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Temporal Memory Dysfunction and Alterations in Tyrosine Hydroxylase Immunoreactivity in Adult Rats Following Neonatal Exposure to Domoic Acid

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ABSTRACT

The purpose of the present study was to determine whether early alterations in glutamate signaling, via daily injections of the glutamate agonist, domoic acid (DOM; 20 μg/kg), during a critical period of CNS development (PND 8 - 14), would result in temporal memory deficits and/or alterations in tyrosine hydroxylase (TH) immunoreactivity. As adults, subjects were assessed for temporal memory ability using a recency discrimination paradigm. Both number and dura- tion of exploratory contacts directed at familiar objects, differing by one hour in recall delay, were measured. Analyses revealed that DOM-treated females demonstrated temporal memory dysfunction, as evidenced in a significantly lower proportion of total exploratory behaviour directed toward the remote object. Integrity of the dopamine system was as- sessed using immunohistochemistry to examine TH immunoreactivity in the prefrontal cortex (PFC) and nucleus accumbens (NAcc). Sections obtained from DOM-treated males had significantly less TH immunoreactivity in the right mPFC, while DOM-treated females had significantly greater TH immunoreactivity in the left core and right shell of the NAcc. These findings are discussed in context of early alterations to glutamate signaling in the development of human neuropsychiatric disorders.

Keywords: Domoic Acid; Glutamate; Schizophrenia; Animal Models; Neurodevelopmental Disorders

1. Introduction

Glutamate (Glu) is the major excitatory neurotransmitter in the mammalian CNS and approximately half of all neurons in the brain are classified as glutamatergic [1]. Glutamate acts on both ionotropic (NMDA, AMPA and kainate [KA] receptors) and G protein coupled me- tabotropic receptors [1-4]. Proper levels of Glu signaling in the developing brain are critical as Glu has been shown to play an important role in CNS maturation, regulating neuronal survival, differentiation, and synap- togenis [5]. While appropriate Glu signaling is essential for a variety of neurophysiological processes, aberrant Glu signaling has been implicated in a variety of neuropathological processes, including disorders such as schizophrenia [6-9].

While much research has focused on identifying the role of the NMDAR in neurodevelopmental disorders such as schizophrenia, less is known about the influence of the KA receptors (KAR). However, the role of KAR in schizophrenia has been postulated, as KAR regulate the mesocorticolimic DA system at various levels. For instance, KARs directly modulate mesoacccumbens neu- rons and serve a modulatory function for DA release in the medial prefrontal cortex (mPFC) [10,11].

We have previously demonstrated that chronic exposure to low doses of domoic acid (DOM; a selective KAR agonist) during a critical period of brain development produces adult animals that demonstrate social withdrawal [12], deficient pre-pulse inhibition (PPI) [13] and latent inhibition (LI) [14], and increases in respon- siveness to novelty [15], which may arguably reflect negative, cognitive, and positive symptoms of schizo- phrenia, respectively. These observations, in conjunction with additional previously published data from our labo- ratory suggesting that early postnatal exposure to low-doses of DOM produce alterations in the functional in- tegrity of the mesocorticolimbic pathway [15-17] and altered cognitive functioning [17], are largely consistent with both clinical manifestations of schizophrenia and with those changes reported in existing animal models [18-22].

The purpose of the present experiment was to further characterize the effects of early exposure to DOM as they pertain to addressing issues of face validity (e.g., refer- ring to how well a model resembles the clinical syndrome in terms of its symptomatology) and construct validity (e.g. referring to similarities in underlying mo- lecular and cellular processes) in an attempt to determine the

prospective utility of this early toxin-treatment re- gime in producing a neurodevelopmental animal model with relevance to schizophrenia. More specifically, the study aimed to evaluate cognitive functions in adult ani- mals using a temporal memory paradigm (reportedly altered in schizophrenia [23]) and to explore for associated DA alterations in the mesocorticolimbic system. [24,25].

