Effects of Methylphenidate and Atomoxetine Treatment on Purkinje Cell Number in Juvenile Rats

Tolgahan Acar, Ph.D., **Berrin Zuhal Altunkaynak,** Ph.D., **Kıymet Kübra Yurt,** MS, **Murat Yuce,** M.D., **Habib Khoshvaghti,** MS, **Elfide Gizem Kıvrak,** MS, **Ebru Elibol,** Ph.D., **Mehmet Emin Onger,** Ph.D., and **Süleyman Kaplan,** Ph.D.

OBJECTIVE: To detect the effects of methylphenidate (MPH) and atomoxetine (ATX) on the cerebellum.
STUDY DESIGN: Male juvenile rats were separated into MPH, ATX, and control groups. The amount of Purkinje cells and the histological structure of the cerebellum were investigated.
RESULTS: In the MPH group a significant increase was noted in the number of Purkinje cells as compared with the other groups. Also, it was observed that the number of Purkinje cells increased in the ATX groups. The histopathological results showed that MPH and especially ATX caused pyknotic nuclei and irregular cell boundaries in some Purkinje cells.
CONCLUSION: MPH and ATX can be used to reduce attention-deficit hyperactivity disorder symptoms. We detected that MPH administration caused an increase but ATX caused a decrease in Purkinje cell numbers. Our data provides evidence to answer questions about the effects of MPH and ATX on the cerebellum. At this point, this study will be useful for contributing to the current literature and furthering the research in this field. (Anal Quant Cytopathol Histpathol 2016; 38:000–000)

Keywords: ADD, ADDH, ADHD, adolescence, adolescent psychology, atomoxetine, atomoxetine hydrochloride, attention deficit disorder, attention deficit disorder with hyperactivity, attention deficit hyperactivity disorder, cerebellum, methylpheni date, methylphenidatehydrochloride, N-methyl-gamma-(2-methylphenoxy)benzenepropanamine hydrochloride, neurodevelopmental disorders, Purkinje cells, stereology.

Attention-deficit hyperactivity disorder (ADHD) is the one of the most common disorders occurring

From the Department of Anatomy, Medical School, Şifa University, İzmir; the Departments of Histology and Embryology and of Child Psychiatry, Medical Faculty, Ondokuz Mayıs University, Samsun; and the Department of Histology and Embryology, Medical Faculty, Adıyaman University, Adıyaman, Turkey.

Dr. Acar is Assistant Professor, Department of Anatomy, Medical School, Şifa University.

Dr. Altunkaynak is Associate Professor, Department of Histology and Embryology, Medical Faculty, Ondokuz Mayıs University.

Miss Yurt, Mr. Khoshvaghti, and Miss Kıvrak are Research Assistants, Department of Histology and Embryology, Medical Faculty, Ondokuz Mayıs University.

Dr. Yuce is Associate Professor, Department of Child Psychiatry, Medical Faculty, Ondokuz Mayıs University.

Drs. Elibol and Onger are Assistant Professors, Department of Histology and Embryology, Medical Faculty, Adıyaman and Ondokuz Mayıs University.

Dr. Kaplan is Professor, Department of Histology and Embryology, Medical Faculty, Ondokuz Mayıs University.

Supported by the Scientific Project Foundation of Ondokuz Mayıs University, Samsun, Turkey.

Address correspondence to: Berrin Zuhal Altunkaynak, Ph.D., Department of Histology and Embryology, Medical Faculty, Ondokuz Mayis University, 55139 Samsun, Turkey (berrinzuhal@gmail.com).

Financial Disclosure: The authors have no connection to any companies or products mentioned in this article.

0884-6812/16/3800-0000/$18.00/0

during childhood. This psychopathology is related with genetic factors, but it has not been identified specifically which genes play a role in the disorder.[1] ADHD is a highly familiar neurodevelopmental disorder with symptoms such as inattention, impulsivity, and hyperactivity.[2] Increasingly, diagnosis and treatment of this disease is by psychostimulant medication.

