 and -3.5mm. Dorsoventral=-5.0mm from the skull, relative to bregma. The needle was left in place for 2-4 minutes after injection to prevent backflow. The skin was sutured with Ethicon 4.0-6.0 sutures (Stoelting co, Illinois, USA). The animals were placed under a warming lamp. They were returned to their mothers until they regained their normal activity and consciousness.

At PD=56-58, the animals underwent 90 minutes of stereotypic movement tests. The movements of grooming, head twitching, head weaving, rearing, turning, self licking, and back pedaling were measured. The stereotyped behaviors were rated as follows: rearing (number of times the animal stood on its hind legs, head weaving (number of times the animal made slow, side to side head movements), head twitching (number of times the animal exhibited rapid lateral twitching of the head similar to the pinned reflex), back pedaling (number of times the animal made backward locomotion) and turning (number of times the animal circled laterally to the left or right over 360 degrees within a relatively small area). Rating of stereotyped behaviors was made over a 90 minutes observation period.[5] After this test, the animals were isolated for 7 days. In the social interaction test, the rats were normally singly housed for a short period before the test. The social activities like following each other, investigating each other, licking each other, climbing on and climbing down (nonaggressive behaviors) and aggressive behaviors like: playing, threatening, and biting were calculated.[1] This research was carried out between October 2009 and March 2010 in the Anatomical Department of Tehran University of Medical Sciences, Tehran, Iran. Our study limitations were the rat strain and and set the Stereotaxic apparatus for neonate.

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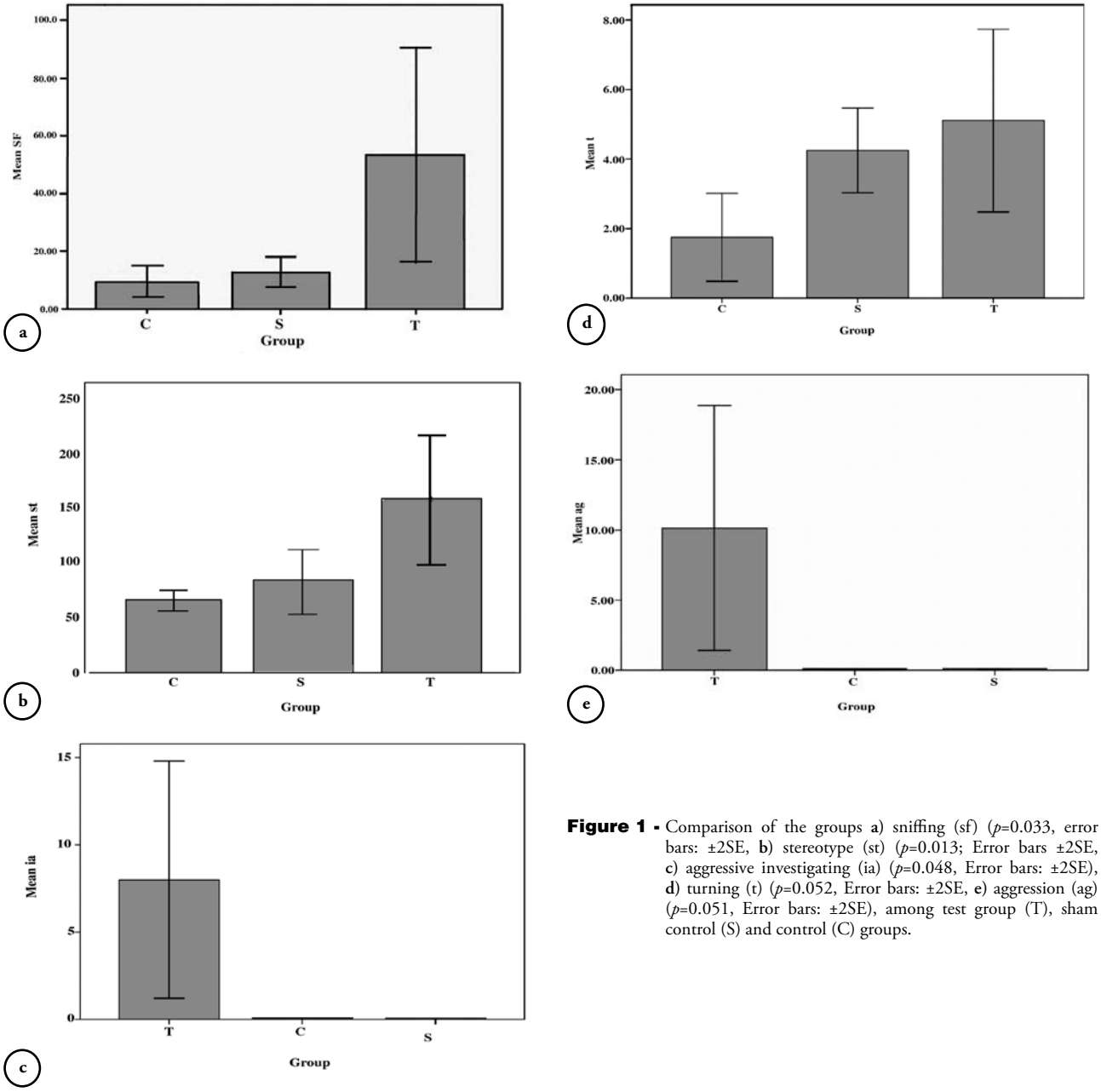