2. Methods

2.1. Experimental Animals

Experiments were conducted on the offspring (n = 40) of twelve untimed pregnant Sprague-Dawley rats (Charles River Laboratories, St Constant, QC). Dams were left undisturbed until the day of birth which was defined as postnatal day (PND) 0. Within 24 hours of birth, litters were culled to 10 pups (5 male and 5 female, when possible). Pups from each litter were then pseudo-randomly assigned to either saline (n = 20) or DOM (n = 20) treatments with an equal number of males and females in each treatment and with each treatment balanced within each litter.

2.2. Neonatal Toxin Treatment

Domoic acid, obtained from Bio Vectra dcl. (Charlottetown, PE), was dissolved in sterile saline and subcutaneous (s.c.) injections were administered in a volume of 10 ml/kg. From postnatal day (PND) 8 - 14, pups were weighed, marked for identification (*i.e.*, tail-marked with a non-toxic marker), and given a single daily subcutaneous injection of either 20 μg/kg DOM or equal volume of saline. Previous work in our laboratory has shown that at this dose, no overt signs of behavioural toxicity are apparent [26,27]. Pups were weaned on PND 21 and were group housed in a pseudo-random manner such that 2 - 3 rats of the same treatment and sex, but not from the same litter, were cage mates. Animals were housed in a colony room which was maintained at approximately 22˚C on a 12 hour reverse light cycle with lights turning on at 07:00

h. All animals received water and food (Purina Lab Chow) *ad libitum*. All procedures were conducted ac- cording to the guidelines established by the Canadian Council on Animal Care and in accordance with the Animal Care Committee at the University of Prince Ed- ward Island.

2.3. Testing Procedures

All behavioural testing was conducted with the experimenter blind to treatment and occurred during the dark phase of the light/dark cycle. Test trials were video-recorded with digital cameras mounted over the arenas for subsequent scoring and analyses.

Temporal Memory Task

Temporal memory performance was assessed in rats at 8 9 months of age. Testing was conducted in a black Plexiglas arena (110 × 40 cm) following a previously described procedure [28] outlined below. Subjects were given a one hour habituation period in the arena in which they were to be tested one day prior to the testing proce- dure. During trials, the objects were centered at each end of the arena, approximately 5 cm from the end walls. Objects were never re-used in more than one test procedure for any subject and both the objects, and the arena was cleaned between trials to eliminate odour cues.

The temporal memory task consisted of two sample trials and a test trial, each lasting five minutes. In the first sample trial, subjects explored two distinct objects placed in the arena, one at each end. Following a one hour delay period, in which subjects were returned to their home cages, a second sample trial was conducted wherein subjects explored two novel, distinct objects (*i.e.*, differing from the original two objects). Following a delay interval of either 1 hour, 24 hours or 96 hours, the test trial was then conducted. During the test trial, subjects were given five minutes to explore two familiar objects, one from the first sample trial (*i.e.*, designated as the remote object) and one from the second sample trial (*i.e.*, designated as the recent object). Each subject underwent the testing procedure three separate times (*i.e.*, corresponding to each of the delay intervals, with each testing procedure separated by one week), and with the delay order counterbalanced across subjects. Dependent measures included the percentage of exploratory behaviour directed at the remote object (*i.e.*, both duration of contact and number of contacts). Exploration was defined as sniffing directed at the object and/or contact between forelimbs and the object. It has been established that rats with intact temporal memory direct more exploratory behaviour at more remote objects. Thus, temporal memory dysfunction would be demonstrated if subjects direct a lower percentage of total exploratory behaviour at the more remote objects [28].

2.4. Tissue Processing

Following the completion of behavioural testing, rats were transcardially perfused using 4% paraformaldehyde.
Brains were immediately dissected from the skull and post-fixed in 4% paraformaldehyde for 24 hours prior to being stored in 0.08% sodium azide in phosphate buffered saline (PBS). A vibratome was used to cut 50 m coronal sections in the area of the PFC and NAcc (3.7 to

2.2 mm and 1.6 to 1.0mm, respectively) [29]. In addition, control sections and serial sections for cresyl violet counter-staining were kept and processed for each brain.

