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**E-mail: submit@mutagens.co.in**
**researchsubmission@hotmail.com**

***Research Paper***

# NEONATAL MATERNAL DEPRIVATION DOWN REGULATES HIPPOCAMPAL GIRK2 CHANNELS AND RELIEVES ANXIETY IN ADULT RAT

Ahmed M. Shehata[1], and Rizk H. A.[2]

[1] Physiology Department,
[2] Pharmacology Department,
National Organization for Drug Control and Research.
6 Abu Hazem Street- Pyramid Ave- Giza- Egypt

**Abstract**

Early postnatal maternal deprivation (MD) leads to a variety of biochemical, molecular and behavioral alteration in the offspring during adulthood. The study aimed to examine the effects of neonatal maternal deprivation (NMD) on hippocampal activity during adulthood in rats. Rats were subjected to daily maternal deprivation for one hour from postnatal day 2 to 14. The animals were returned to their mothers cages and kept undisturbed until weaning in day 21 and separated into males and females in separate cages until day 70. The male animals were only used in the study. Hippocampal extracelluar DA and 5-HT were *in vivo* determined using microdialysis technique with HPLC –EC detection. The behavioral performance was evaluated using open- field test (OFT) connected with computerized video- tracking system. In addition, the level of expression of hippocampal mRNA of G protein inwardly rectified potassium ($GIRK_2$) channel was determined using PCR technique. **Results** Microdialysis results revealed no change in the level of extracellular dopamine and serotonin, higher center time in OFT and significantly decreased in the level of $GIRK_2$ expression of NMD group in comparison to control animals. The study indicates that NMD has an anxiolytic effect, where it minimizes the stressful effect of OFT, possibly, due to GIRK2 down regulation.

Key words: Hippocampus, Neurotransmitters, Maternal deprivation, Stress, Receptor, Rat.

## INTRODUCTION

Stress has been described as a potential threat, arising from outside or from within the organism [1,2] and has also been defined as a physiological or psychological threat that activates the "Stress- response machinery [3]. Early postnatal maternal deprivation (MD) leads to a variety of biochemical, molecular and behavioral alteration in the offspring during adulthood and modifies the response of MD individuals toward some neuroactive compounds [2, 4-8]. Moreover, early MD in rodents has been proposed as an animal model for certain aspects of schizophrenic and affective psychopathologies [9-11].

Several findings relate the hippocampal formation to the behavioral consequences of stress, where various major neurotransmitter systems in the hippocampus are involved in these effects [12-14]. In addition, neonatal maternal deprivation has been found to alter the bidirectional

neural plasticity in the amygdala and modify the memory consolidation and information processing in the hippocampus [11,15].

G-protein-coupled inward rectifier $K^+$ (GIRK) channels play an important role in regulating cellular excitability and synaptic transmission in the nervous system and in many tissues [16]. Stimulation of $G_i$-coupled receptors activates the G protein gated inward rectifier $K^+$ channels, leading to hyperpolarization and reduction of membrane excitability [17]. Although the receptor- activated GIRK currents vary according to the receptor subtypes stimulated and cell types examined, they generally are biphasic, with a rapid activation followed by a slower desensitization [18]. In the hippocampus, $GIRK_1$ and $GIRK_2$ subunits are primarily localized to postsynaptic compartments and particularly found in peri- and extrasynaptic regions of dendritic spines of CA1 pyramidal neurons [19,20]. However, the high hippocampal GIRK expression indicates the importance that GIRK channels may have in modulating learning and memory [20].

The study aimed to examine the effect of neonatal deprivation (NMD) on brain activity during adulthood in rats. This was achieved through the determination of extracellular DA and serotonin, and $GIRK_2$ channel expression in the hippocampal CA1 region and evaluating the behavioral performance in open field test for the adult animals subjected to neonatal maternal deprivation.