Methylphenidate (MPH) and atomoxetine (ATX) are used commonly as a psychostimulant for the treatment of neurodevelopmental disorders. These drugs show their effect via extracellular catecholamine levels. Additionally, they affect dopamine and norepinephrine functions in the brain regions of the prefrontal cortex, hippocampus, and especially the cerebellum.[3-5]

During active attention tasks, the cerebellum and prefrontal cortex make connections with the frontal and parietal lobes. While performing the attention tasks, the cerebellum receives signals from a variety of specific brain regions.[6,7] MPH inhibits the transport of dopamine by binding to synaptic membranes with high affinity; hence, levels of dopamine and norepinephrine increase within the synaptic space.[8-11] MPH has shown a neuroprotective effect with the receptor mechanism.[12] Additionally, changes in catecholamines, which include MPH, can lead to toxic effects. There have been no reports about the direct effects of MPH, but differences in catecholamine levels may cause toxic or protective effects.[13] Additionally, MPH affects energy metabolism and oxidative stress mechanism.[14,15] This is activated via the apoptotic pathway and leads to cell death.[16,17]

ATX is a selective norepinephrine blocker. The prefrontal cortex, thalamus, locus ceruleans, and cerebellum are the regions mainly affected in the central nervous system.[18] Recent studies have shown that both of these drugs are unregulated and normalized left ventrolateral prefrontal cortex and cerebellum.[19]

show activation on the

As is known, the cerebellum is responsible for motor functions, coordination, and movement.[20,21] Recent studies have provided evidence that the cerebellum plays a critical role in hyperactivity. The potential effects of psychostimulants on the cerebellum are still poorly understood. Martins et al stated that these drugs trigger oxidative stress and impair the structure and functions of the central nervous system.[22] Additionally, their long-term use, especially in early childhood, may result in changes in the biochemical structure of the brain.[23,24] Schmitz et al performed an experimental study on juvenile rats and reported that MPH caused harmful effects in the lipid and proteins of the prefrontal cortex, but this harmful effect was not seen in the striatum, hippocampus, and cerebellum.

In this study the effects of MPH and ATX were analyzed[25] on the juvenile rat cerebellum by stereological and histological methods.

Materials and Methods

Experimental Design

Juvenile male Wistar albino rats (28 days old) were obtained from the Animal Research Center at Ondokuz Mayıs University, Samsun, Turkey. All experimental work was approved by the ethics committee (approval No. 2011/62). Three groups were formed randomly as follows (n=6 rats per group): Control group (physiological saline treated), MPH group (1 mg/kg, MPH treated), and ATX group (1 mg/kg ATX treated). Substances were administered for 14 days. Drugs and saline solution were ceased at the end of the 14th day, and the experimental animals were monitored drug-free for a further 45 days. Food pellets and tap water were provided ad libitum. MPH and ATX were prepared freshly, dissolved in 0.9% saline solution, and MPH, ATX, and saline were given by gastric lavage in a volume of 1 mL/kg of body weight. The administration of all drugs and saline were performed at 12 h intervals in 2 divided doses.

Histological Examination and Stereological Analysis

All animals were perfused intracardially with physiological saline at the end of the experimental procedure (60th day). The routine histological staining techniques were used in the processing of the cerebellum tissues. A microtome (Leica RM 2135, Leica Instruments, Nussloch, Germany) was used in taking the sections (20 µm), and they were stained with 0.1% Cresyl fast violet dye. Following the histological processing, stereological analysis was performed using the optical dissector method. Stereological methods allow the analysis of biological tissue in 3-D, allowing the use of theoretically unbiased sampling, and these estimation methods assess the first order stereological parameters—volume, surface area, length, and number. In the current study the estimation of the total amount of neurons in the cerebellum was carried out with the Optical Fractionator method[26,27] (Figure 1).