2.4.1. Tyrosine Hydroxylase Immunohistochemistry

Sections were processed free-floating and were stored in 24-well cell culture plates. The immunohistochemical procedure began with a series of rinses (3 × 5 min unless otherwise stated) in phosphate-buffered saline (PBS; pH 7.3) followed by a 30 minute incubation period in 1% hydrogen peroxide in PBS. Sections were then re-rinsed in PBS prior to being incubated for 30 minutes in 1% sodium borohydride in PBS. Another series of rinses in PBS was then conducted prior to a 30 minute incubation period in blocking serum (10% normal horse serum in PBS). Following the incubation period the blocking serum was removed and sections were treated with the primary antibody to TH (mouse anti-TH, monoclonal) at a working dilution of 1:2000 in PBS for approximately 60 hours at 4˚C. After the incubation period a series of rinses (5 × 5 min) in Tris buffered saline (TBS; pH 7.3) was performed before sections were incubated for 2 hours in biotinylated horse anti-mouse IgG (Vector Laboratories) in TBS in a dilution of 1:100. Tissue was then rinsed in TBS prior to a 2-hour incubation period in avidin-biotin complex, after which the final set of rinses in TBS was performed. Sections were then floated in PBS onto slides before the peroxidase reaction was developed using 0.2% hydrogen peroxide in diaminobenzidine (DAB) for 8 minutes. To quench the reaction, following treatment with DAB sections were rinsed with approximately 2 mL of both PBS and distilled water be- fore being left to dry overnight. The next day, sections were cover-slipped using Permount.

2.4.2. Quantification

Sections containing the mPFC and NAcc (core and shell), from the left and right hemisphere were photographed (250X magnification) using a digital Cannon Rebel EOS xSi camera mounted on a MEIJI trinocular ML5000 series microscope. For projection areas, instances of immunoreactivity were counted as a measure of TH and were operationally defined as dark, punctated dots which represented sites of TH localization. Counts were conducted using a standard counting square that, for the mPFC, was positioned in layer VI of both the left and right hemispheres, approximately 700 μm from the pial surface. For the NAcc, measured were obtained from four standard locations in the left and right core and from two standard locations in the left and right shell regions. For dopaminergic nuclei, immunoreactivity was determined by performing cell counts on immunopositive somata.

3. Results

3.1. Temporal Memory Task

Data obtained from male and female rats were analyzed using separate independent t-tests. A preference ratio was calculated for the remote objects (*i.e.*, determined by the percentage of the total duration and number of exploratory contacts that were directed at the remote object). Analysis of data obtained from female rats demonstrated that DOM-treated rats directed a lower percentage of exploratory contact toward the remote object at 96 hrs [$t(17) = 1.928$, $p = 0.035$] (**Figure 1(a)**), an effect not present in male counterparts (**Figure 1(b)**).

3.2. TH Immunohistochemistry

3.2.1. Prefrontal Cortex

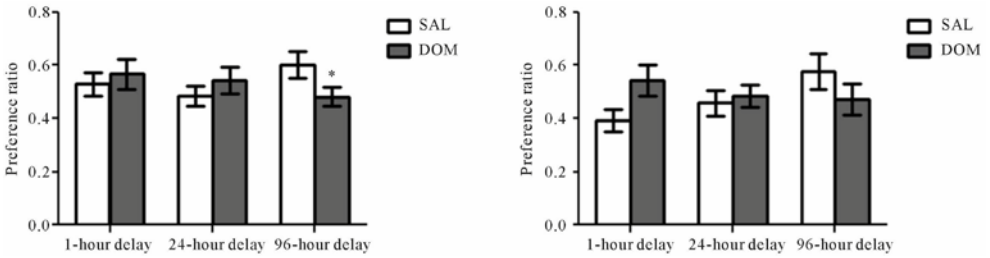
An analysis of immunoreactivity in the right mPFC of male rats revealed a statistically significant treatment effect with DOM-treated rats exhibiting less TH staining than SAL-treated counterparts [$t(9) = -2.392$, $p = 0.02$] (**Figure 2(b)**), an effect not present in female DOM-treated rats (**Figure 2(a)**). No other statistically significant differences in terms on immunoreactivity were found for either sex in either hemisphere of mPFC.