## MATERIALS AND METHODS

Experimental animals: Female pregnant Sprague Dawley rats (150-200 g) were kindly provided from our breeding center at NODCAR and kept for a week for acclimatization under normal conditions and constant temperature (25±1C°) with *ad libitum* water and food until starting the experiment. All litters were born within one- day period. Maternal deprivation started from PND 2 until PND 14 between 9.00- 12.00 am by removing the mother from the homecage containing the pups for 60 min. After PND 14, the rats remained with their mothers continuously until weaning (21 days of age), at which time female littermates discarded , male littermates were retained for the study and housed six to a cage. All subsequent experiments were performed in adulthood (60-75 days). The maternally deprived animals were divided into two groups each of 10 rats. Group 1 was used for the *in vivo* determination of hippocampal dopamine and serotonin. Group 2 was subjected to open field test then the animals were sacrificed to determine the level of mRNA expression. Two control groups (naive) ran parallel to the deprived groups The experiments were conducted according to the institutional guidelines for animal care and use of NODCAR, Egypt.

*Chemicals*: All chemicals, unless specified otherwise, were purchased form Sigma-Aldrich.

Guide canulae, probes, HPLC column were purchased from Eicom Co. - Japan. TRIzol reagent and IQ™SYBR GREEN Supermix kit for determination of $GIRK_2$ channel mRNA were purchased from Invitrogen Co. and BIO-RAD Co. CA, USA.

## *IN VIVO* DETERMINATION OF HIPPOCAMPAL DA AND 5-HT.

The rats were anesthetized with pentobarbital (50.0 mg/kg, i.p.) and mounted on stereotaxic frame and implanted with guide canulae in CA1 in hippocampus. The stereotaxic coordinate were 3.8 mm caudal to bregma, 1.6 mm from medial- lateral, and 3.6 mm ventral from the dura surface [21]. Two jeweler's screws were placed in the skull surrounding the cannula and cemented in place with dental acrylic. The extracellular level of CA1 hippocampal DA and 5-HT were determined in the animals by perfusing the implanted probe at constant flow rate of 2µl/min with artificial CSF (composed of 145 mM NaCl, 3 mM KCl, 126 mM $CaCl_2$, and 1mM $MgCl_2$, buffered at pH 7.4 with 2 mM sodium phosphate buffer). The perfusate was collected every six min. and automatically injected and assayed by HPLC with electrochemical detection. After 1 hour of stabilization, to reach uniform concentration of DA and serotonin in dialysate, ten samples were collected from each animal to measure the extracellular levels of DA and 5-HT. Following determining hippocampal dopamine and serotonin, probe positioning was histologically verified [22,23]. Results are only taken from animals where the probes were correctly placed in the CA1 hippocampus.

**Open field test**

The open field test (OFT) is a widely used procedure for examining the behavioral effects of drugs and anxiety. Open field has been used to measure anxiety and depression reliably in rodents [24]. Each rat was placed in the center of 100x 100 $cm^2$ field, with a black arena divided in the monitoring system into 10x10 squares, surrounded by 50 cm high wall under dim light. Activity was measured for 5 min to assess total distance traveled, number of square crossing and time spent in the central area (area > 20 cm from the walls). Open field data were recorded using tracking system and automatically analyzed by a computerized software system. The field was cleaned with water containing ethanol between trials to eliminate any lingering olfactory cues. One day later, the animals were sacrificed and the brains were dissected to retrieve hippocampal region.

**Quantitative analysis of $GIRK_2$ mRNA by using real-time PCR**

Total RNA was extracted from isolated hippocamus using a TRIzol® Reagent, (Invitrogen) according to standard method [25]. cDNA was synthesized from 200 ng of total RNA Synthesis Kit, following the suppliers protocol (Bio-Rad). Levels of $GIRK_2$ mRNA were determined using iQ™ SYBR® Green Supermix (Bio-Rad) kit and reverse transcription- real-time PCR using a Chromo 4™ (Bio-Rad) instrument. Primers for $GIRK_2$ were constructed using Primer3 software. Primers for GIRK2 were 5'-GCAAGCTTATGACAA-TGGCCAA- GTTAAC-3' and 5'-GCTCTAGAAT-CACCCATTCCTCTCCGTC-3'. For standardization of quantification, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was amplified simultaneously. The change of reporter fluorescence from each reaction tube was monitored by Chromo 4™. The threshold cycle of each gene was determined as PCR cycles at which amplification efficiency in reporter fluorescence is almost 100 % as much as possible. The level of original mRNA expression of target gene and GAPDH was calculated according to a standard curve made by total RNA extracted from whole brain. In order to reduce the variation between individuals, the expression level of target gene was normalized by that of GAPDH.