The Optical Fractionator methods used for par-

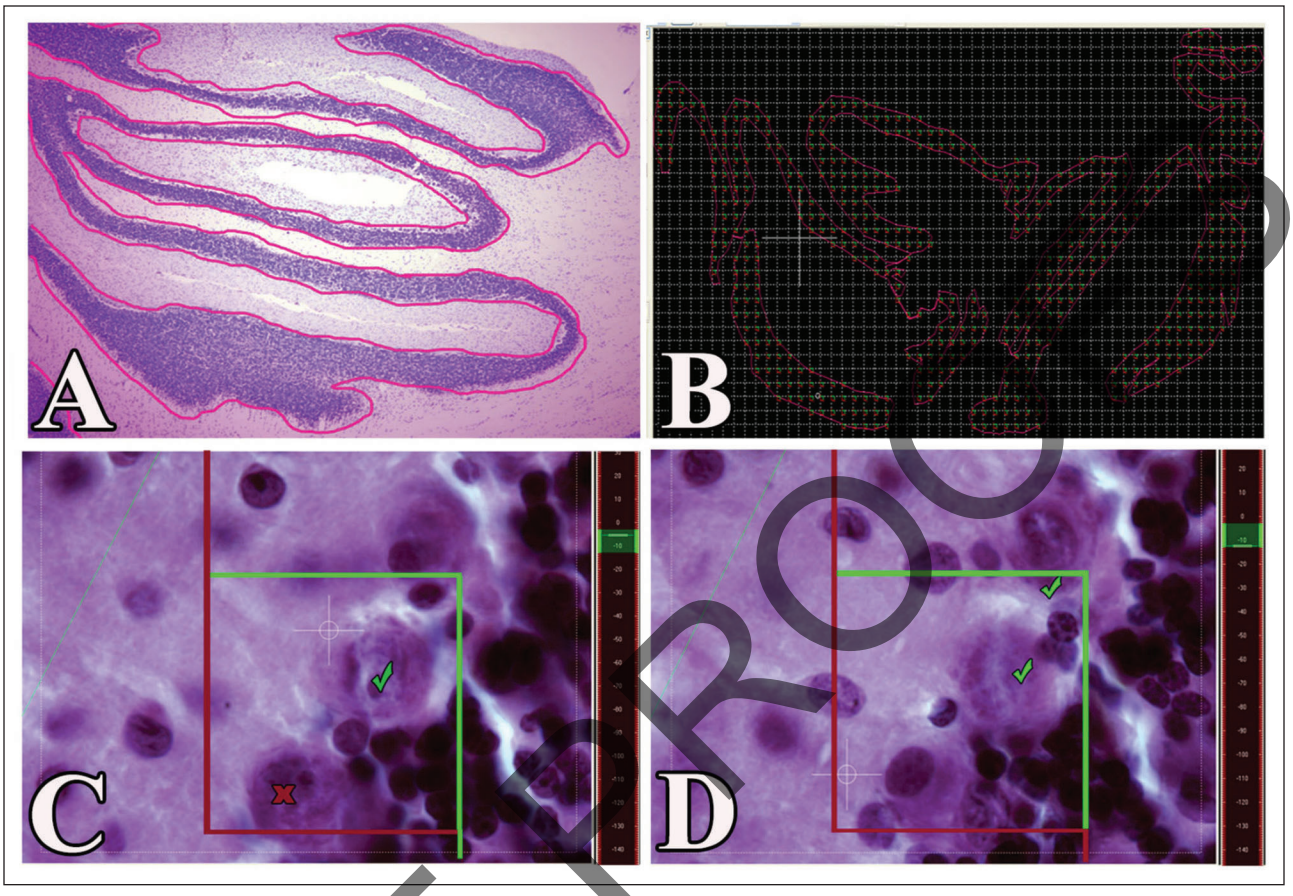

Figure 1 The Optical Fractionator method (A–D). Tracing of the counting area of the cerebellum to estimate total Purkinje cell number (A). Following the contour, the stereological counting probe was determined as 62,500 μm² using uniform distances between the lines in directions X and Y (B). Micrographs at C and D show a high-magnification image of the microscopic field indicated in a section for different thickness. One Purkinje cell is found at C, and 2 Purkinje cells are found at D. The microscopic field is superimposed with a counting frame with dimensions 45×45 μm. Purkinje cells located inside the counting frame or intersecting the green (inclusion) line were counted. However, Purkinje cells that intersected the red line were excluded (C–D). Original magnifications ×50 (A–B) and ×100 (C–D).