3.2.2. Nucleus Accumbens

Analyses revealed statistically significant treatment effects with female DOM-treated rats exhibiting more TH staining than SAL-treated counterparts in the left core [$t(10) = -2.691$, $p = 0.012$], and the right shell [$t(9) = -3.405$, $p = 0.004$] (**Figure 3(a)**); an effect not present in male rats (**Figure 3(b)**). No other statistically significant differences in terms of immunoreactivity were found for either sex in either hemisphere of the shell of core re- gions of the NAcc.

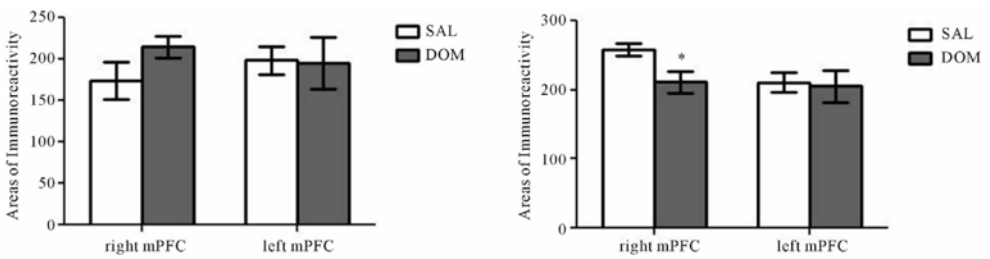
4. Discussion

Results from previous studies in the laboratory have demonstrated that early alterations in Glu signaling through daily injections of the Glu agonist DOM during a critical period of CNS development (e.g. PND 8 -14) results in various behavioural abnormalities [12-17] that are consistent with those exhibited in existing animal models of schizophrenia. Thus, the current study was conducted to evaluate timing behaviour and TH immunoreactivity, two assessments that are relevant in further determining whether this early toxin treatment regime may show potential utility in capturing some endophe



(a) (b)

Figure 1. Mean preference ratio for the remote object for (a) female and (b) male rats during a 5-min test trial. Error bars indicate SEM.



An asterisk denotes a significant difference between DOM-treated and saline-treated rats, p < 0.05.

(a) (b)

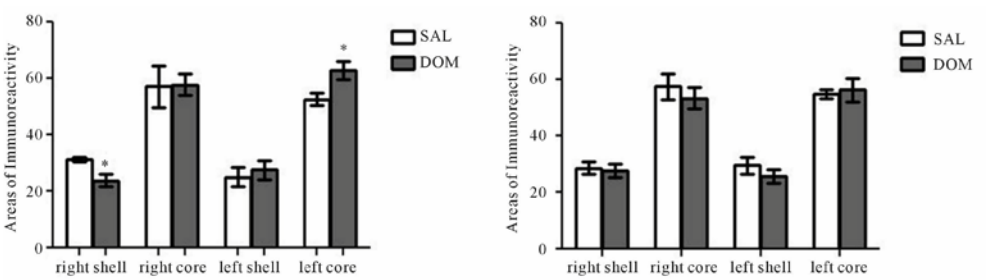


Figure 2. Mean number of immunoreactive areas in the left and right mPFC of (a) female and (b) male rats. Error bars indicate SEM. An asterisk denotes a significant difference between DOM-treated and saline-treated rats, p < 0.05.

(a) (b)

Figure 3. Mean number of immunoreactive areas in the left and right core and shell of the NAcc of (a) female and (b) male rats. Error bars indicate SEM. An asterisk denotes a significant difference between DOM-treated and saline-treated rats, p < 0.05.

notype of schizophrenia.

Analyses of data obtained during temporal memory testing revealed that adult DOM-treated females tested with the 96-hour delay directed a lower proportion of their total time spent exploring at the remote object, a finding consistent with studies demonstrating temporal memory dysfunction in the clinical population and in rodents following vasopressin 1-b receptor knockout [30], irreversible lesion of the mPFC [28], and reversible le- sions of the hippocampus [31]; all of which have relevance to schizophrenia.