**STATISTICAL ANALYSIS**

Data were analyzed using Student's t test. P values < 0.05 were considered significant.

**RESULTS**

**BEHAVIORAL PERFORMANCE IN OPEN FIELD TEST**

The performance in the open field test showed no significant difference between naïve and NMD groups regarding the number of square crossing and total distance, whereas, NMD group showed higher center time in comparison to naive group (Figure 1, 2& 3).

**NEUROCHEMICAL AND MOLECULAR PARAMETERS**

Neonatal maternal deprived animals showed no statistical difference in the extracellular levels of both dopamine and serotonin in comparison to levels of naive animals. Hippocampal dopamine and serotonin levels of control animals were 0.660 ±0.065 and 2.176 ± 0.114 f mol/12µl respectively (Figure 4). On the other hand, NMD adult animals showed significant decrease in the expression level of $GIRK_2$ channel in comparison to control group (Figure 5).

**DISCUSSION**

The present data revealed that neonatal maternal deprivation (NMD) for 1 hour from PND 2-14 didn't induce significant change in the extracelluar level of hippocampal dopamine and serotonin in adulthood. This could interpreted that NMD has no effect on the rate of transmitters release. Also, this might indicate that the present NMD stress model was not enough to elicit a persistent neurochemical effects during adulthood. It is worthy to note that, while stressors reliably influence various neuroendocrine and central neurotransmission, the nature and magnitude of these effects may be related to the characteristics of the stressors (like severity, duration, chronicity and controllability), as well as several experiential (previous stress exposure) and organismic factors ( age, strain and species) [26]. In accordance,

hippocampal neurochemistry is involved in the behavioral changes that result from early stressful life events [27]. In addition, motivational behavior in an operant conditioning task is greatly influenced by hippocamal homer1 level [28].
Regarding the open field performance, the study showed that NMD animals didn't differ from naive animals in locomotor activity but had higher center time in OFT indicating an anxiolytic effect. It is worthy to note that OPT represents in a stressful situation which leads to anxiety [29]. In accordance to this interpretation, the increased center time is considered a behavioral outcome associated with reduced anxiety-like behavior [30].

It might seem paradox, that while NMD modulated behavioral performance, it did not induce noticeable change in the level of extracelluar hippocampal transmitters (DA and 5-HT) in comparison to naïve animals. Hence, in the light of decreased expression of GIRK2 receptor, it might be plausible that the anxiolytic effect of NMD might be due to change in brain neurotransmission due to change in receptor density and/ or modulation of the efficiency of the cellular transduction system rather than just increased or decreased release of brain transmitters. Nevertheless, a previous study indicated that MD stress gives rise to persistent changes in the function, but not the density or mRNA expression of central 5-$HT_{1A}$ receptors and/or 5-HT transporters [31].

Despite that several studies have shown increased incidence of schizophrenia and other mental illnesses in individuals subjected to different forms of prenatal or early postnatal stress [10,32]. These effects might present a negative effect. On the other hand, in the present study, NMD stress didn't cause noticeable neurochemical or behavioral abnormalities, except for center time, indicating ant- anxiety activity, which might be a positive and beneficial effect. It is important to note that the effectiveness of any stress is dependent on its kind, severity, duration and surrounding environmental factors. Accordingly, early life stress effects might be beneficial or detrimental. In agreement with this revelation, the ”two- hit” theory postulates that first hit consists of early prenatal or postnatal stress and the “second hit” consists of one or more environmental factors, such as drug abuse or social stress, and combination of the two hits produces the symptoms of diseases [33].

In accordance to the present findings, a previous study indicated that neither brief maternal deprivation (15 min) nor long maternal separation (180 min), from postnatal day 2-14, produced major impact on learning or memory tasks in male Wister rats [34]. Moreover, it has been found that periodic or single maternal deprivation had little effect on the growth of developing rats and stress response of the HPA axis to the novel stress in the adulthood [35]. On the other hand, several studies indicated that neonatal maternal deprivation is a potent stressor which results in permanent behavioral alterations associated with a variety of modifications of neuroendocrine functions [36]. Among these functions, the hypothalamic- pituitary-adrenal (HPA) axis has been largely investigated in rats and it was established that neonatal stress enhances the adrenocortical response to novelty in adult rats.[37].