ticle counting are especially useful in the neurosciences. In this technique, particles are counted with an optical dissector in a uniform and systematic sample that constitutes a specific fraction of the region that is to be analyzed.[28] The optical dissector begins with a look up section at the top of the optical dissector and a reference section at the bottom of the optical dissector. According to a pilot study conducted previously, it has been reported to sample every sixth section (ssf) through to the systematic random sampling procedure. Approximately 15–20 sections taken from each brain are known to be sufficient in the estimation of the total amount of neurons when using the Optical Fractionator method for counting the cells.[28-30] Each dissector probe is actually a virtual counting box located in every counting field, and its height is less than the section thickness. This means that in every counting field a known fraction of section thickness (dissector height/section thickness) was chosen for particle counting. For the present study the unbiased counting frame area was 2,025 μm². The area sampling fraction (asf) was 2,025 μm²/62,500 μm². The dissector height was 12 μm, and a guard zone of 3 μm at the topmost part of the section was left out of the analysis at each step as an upper guard zone. In this case there is a third sampling step which is called *thickness sampling fraction* (TSF) (TSF=20/mean section thickness for each animal). About 16–20 sections were estimated for

each cerebellum using systematic random sampling.

In a stereology workstation (Stereo Investigator 9.0, MicroBrightField, Inc., Williston, Vermont, USA) stereological analysis was done by using a light microscope (Leica M 4000 B, Germany) with an attachment of digital color. The estimation of the total Purkinje cell number in the cerebellum was performed by using the following formula:

$$N = \sum Q \cdot (1/ssf) \cdot (1/asf) \cdot (1/tsf),$$

where ∑Q represents the total amount of Purkinje cells counted in all optically sampled fields of the cerebellum as dissector particles, ssf represents the section sampling fraction (1/6), asf represents the area sampling fraction (2,025/62,500), and tsf represents the thickness sampling fraction (defined by dissector height [20 µm] divided by the estimated mean section thickness).[27] The sufficiency of sampling and the proper number of sampled cells was coefficient of error (CE), and the coefficients of variation (CV) were observed in an acceptable level.[31,32] When the stereological examination was performed, we focused on Purkinje neuron numbers in the Purkinje cell layer. All discussed layers are shown in Figure 1.

Statistical Analysis

Before analysis the test of normality was applied to all groups. Data were found to be homogeneous. The mean cell amounts in the cerebellum were compared by groups using the one-way analysis of variance (ANOVA) (Tukey's Post-Hoc Test), and the homogeneity test was performed using by Levene test. Results were stated as the mean ± SEM. Statistical analyses were done on SPSS 20.0 for Mac (IBM Corporation). p Values of 0.05 were accepted as statistically significant.

Results

Stereological Results

There were significant differences among the 3 groups: Control, MPH, and ATX ($p < 0.05$) (Table I) (Figure 2). In the MPH group the number of Purkinje cells was significantly increased as compared to that in the Control and ATX groups ($p < 0.05$). A significant decrease in the amount of Purkinje cells was noted in the ATX group as compared to the MPH and Control groups ($p < 0.05$).

Histopathological Examination

We stereologically and histologically examined tissue samples by Cresyl violet dye. Normal-looking Purkinje cells in the cerebellum had healthy cytoplasm, light nuclei, and prominent nucleoli (Figure 3). Some Purkinje cells had pyknotic nuclei, dark-stained cytoplasm, or irregular cell boundaries in the light micrographs of the MPH group and, especially, the ATX group. Additionally, we detected cellular debris in the ATX group (Figure 3).

Table I *Comparison of Total Purkinje Numbers for Control, MPH, and ATX Groups*

Group	Mean ± standard error of the mean	Coefficient of variation value
Control	517,000 ± 5,400	0.01
MPH	628,000 ± 15,000	0.04
ATX	472,000 ± 9,200	0.03

Mean cell number and standard error of the mean values were represented below for each group. Coefficient of variation values were obtained from stereological analysis.

Discussion

The main purpose of the present research was to assess the effects of MPH and ATX on the cerebellum. As is known, ADHD is a neuropsychological and pediatric disorder with the symptoms of hyperactivity, inattentiveness, and impulsiveness. It starts in childhood and continues during ado-