Immunohistochemical analyses revealed alterations in both the nuclei and projection areas of the mesocorticolimbic DA system, with DOM-treated male rats demonstrating significantly less TH in the right mPFC, while female subjects had significantly more TH in the left core and right shell of the NAcc. Such alterations are likely to be the result of either alterations in the number of DA somata and/or altered expression of TH. Regardless of the source of dopaminergic alterations, these results are consistent with studies of the clinical population which have shown decreased density of TH-immunoreactive axons in layers III and VI of the entorhinal cortex and in layer VI of dorsomedial PFC of individuals with schizophrenia [32,33]. Also, rodents injected with the compete- tive NMDAR antagonist CGP 40116, a treatment that is hypothesized to have relevance to schizophrenia, have been found to exhibit decreased the density of TH-immunoreactive axons in both the deep layers (V/VI) and superficial layers (II/III) of the mPFC [34]. Significant increases in TH in the NAcc have also been observed in adult pups that were exposed to prenatal inflamationinduced hypoferremia; another treatment with relevance to schizophrenia [35].

Significantly, these neurochemical findings, thought modest, are consistent with behavioural abnormalities following neonatal exposure to DOM. The observation of increased TH in the left core and right shell of the NAcc in DOM-treated females is consistent with the previous finding of increased sensitivity to the rewarding proper- ties of nicotine in DOM-treated females using a condi- tioned place preference paradigm [16]. This increase in sensitivity to the rewarding properties of nicotine is significant as heightened responsiveness to reward is likely to result of increased activity of the mesolimbic DA pathway which terminates in the NAcc, a finding that is consistent with increased TH in both the left core and right shell of DOM-treated adult females. Also, it is proposed that nicotine dependence is dependent on plasticity of the mesocorticolimbic DA system [36], thus suggesting that the functional integrity of the mesolimbic DA system has been altered by neonatal treatment with DOM. Additionally, the finding of decreased TH in the right mPFC of DOM-treated males is consistent with and pre- vious findings of abnormal social interaction, a negative symptom of schizophrenia which is associated with hypodopaminergia in the prefrontal cortex, in DOM-treated males [12].

Notably, there were numerous sex differences found in the immunohistochemical and temporal memory paradigms. These sex differences are consistent with the re- sults of various studies in our laboratory that have shown sex-specific alterations in behavioural tests of social withdrawal [12], PPI [13], LI [14], and emotionality [37]. However, while sex differences have been consistently observed, the source of these differences is, as of yet, undetermined. Such differences may be the result of differences in developmental time-lines for each sex with males appearing to have a larger window of vulnerability to insults [38], females appearing to be more vulnerable in early post-natal period as compared to males [39], and developmental time-points may not be equivalent (*i.e.*, females appear to mature faster than males) [40].

5. Conclusion

Results from the current study are of importance in that both behavioural and neurochemical alterations were detectable following neonatal treatment with a very low dose of a KAR agonist during a critical period of brain development. These findings are characteristic of schizophrenia and thereby further validate a novel neurodevelopmental animal model of schizophrenia, a model which may have applicability in advancing out understanding of

this devastating neuropsychiatric disorder.

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REFERENCES

[1] S. Ozawa, H. Kamiya and K. Tsuzuki K, "Glutamate Receptors in the Mammalian Central Nervous System," *Pro-gress in Neurobiology*, Vol. 54, No. 5, 1998, pp. 581-618. doi:10.1016/S0301-0082(97)00085-3

[2] B. Bettler and C. Mulle, "Review: Neurotransmitter Receptors II: AMPA and Kainate Receptors," *Neuropharmacology*, Vol. 34, No. 2, 1995, pp. 123-139. doi:10.1016/0028-3908(94)00141-E

[3] M. Jorgensen, C. K. Tygesen and P. H. Andersen, "Iono- tropic Glutamate Receptors-Focus on Non-NMDA Receptors," *Pharmacology & Toxicology*, Vol. 76, No. 5, 1995, pp. 312-319. doi:10.1111/j.1600-0773.1995.tb00153.x

[4] D. Bleakman and D. Lodge, "Neuropharmacology of AMPA and Kainate Receptors," *Neuropharmacology*, Vol. 37, No. 10-11, 1998, pp. 1187-1204. doi:10.1016/S0028-3908(98)00139-7

