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# Effect of Prenatal Ketamine Exposure on GFAP Marker Expression in Mice Prefrontal Cortex Mice Prefrontal Cortex

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***Abstract---*** *Ketamine, N-Methyl-D-Aspartate receptor antagonist agent, is widely used clinically for anesthesia, particularly in the developing countries. About 2% of pregnant women in the United States required surgeries because of the problems related to the pregnancy itself or due to other medical conditions, and ketamine regarded the first choice of anesthesia. This percentage is increasing, relatively because of laparoscopic procedures and fetal surgery.*

***Keywords---*** *Prenatal Ketamine, GFAP marker, Prefrontal cortex, Fluorescent Immunohistochemistry.*

## I. INTRODUCTION

Ketamine, N-Methyl-D-Aspartate receptor antagonist agent, is widely used clinically for anesthesia, particularly in the developing countries (Craven, 2007). About 2% of pregnant women in the United States required surgeries because of the problems related to the pregnancy itself or due to other medical conditions, and ketamine regarded the first choice of anesthesia. This percentage is increasing, relatively because of laparoscopic procedures and fetal surgery (Cheek and Baird, 2009).

In rodents and primates, some anesthetics used in early-life might impair cognitive function, an effect that can last into adulthood (Zheng *et al*., 2013). These findings are supported by retrospective clinical data which indicate that the use of anesthetics during early life may be associated with late-onset learning disabilities.

Laboratory mice exhibit specific anatomic and physiologic peculiarities that influence the effects of anesthetic agents. Due to their small body size, drug metabolism and excretion are really fast, reducing the half-life of injectable drugs and interpreting the duration of anesthesia a more critical factor compared with larger species. In addition, their high oxygen consumption rate reduces the survival rate for hypoxemia. In fact, irreversible central nervous system (CNS) damage occurs only a few seconds after respiratory arrest in mice (Abou-Madi, 2006).

This study is planned to investigate the effect of N- methyl D- aspartate receptor blocking agent (Ketamine) on the immunohistochemical changes (Anti-GFAP antibody) affected by NMDA receptor blocking agent (Ketamine) on prefrontal cerebral cortex.

## II. MATERIALS AND METHODS

In this study 60 pregnant mice were divided into three groups (20 mice in each group). Each group was divided into two equal subgroups; control and experimental. The control groups were injected with distilled water

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intraperitoneally six times a week at the first, second and third week of pregnancy respectively. The experimental groups were injected intraperitoneally with anesthetic dose of ketamine hydrochloride (50 mg/kg) (Hahn *et al*., 2005) during the first, second and third week of pregnancy respectively. Each group was injected six times at 24 hr. interval at day 1, 2, 3, 4, 5 and 6 of first, second and third week respectively using a 30-gauge needle and they were returned to their dam between injections to provide warmth and minimize potential stressors.

Mice were weighed before each injection due to increase in weight during pregnancy. The injection of all animals was done at 9-10 o'clock of morning. Each pregnant mouse delivered (5-10) neonates, and after delivery the males and females were chosen randomly.

Mice neonates were sacrificed by decapitation at age of one day by using scalpel to be immersed in the fixative solution to the next day and then the skin was removed by scissors and forceps, maintaining the skull to preserve the delicate brain tissue.

Routine histological tissue processing was done; fixation in 10% neutral buffered formalin, PH=7, dehydration in ascending concentrations of ethanol alcohol (70%, 90%, 100%), clearing in xylene for one hour, infiltration in paraffin wax 60°C for two hours, then embedding in paraffin wax. The heads were placed at lateral side at blocking. After hardening of the blocks, separate them from the molds and were kept in a refrigerator at 4°C until they were sectioned. Paraffin blocks were sectioned sagittaly at 5µm thickness by using microtome.

The immunohistochemical staining was started with antigen retrieval. The Slides were put in a coplin jar containing sodium citrate buffer (Antigen retrieval solution) which was prepared according to (Taylor *et al.*, 2006) as follows:

Solution A: Citric acid monohydrate (C6H8O7.H2O or C6H10O8) 10.505 g. in 500 ml. of distilled water (0.1 M).

Solution B: Sodium citrate (Na3C6H5O7.H2O) 14.704 g. in 500 ml. of distilled water (0.1 M). 9 ml. of solution A + 41 ml. of solution B mixed in 500 ml. of distilled water and the pH of the solution was adjusted to 6. Then the coplin jar was transported to an autoclave (120 °C., 1.2 Bar) for 30 minutes and left to cool down to room temperature for 20 minutes (according to trial optimization). Slides were pounded in running tap water for 5 minutes, distilled water for 5 minutes, Tris buffer-Tween 20 (pH 8) for 5 minutes and lastly rinsed three times with Tris buffer (pH 8).