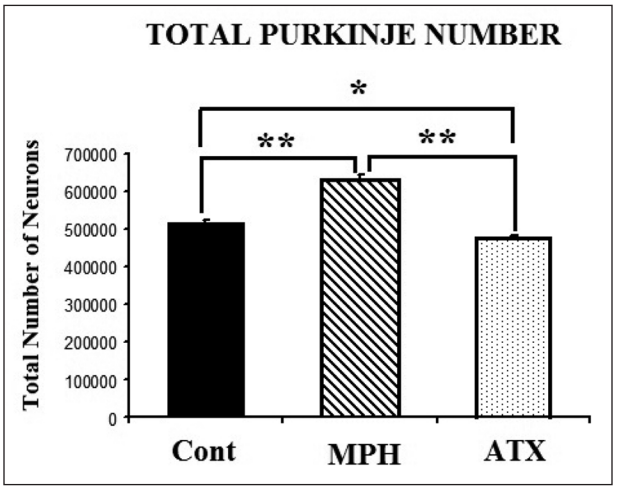

Figure 2 Comparison of total Purkinje number in the cerebellum sections for each group. An increase in cellular density in the MPH as compared to Control and ATX groups is noted ($p < 0.01$). A significant difference between the Control and ATX groups was also noted ($p < 0.05$).

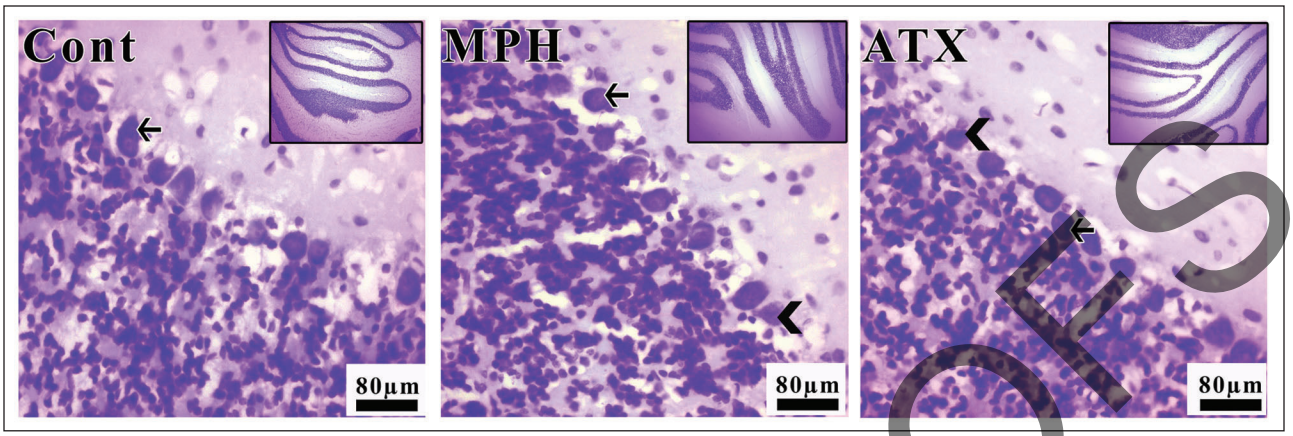

Figure 3 The histological images of the Control group (A), MPH group (B), and ATX group (C) are shown at high magnification (original magnification ×400). The images show dark-stained and pyknotic nuclei (head of black arrows) in the ATX group when Cresyl violet–stained sections were examined (A–C). Note the cellular organization and increase in cellular profiles in MPH. Scale bars = 80 µm.

lescence.[33] Changing the extracellular dopamine levels treats the disorder. ATX and MPH are the most often used psychostimulant drugs, affecting the catecholamine levels. Dopamine neurotransmission is absolutely necessary to motor control, which is thought to be disrupted in children with both attention-deficit and hyperactivity disorder.[34] Our study was undertaken to investigate the effects of the change in the dopaminergic system on the cerebellum following use of ATX and MPH in childhood. The cerebellum of primates includes a plurality of the dopamine transporter and is in communication as high as with other encephalic regions.[35] The cerebellum has a high level of nitric oxide synthase activity. Furthermore, nitric oxide mediates the neurotoxic effects of a considerable number of N-methyl-D-aspartate receptors in the cerebellum.[36]

All of the scientific data on these commonly used childhood medications suggest that the stimulant MPH and the nonstimulant ATX may have negative effects on the cerebellum. The cerebellum plays a significant role in motor and cognitive behaviors, and these drugs may lead to negative effects on the Purkinje cells in the cerebellum. The cerebellar Purkinje cells are major efferent neurons which manage the integration of the formation response and motor function.[37,38]