Figure 1 - Comparison of the groups **a**) sniffing (sf) (p=0.033, error bars: ±2SE, **b**) stereotype (st) (p=0.013; Error bars ±2SE, **c**) aggressive investigating (ia) (p=0.048, Error bars: ±2SE), **d**) turning (t) (p=0.052, Error bars: ±2SE, **e**) aggression (ag) (p=0.051, Error bars: ±2SE), among test group (T), sham control (S) and control (C) groups.

The statistical analysis was based on analysis of variance (ANOVA) and Minitab software. The significance threshold was set at 0.05.

Stereotypic movement test. An ANOVA revealed a significant increase of stereotypic movements (p=0.013) among the test group and sham control and control groups (**Figures 1a-1e**). Among the variables of stereotypic movements, sniffing and turning revealed a significant increase in the test group as follows, for sniffing the p-value among the test group relative to the sham control group, and sham control group relative to the control group is 0.033 (**Figure 1a**). For turning, the p-value between the test and control groups is 0.052 (**Figure 1**d)

Social behavioral test. An ANOVA revealed a significant increase in the sham control group in non-aggressive behavior relative to the test group (p=0.048). The other ANOVAs revealed an increase in the test group in aggressive behavior relative to the sham control and control groups (**Figure 1e**) (p=0.051). Among the variables of aggressive behavior, the aggressive behavior of investigating each other (i+a) revealed a significant increase in the test group relative to the sham control and control groups (p=0.048) (**Figure 1c**). These data indicated surgery without neurotoxin stimulates nonaggressive behavior and investigating each other.

In agreement with other investigators, we found a significant increase of stereotypic behaviors (p=0.013) in an animal model of schizophrenia, neonatal ventral hippocampal lesion model.[5] A study investigated Phencyclidine (PCP) induced behavior as an animal model of schizophrenia, we used NVHL.[2] They, like us, reported negative results on rearing in the 5mg/kg PCP induced behavior, but found an increase of vehicle (without any drug) in turning (p=0.052) and sniffing (p=0.033). We can conclude that PCP and NVHL are 2 similar models regarding their stereotypic behavioral characteristics.

In accordance with the study of Becker and Grecksch,[1] NVHL rats have aggressive behavior relative to the control group. We found a significant increase in the sham control group versus the test group in nonaggressive behaviors (p=0.048). This information with a significant increase in aggressive behavior in the test group versus the sham and control groups (p=0.051) confirms Becker and Grecksch findings.[1] In addition, some components of aggressive behavior like investigating each other aggressively (i+a) is the same as Becker et al[1] and Lipska[2] findings.We conclude that NVHL in Wister rats has the same results as Sprague Dawley rats.

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