Immunofluorescent Staining was done after antigen retrieval. Tissue sections were “bounded” using wax pen, rinsed 3 times in Tris buffer (pH 8) and incubated in a moist chamber with the primary unconjugated antibodies for 2 hours at room temperature (37°C). The primary antibodies were diluted at (1:100) in Tris buffer (pH 8).

| Antibody | Host | Isotype | Clonality | Supplier | Dilution |
|---|---|---|---|---|---|
| Anti-GFAP | Rabbit | IgG | polyclonal | Biorbyte® catalog #orb10706 | 1:100 |
| TRITC | Goat anti-Rabbit | IgG | | Biorbyte® catalog # orb27730 | 1:100 |

Then, sections labeled with (Anti-GFAP antibody) rinsed by Tris buffer (pH 8) and incubated in a dark moist chamber for 1 hour with Goat anti-Rabbit (TRITC) secondary antibody.

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After incubation for 1hour, sections rinsed carefully by Tris buffer (pH 8) using micropipette for 3 times and then a small drop of mounting medium containing DAPI were added (Santa Cruz® catalog # SC-24941) to stain nuclei and finally cover slip were placed.

Slides were examined by fluorescent microscope (AXIO-ZEISS®) by Meta System photographing program. Digital camera (AXIO-ZEISS®) mounted to the fluorescent microscope was utilized for photographing, and the pictures were examined by a special software (Image J) for measuring the optical densities of the target regions in the slide that represent the quantitative measurement of marker expression with equal magnification to be further analyzed by statistical package of social sciences SPSS.

## III. RESULTS

***Control Groups*:** Sections stained with fluorescent immunohistochemical marker specific for astrocytes in the control groups examined under fluorescent microscope appeared as uniformly distributed fluorescent reactivity all over the sections, with blue colored nuclear staining and red plaques inside cytoplasm of some cells with reticular reddish background in the intercellular spaces (figure 1).

The spaces with red fluorescence plaques represented the astrocytes processes labeled with the GFAP marker. The reticular faint red hue indicates the GFAP reactivity of the astrocytes processes. The red immunohistochemical reactivity was markedly observed in the most outer and the deeper laminae of the cerebral cortex i.e. layer I and V, VI.

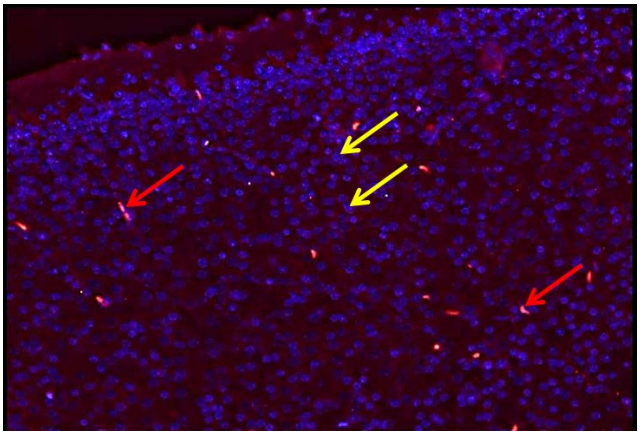

Figure 1: Anti-GFAP fluorescent immunohistochemical staining of the neonate mice prefrontal cerebral cortex of the Control group showing nuclei of neurons and neuroglia (yellow arrows) and astrocytes processes (red arrows) with red fluorescence surrounding (under fluorescent microscope X100)

***Experimental Group 1 (First Week)*:** The prefrontal cerebral cortex of the animal treated with ketamine during the first week of pregnancy showed subjectively less marked fluorescent reactivity indicated by the red hue of all the cortical laminae compared with the control group .

The laminar disproportion of the immunohistochemical reactivity seen in the cortices of the control group was also observed in this group of animals (figure 2).