According to our study, a significant difference was seen in the number of Purkinje cells when comparing the ATX, MPH, and Control groups. Schmitz et al[25] reported that the activities of superoxide dismutase and catalase were increased. However, there was no change in the amount of thiobarbituric acid, the reactive species, and sulfhydryl groups in the cerebellum of rats treated with 2.0 mg/kg MPH. Perhaps this was caused by the antioxidant enzyme situation with its loss of neutralized reactive species. In our study we detected that MPH administration caused an increase in Purkinje cell numbers, but negative effects on Purkinje cell numbers for were seen in the ATX group. Other areas of the brain, such as the striatum, prefrontal cortex, and hippocampus, may be more sensitive to the effects of MPH.[25]

Scherer et al[24] suggested that MPH has specific effects on the encephalic regions. They found that MPH administration caused an increase in the activity of antioxidant enzymes through free radicals in the hippocampus and prefrontal cortex, but their activities were not heightened in the striatum. Many studies indicate that the effect of MPH depends on the brain region. This effect may be due to sensitivity and also differences in the antioxidant capacity of various regions of the brain.[25,39-41]

Some researchers reported that ATX led to an increase in the concentrations of extracellular norepinephrine in the prefrontal cortex, hypothalamus, hippocampus, and cerebellum of the rat.[11,42-44] Swanson et al reported a short-term increase in the extracellular norepinephrine levels in the cerebellum.[11] These results are probably associated with the fact that there is a more efficient auto-

receptor control system in the cerebellum. The administration of the physiological saline in the Control group caused a loss of Purkinje cells in the cerebellum. This situation could be caused by physiological effects of saline or by the stress mechanism. A reliable and unbiased stereological method, an Optical Fractionator, was used in this study for estimating the total number of Purkinje cells, thus giving the closest results to the true value.[31]

In conclusion, MPH and ATX are used to reduce the symptoms of ADHD. MPH usage did not result in a loss of Purkinje cells in the cerebellum. It is possible that the amount of MPH applied was insufficient to create cell damage or that the application time was too short. Thus, new immunohistochemical and molecular studies are required to explain any degeneration mechanism of MPH and/or ATX on Purkinje cells in the cerebellum. We believe that our study will encourage researchers to further investigate the effects of MPH and/or ATX on the cerebellum.