[5] J. W. McDonald and M. V. Johnston, "Physiological and Pathophysiological Roles of Excitatory Amino Acids during Central Nervous System Development," *Brain Research, Brain Research Reviews*, Vol. 15, No. 1, 1990, pp. 41-70. doi:10.1016/0165-0173(90)90011-C

[6] C. G. Parsons, W. Danysz and G. Quack G, "Glutamate in CNS Disorders as a Target for Drug Development: An Update," *Drug News & Perspectivew*, Vol. 11, No. 9, 1998, pp. 523-569. doi:10.1358/dnp.1998.11.9.863689

[7] S. I. Deutsch, R. B. Rosse, B. L. Schwartz and J. Mastro- paolo, "A Revised Excitotoxic Hypothesis of Schizophrenia: Therapeutic Implications," *Clinical Neuropharmacology*, Vol. 24, No. 1, 2001, pp. 43-49. doi:10.1097/00002826-200101000-00008

[8] V. Bubeníková-Valesová, J. Horácek, M. Vrajová and C. Höschl, "Models of Schizophrenia in Humans and Animals Based on Inhibition of NMDA Receptors," *Neuro-science and Biobehavioral Reviews*, Vol. 32, No. 5, 2008, pp. 1014-1023. doi:10.1016/j.neubiorev.2008.03.012

[9] K. Y. Tseng, R. A. Chambers and B. K. Lipska, "The Neonatal Ventral Hippocampal Lesion as a Heuristic Neurodevelopmental Model of Schizophrenia," *Behavioural Brain Research*, Vol. 204, No. 2, 2009, pp. 295 305. doi:10.1016/j.bbr.2008.11.039

[10] H. P. Jedema and B. Moghddam, "Characterization of Excitatory Amino Acid Modulation of Dopamine Release in the Prefrontal Cortex of Conscious Rats," *Journal of Neurochemistry*, Vol. 66, No. 4, 1996, pp. 1448-1453.

doi:10.1046/j.1471-4159.1996.66041448.x

[11] W. R. Wu, N. Li and B. A. Sorg, "Regulation of Medial Prefrontal Cortex Dopamine by Alphaamino-3-Hydroxy-5-Methylisoxazole-4-Propionate/Kainate Receptors," *Neuro- science*, Vol. 114, No. 2, 2002, pp. 507-516. doi:10.1016/S0306-4522(02)00276-2

[12] C. L. Ryan, M. A. Robbins, M. T. Smith, I. C. Gallant, A. L. Adams-Marriott and T. A. Doucette, "Altered Social Interaction in Adult Rats Following Neonatal Treatment with Domoic Acid," *Physiology & Behavior*, Vol. 102, No. 3-4, 2011, pp. 291-295. doi:10.1016/j.physbeh.2010.11.020

[13] A. L. Adams, T. A. Doucette and C. L. Ryan, "Altered Pre-Pulse Inhibition in Adult Rats Treated Neonatally with Domoic Acid," *Amino Acids*, Vol. 35, No. 1, 2008, pp. 157-160. doi:10.1007/s00726-007-0603-3

[14] A. L. Marriott, C. L. Ryan and T. A. Doucette, "Neonatal Domoic Acid Treatment Produces Alterations to Prepulse Inhibition and Latent Inhibition in Adult Rats," *Pharma-cology, Biochemistry and Behavior*, Vol. 103, No. 2, 2012, pp. 338-344. doi:10.1016/j.pbb.2012.08.022

[15] M. A. Burt, C. L. Ryan and T. A. Doucette, "Altered Responses to Novelty and Drug Reinforcement in Adult Rats Treated Neonatally with Domoic Acid," *Physiology & Behavior*, Vol. 93, No. 1-2, 2008, pp. 327-336. doi:10.1016/j.physbeh.2007.09.003

[16] M. A. Burt, C. L. Ryan and T. A. Doucette, "Low Dose Domoic Acid in Neonatal Rats Abolishes Nicotine Induced Conditioned Place Preference during Late Adoles- cence," *Amino Acids*, Vol. 35, No. 1, 2008, pp. 247-249. doi:10.1007/s00726-007-0584-2