Data showed that NMD stress significantly decreased $GIRK_2$ channel expression, which might indicate that they facilitate excitatory neuronal transmission. The observation that although stress significantly decreased $GIRK_2$ channel expression, it did not affect the release of serotonin and dopamine, might indicate that stress modulates multiple sites with different outputs. In agreement to this interpretation, a previous study indicated that G protein-coupled inwardly rectifying $K^+$ channels (GIRKs) mediate postsynaptic but not presynaptic transmitter actions in hippocampal neurons and that that the same receptor can couple to different effector systems according to its sub-cellular location in the neuron [38]. Accordingly, several studies indicated that changes in expression of $GIRK_2$ containing channels have functional consequences that likely affect the balance between excitatory and inhibitory neuronal transmission [20,39]. The observation that various antidepressant drugs cause the inhibition GIRK channels expression [40,41], may support this interpretation.

## CONCLUSION

It is suggested that NMD effect is mediated by altering the expression of $GIRK_2$ channels coupled with presynaptic 5-$HT_{1A}$. Consequently GIRK channel downregulating leads to reduction in the inhibitory inputs from a number of metabotropic receptors and increases the excitability of serotonergic and presumably dopaminergic neurons. These effects eventually lead to the imbalance between excitatory and inhibitory neuronal transmission in favor of excitatory neurotransmission leading to the anxioltyic effect of NMD on OFT .

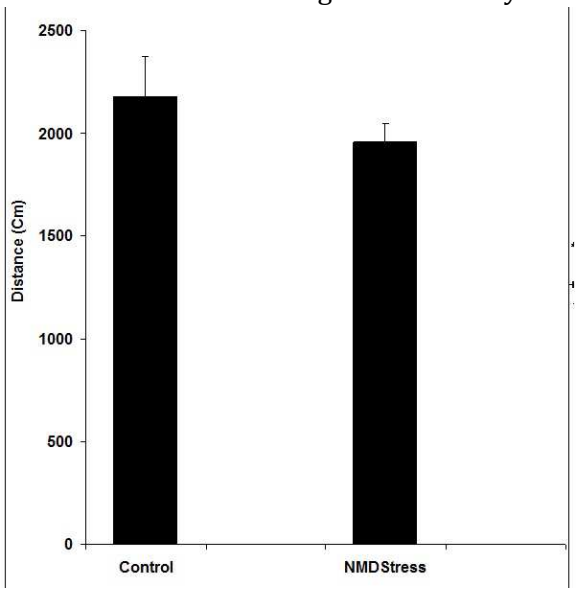


Figure 1 - Effect of Neonatal Maternal Deprivation (NMD) Stress on Locomotor Activity in Open Field Test in Adult Male Rat.

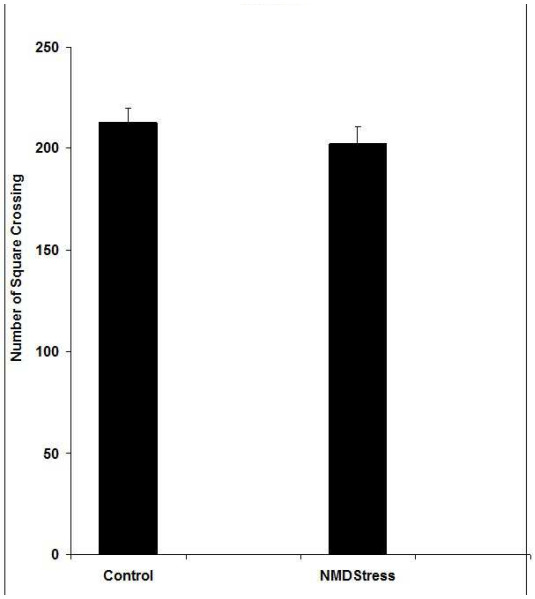


Figure 2 - Effect of Neonatal Maternal Deprivation (NMD) Stress on Number of Square Crossing in Open field Test in Adult Male Rat.