References

1. Faraone SV, Perlis RH, Doyle AE, Smoller JW, Goralnick JJ, Holmgren MA, Sklar P: Molecular genetics of attention-deficit/hyperactivity disorder. Biol Psychiatry 2005;57:1313-1323
2. Goetz M, Vesela M, Ptacek R: Notes on the role of the cerebellum in ADHD. Austin J Psychiatry Behav Sci 2014;1: 1013
3. Townsend J, Westerfield M, Leaver E, Makeig S, Jung TP, Pierce K, Courchesne E: Event-related brain response abnormalities in autism: evidence for impaired cerebello-frontal spatial attention networks. Brain Res Cogn Brain Res 2001;11: 127-145
4. Ojeda N, Ortuño F, Arbizu J, López P, Martí-Climent JM, Peñuelas I, Cervera-Enguix S: Functional neuroanatomy of sustained attention in schizophrenia: Contribution of parietal cortices. Hum Brain Mapp 2002;17:116-130
5. Barrett NA, Large MM, Smith GL, Karayanidis F, Michie PT, Kavanagh DJ, Fawdry R, Henderson D, O'Sullivan BT: Human brain regions required for the dividing and switching of attention between two features of a single object. Brain Res Cogn Brain Res 2003;17:1-13
6. Schmahmann JD, Pandya DN: The cerebrocerebellar system. Int Rev Neurobiol 1997;41:31-60
7. Middleton FA, Strick PL: Basal ganglia and cerebellar loops: Motor and cognitive circuits. Brain Res Rev 2000;31:236-250
8. Schweri MM, Skolnick P, Rafferty MF, Rice KC, Janowsky AJ, Paul SM: [3H] Treo-(+/−)-methylphenidate binding to 3,4-dihidroxyphenylethylamine uptake sites in corpus striatum; correlation with the stimulant properties of ritalinic acid esters. J Neurochem 1985;45:1062-1070
9. Heron C, Costentin J, Bonnet JJ: Evidence that pure uptake inhibitors including cocaine interact slowly with the dopamine neuronal carrier. Eur J Pharmacol 1994;264:391-398
10. Schenk JO: The functioning neuronal transporter for dopamine: Kinetic mechanisms and effects of amphetamines, cocaine and methylphenidate. Prog Drug Res 2002;59:111-131
11. Swanson JM, Volkow ND: Pharmacokinetic and pharmacodynamics properties of stimulants: Implications for the design of new treatments for ADHD. Behav Brain Res 2002; 130:73-78
12. Kitamura Y, Kakimura J, Taniguchi R: Antiparkinsonian drugs and their neuroprotective effects. Biol Pharm Bull 2002;25:284-290
13. Ziv I, Melamed E, Nardi N, Luria D, Achiron A, Offen D, Barzilai A: Dopamine induces apoptosis-like cell death in cultured chick sympathetic neurons: A possible novel pathogenetic mechanism in Parkinson's disease. Neurosci Lett 1994;170:136-140
14. Gomes KM, Inácio CG, Valvassori SS, Réus GZ, Boeck CR, DalPizzol F, Quevedo J: Superoxide production after acute and chronic treatment with methylphenidate in young and adult rats. Neurosci Lett 2009;465:95-98
15. Réus, GZ, Scaini G, Furlanetto CB, Morais MO, Jeremias IC, Mello-Santos LM, Freitas KV, Quevedo J, Streck EL: Methylphenidate treatment leads to abnormalities on krebs cycle enzymes in the brain of young and adult rats. Neurotoxic Res 2013;24:251-257
16. Hyman BT, Yuan J: Apoptotic and non-apoptotic roles of caspases in neuronal physiology and pathophysiology. Nat Rev Neurosci 2012;13:395-406
17. Lagace DC, Yee JK, Bolaños CA, Eisch AJ: Juvenile administration of methylphenidate attenuates adult hippocampal neurogenesis. Biol Psychiatry 2006;60:1121-1130
18. Takano A, Gulyas B, Varrone A, Maguire RP, Halldin C: Saturated norepinephrine transporter occupancy by atomoxetine relevant to clinical doses: A rhesus monkey study with (S,S)-[(18)F]FMeNER-D (2). Eur J Nucl Med Mol Imaging 2009;36:1308–1314
19. Cubillo A, Smith AB, Barrett N, Giampietro V, Brammer MJ, Simmons A, Rubia K: Shared and drug-specific effects of atomoxetine and methylphenidate on inhibitory brain dysfunction in medication-naive ADHD boys. Cereb Cortex 2014;24:174-185
20. Horne MK, Butler EG: The role of the cerebello-thalamocortical pathway in skilled movement. Prog Neurobiol 1995; 46:199-213
21. Morton SM, Bastian AJ: Cerebellar control of balance and locomotion. Neuroscientist 2004;10:247-259
22. Martins MR, Reinke A, Petronilho FC, Gomes KM, Dal-Pizzol F, Quevedo J: Methylphenidate treatment induces oxidative stress in young rat brain. Brain Res 2006;1078:189-197
23. Fagundes AO, Rezin GT, Zanette F, Grandi E, Assis LC, Dal-Pizzol F, Quevedo J, Streck EL: Chronic administration of methylphenidate activates mitochondrial respiratory chain in brain of young rats. Int J Dev Neurosci 2007;25:47-51
24. Scherer EB, Matté C, Ferreira AG, Gomes KM, Comim CM, Mattos C, Quevedo J, Streck EL, Wyse AT: Methylphenidate treatment increases Na(+), K (+)- ATPase activity in the cerebrum of young and adult rats. J Neural Transm (Vienna) 2009;116:1681-1687

25. Schmitz F, Scherer EB, Machado FR, da Cunha AA, Tagliari B, Netto CA, Wyse AT: Methylphenidate induces lipid and protein damage in prefrontal cortex, but not in cerebellum, striatum and hippocampus of juvenile rats. Metab Brain Dis 2012;27:605-612

26. Odaci E, Cihan OF, Aslan H, Ragbetli MÇ, Kaplan S: Prenatal diclofenac sodium administration increases the number of Purkinje cells in female rats: A stereological study. Int J Devl Neuroscience 2010;28:145-151