[17] A. L. Adams, T. A. Doucette, R. James and C. L. Ryan, "Persistent Changes in Learning and Memory in Rats Following Neonatal Treatment with Domoic Acid," *Physiology & Behavior*, Vol. 96, No. 4-5, 2009, pp. 505-512. doi:10.1016/j.physbeh.2008.11.019

[18] J. M. Gold, C. Carpenter, C. Randolph, T. E. Goldberg, and D. R. Weinberger, "Auditory Working Memory and Wisconsin Card Sorting Test Performance in Schizophrenia," *Archives of General Psychiatry*, Vol. 54, No. 2, 1997, pp. 159-165. doi:10.1001/archpsyc.1997.01830140071013

[19] R. S. E. Keefe, S. E. Lees-Roitman and R. L. Dupre, "Performance of Patients with Schizophrenia on a Pen and Paper Visuospatial Working Memory Task with Short Delay," *Schizophrenia Research*, Vol. 26, No. 1, 1997, pp. 9-14. doi:10.1016/S0920-9964(97)00037-6

[20] G. W. Dalack, D. J. Healy and J. H. Meador-Woodruff, "Nicotine Dependence in Schizophrenia: Clinical Phe- nomena and Laboratory Findings," *The American Journal of Psychiatry*, Vol., 155, No. 11, 1998, pp. 1490-1501.

[21] R. A. Chambers and D. W. Self, "Motivational Responses to Natural and Drug Rewards in Rats with Neonatal Ventral Hippocampal Lesions: An Animal Model of Dual Diagnosis Schizophrenia," *Neuropsychopharmacology*, Vol. 27, No. 6, 2002, pp. 889-905.

doi:10.1016/S0893-133X(02)00365-2

[22] B. K. Lipska, J. M. Aultman, A. Verma, D. R. Wein- berger and B. Moghaddam, "Neonatal Damage of the Ventral Hippocampus Impairs Working Memory in the Rat," *Neuropsychopharmacology*, Vol. 27, No. 1, 2002, pp. 47-54. doi:10.1016/S0893-133X(02)00282-8

[23] B. Eleveg, G. D. A. Brown, T. McCormack, J. I. Vousden and T. E. Goldberg, "Identification of Tone Duration, Line Length, and Letter Position: An Experimental Approach to Timing and Working Memory Deficits in Schizophrenia," *Journal of Abnormal Psychology*, Vol. 113, No. 4, 2004, pp. 509-521.
doi:10.1037/0021-843X.113.4.509

[24] B. L. Schwartz, L. H. Deutsch, C. Cohen, D. Warden and
S. I. Deutsch, "Memory for Temporal Order in Schizophrenia," *Biological Psychiatry*, Vol. 29, No. 4, 1991, pp. 329-339. doi:10.1016/0006-3223(91)90218-B

[25] F. A. V. Waters, M. T. Maybery, J. C. Badcock and P. T. Michie, "Context Memory and Binding in Schizophrenia," *Schizophrenia Research*, Vol. 68, No. 2-3, 2004, pp. 119-125. doi:10.1016/S0920-9964(03)00221-4

[26] T. A. Doucette, S. M. Strain, G. V. Allen, C. L. Ryan and
R. A. R. Tasker, "Comparative Behavioural Toxicity of Domoic Acid and Kainic Acid in Neonatal Rats," *Neurotoxicology and Teratology*, Vol. 22, No. 6, 2000, pp. 863
869. doi:10.1016/S0892-0362(00)00110-0

[27] T. A. Doucette, P. B. Bernard, P. C. Yuill, R. A. Tasker and C. L. Ryan, "Low Doses of Non-NMDA Glutamate Receptor agonists Alter Neurobehavioural Development in the Rat," *Neurotoxicology and Teratology*, Vol. 25, No. 4, 2003, pp. 473-479.
doi:10.1016/S0892-0362(03)00034-5

[28] J. B. Mitchell and J. Laiacona, "The Medial Frontal Cortex and Temporal Memory: Tests Using Spontaneous Exploratory Behaviour in the Rat," *Behavioural Brain Research*, Vol. 97, No. 1-2, 1998, pp. 107-113.
doi:10.1016/S0166-4328(98)00032-1

[29] G. Paxinos and C. Watson C, "The Rat Brain in Stereotaxic Coodinates," 4th Editon, Academic Press, Toronto, 1998.