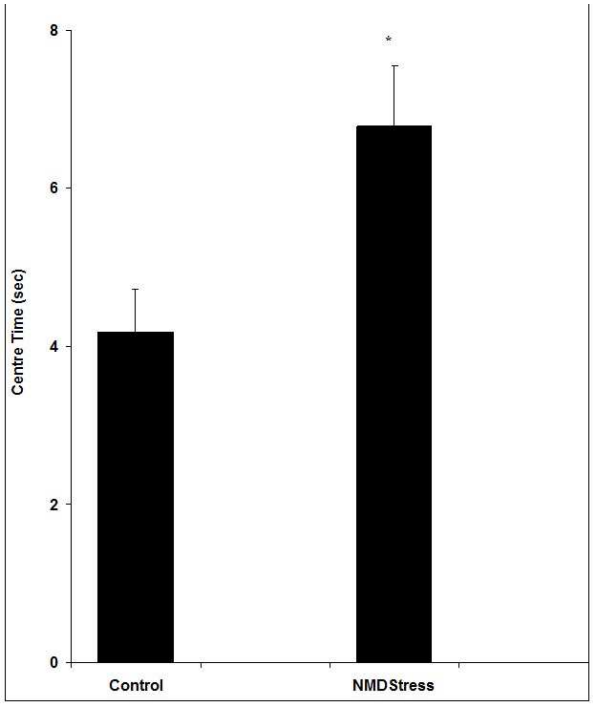


Figure 3 - Effect of Neonatal Maternal Deprivation (NMD)Stress on Centre Time in Open Field Test in Adult Male Rat.

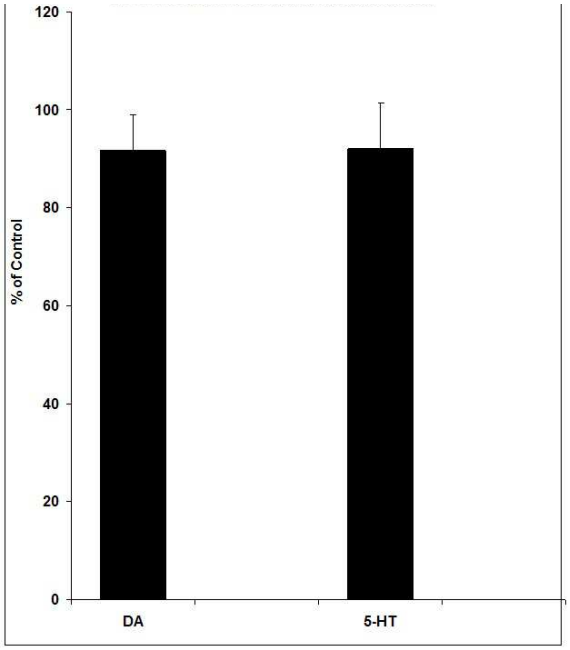


Figure 4 - Effect of Neonatal Maternal Deprvation (NMD) Stress on Extracellular Dopamine (DA) and Serotonin (5-HT) Levels in Dorsal Hippocamapal Area in Adult Male Rat.

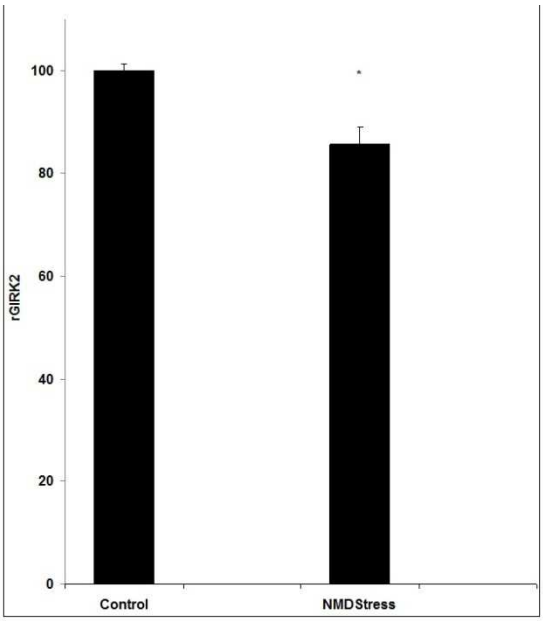


Figure 5 - Effect of Neonatal Maternal Deprivation (NMD)Stress on Hippocampal GIRK2 channels Expression in Adult Male Rat.

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