27. Aydın K, Çokluk C, Ayas B, Onger ME, Keskin I, Özyasar A, Aslan H, Kaplan S: Hippocampal cell loss after an anterior and posterior anastomotic vein occlusion model in rats. Int J Devl Neurosci 2011;29:717-722

28. Tunc MJ, Slomianka L, Gundersen HJ: Unbiased stereological estimation of the total number of neurons in the subdivisions of the rat hippocampus using the optical fractionator. Anat Rec 1991;231:482-497

29. Gundersen HJ: Stereology of arbitrary particles: A review of unbiased number and size estimators and the presentation of some new ones, in memory of William R. Thompson. J Microsc 1986;143:3-45

30. Tunc AT, Turgut M, Aslan H, Sahin B, Yurtseven ME, Kaplan S: Neonatal pinealectomy induces Purkinje cell loss in the cerebellum of the chick: A stereological study. Brain Res 2006;1067:95-102

31. Kaplan S, Odacı E, Canan S, Onger ME, Aslan H, Ünal B: The dissector counting technique. Neuro Quantology 2012; 10:44-53

32. Kaplan S, Esrefoglu M, Aktas A, Gül M, Onger ME, Altunkaynak ME, Ulkay MB, Ragbetli MÇ: The effect of prenatal exposure of a non-steroidal anti-inflammatory drug on the optic nerve of female rats: A stereological, histological, and electron microscopic study. J Matern Fetal Neonatal Med 2013;26:1860-1864

33. Swanson J, Baler RD, Volkow ND: Understanding the effects of stimulant medications on cognition in individuals with attention-deficit hyperactivity disorder: a decade of progress. Neuropsychopharmacology 2011;36:207-226

34. Goldman-Rakic PS, Muly EC, Williams GV: D(1) receptors in prefrontal cell and circuits. Brain Res Brain Res Rev 2000; 31:295-301

35. Andersen SL: Stimulants and the developing brain. Trends Pharmacol Sci 2005;26:237-243

36. Bredt DS, Snyder SH: Isolation of nitric oxide synthase, a calmodulin-requiring enzyme. Proc Natl Acad Sci USA 1990; 87:682-685

37. Bonfoco E, Leist M, Zhivotovsky B, Orrenius S, Lipton SA, Nicotera P: Cytoskeletal breakdown and apoptosis elicited by NO donors in cerebellar granule cells require NMDA receptor activation. J Neurochem 1996;67:2484-2493

38. Gültürk S, Kozan R, Bostancı MO, Sefil F, Bağırıcı F: Inhibition of neuronal nitric oxide synthase prevents iron-induced cerebellar Purkinje cell loss in rat. Acta Neurobiol Exp (Wars) 2008;68:26-31

39. Kuczenski R, Segal DS: Exposure of adolescent rats to oral methylphenidate; Preferential effects on extracellular norepinephrine and absence of sensitization and cross-sensitization to methamphetamine. J Neurosci 2002;22:7264-7271

40. Drouin C, Page M, Waterhouse B: Methylphenidate enhances noradrenergic transmission and suppresses mid- and long-latency sensory responses in the primary somatosensory cortex of awake rats. J Neurophysiol 2006;96:622-632

41. Arnsten AF: Toward a new understanding of attention-deficit hyperactivity disorder pathophysiology: An important role for prefrontal cortex dysfunction. CNS Drugs 2009; 23:33-41

42. Bymaster FP, Katner JS, Nelson DL, Hemrick-Luecke SK, Threlkeld PG, Heiligenstein JH, Morin SM, Gehlert DR, Perry KW: Atomoxetine increases extracellular levels of norepinephrine and dopamine in prefrontal cortex of rat; A potential mechanism for efficacy in attention deficit/hyperactivity disorder. Neuropsychopharmacology 2002;27:699-711

43. Achterberg EJ, van Kerkhof LW, Damsteegt R, Trezza V, Vanderschuren LJ: Methylphenidate and atomoxetine inhibit social play behavior through prefrontal and subcortical limbic mechanisms in rats. J Neurosci 2015;35:161-169

44. Navarra RL, Clark BD, Zitnik GA, Waterhouse BD: Methylphenidate and atomoxetine enhance sensory-evoked neuronal activity in the visual thalamus of male rats. Exp Clin Psychopharmacol 2013;21:363–374