[30] L. M. DeVito, R. Konigsberg, C. Lykken, M. Sauvage, W.
S. Young and H. Eichenbaum, "Vasopressin 1b Receptor Knock-Out Impairs Memory for Temporal Order," *The Journal of Neuroscience*, Vol. 29, No. 9, 2009, pp. 26762683. doi:10.1523/JNEUROSCI.5488-08.2009

[31] J. G. Howland, R. A. Harrison, D. K. Hannesson and A.
G. Phillips, "Ventral Hippocampal Involvement in Temporal Order, but Not Recognition, Memory for Spatial Information," *Hippocampus*, Vol. 18, No. 3, 2008, pp. 251
257. doi:10.1002/hipo.20396

[32] M. Akil, C. L. Edgar, J. N. Pierri, S. Casali and D. A. Lewis, "Decreased Density of Tyrosine Hydroxylase-Immunoreactive Axons in the Entorhinal-Cortex of Schizophrenic Subjects," *Biological Psychiatry*, Vol. 47, No. 5, 2000, pp. 361-370.
doi:10.1016/S0006-3223(99)00282-6

[33] M. Akil, J. N. Pierri, R. E. Whitehead, C. L. Edgar, C.

Mohila, A. R. Sampson and D. A. Lewis, “Lamina-Specific Alterations in the Dopamine Innervation of the Pre-frontal Cortex in Schizophrenic Subjects,” *The American Journal of Psychiatry*, Vol. 156, No. 10, 1999, pp. 15801589.

[34] K. Wedzony, K. Fijal and A. Chocyk, “Blockade of NMDA Receptors in Postnatal Period Decreased Density of Tyrosine Hydroxylase Immunoreactive Axonal Arbors in the Medial Prefrontal Cortex of Adult Rats,” *Journal of Physiology and Pharmacology*, Vol., 56, No. 2, 2005, pp. 205-221.

[35] A. Aguilar-Valles, C. Flores and G. N. Luheshi, “Prenatal Inflammation-Induced Hypoferremia Alters Dopamine Function in Adult Offspring in Rat: Relevance for Schizophrenia,” *PloS One*, Vol. 5, No. 6, 2010, e10967. doi:10.1371/journal.pone.0010967

[36] D. J. Balfour, “Neuroplasticity within the Mesoaccumbens Dopamine System and Its Role in Tobacco Dependence,” *Current Drug Targets, CNS and Neurological Disorders*, Vol. 1, No. 4, 2002, pp. 413-421. doi:10.2174/1568007023339076

[37] T. A. Doucette, C. L. Ryan and R. A. Tasker, “Gender- based Changes in Cognition and Emotionality in a New Rat Model of Epilepsy,” *Amino Acids*, Vol. 32, No. 3, 2007, pp. 317-322. doi:10.1007/s00726-006-0418-7

[38] J. L. Nunez and M. M. McCarthy, “Evidence for an Ex- tended Duration of GABA-Mediated Excitation in the Developing Male versus Female Hippocampus,” *Developmental Neurobiology*, Vol. 67, No. 14, 2007, pp. 18791890. doi:10.1002/dneu.20567

[39] A. Kyrozis, O. Chudomel, S. L. Moshe and A. S. Galanopoulou, “Sex-Dependent Maturation of GABA$_A$ Receptor-Mediated Synaptic Events in Rat Substantia Nigra Reticulate,” *Neuroscience Letters*, Vol. 398, No. 1-2, 2006, pp. 1-5. doi:10.1016/j.neulet.2005.12.018

[40] R. K. Lenroot, N. Gogtay, D. K. Greenstein, E. Molloy- Wells, G. L. Wallace, L. S. Clasen, J. D. Blumenthal, J. Lerch, A. P. Zijdebnos, A. C. Evans, P. M. Thompson and J. N. Giedd, “Sexual Dimorphism of Brain Developmental Trajectories during Childhood and Adolescence,” *NeuroImage*, Vol. 36, No. 4, 2007, pp. 1065-1073. doi:10.1016/j.neuroimage.2007.03.053

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