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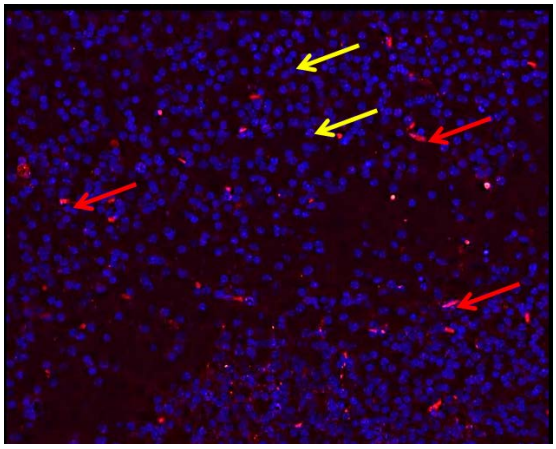

Figure 2: Anti-GFAP fluorescent immunohistochemical staining of the neonate mice prefrontal cerebral cortex of the Experimental group 1 showing nuclei (yellow arrows) and astrocytes processes (red arrows) with red fluorescence surrounding (under fluorescent microscope X100)

***Experimental Group 2 (Second Week)*:** Immunohistochemical staining appeared subjectively with a less fluorescent reactivity compared to that of the control group and first experimental group. The laminar arrangement of the cerebral cortex could not be discriminated under the fluorescent microscope in the cortices of the animals of this group, this finding interferes with discrimination of the laminar immunohistochemical variability in this group, however, the subpial lamina still showing intense immunohistochemical expression (figure 3).

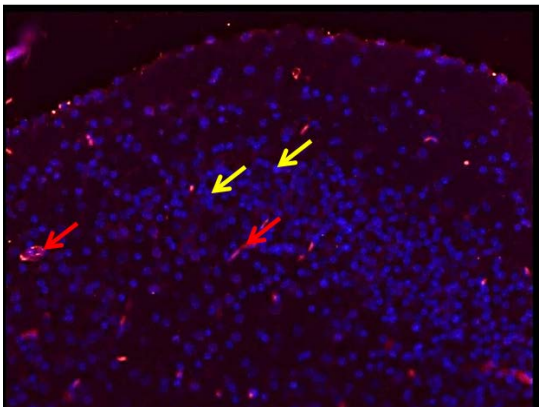

Figure 3: Anti-GFAP fluorescent immunohistochemical staining of the neonate mice prefrontal cerebral cortex of the Experimental group 2 showing neurons (yellow arrows) and astrocytes processes (red arrows) with red fluorescence surrounding (under fluorescent microscope X100)

***Third Experimental Group (Third Week)*:** Prefrontal cerebral cortex stained with fluorescent immunohistochemical marker of Anti-GFAP revealed low fluorescent reactivity less than the control and the previous experimental groups with hazy reddish colored cells. The subpial layer showed marked red immunohistochemical GFAP reactivity, such judgment could not be evaluated at the deeper laminae (figure 4).

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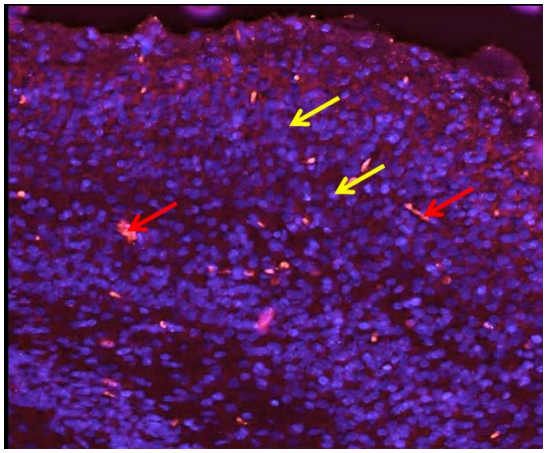

Figure 4: Anti-GFAP fluorescent immunohistochemical staining of the neonate mice prefrontal cerebral cortex of the Experimental group 3 showing nuclei (yellow arrows) and astrocytes processes (red arrows) with red fluorescence surrounding (under fluorescent microscope X100)

**Image J Analysis of the results of GFAP Immunofluorescent marker optical density:** Measuring the optical density of fluorescent GFAP marker in the prefrontal cortex of the control and the experimental groups by image J analysis software, the gray scale level was standardized according to a standard optical density step tablet (Ferreira and Rasband, 2012).

For the control group the calibration curve showed in figure (5), with tracing of each target area (circled area), the Mean Gray Value within the selection (the sum of the gray values of all the pixels in the selection divided by the number of pixels) was reported in calibrated units (optical density). For red-green-blue (RGB) colored images, the mean was calculated by converting each pixel to gray scale using the formula: Gray = (Red + Green + Blue) / 3.

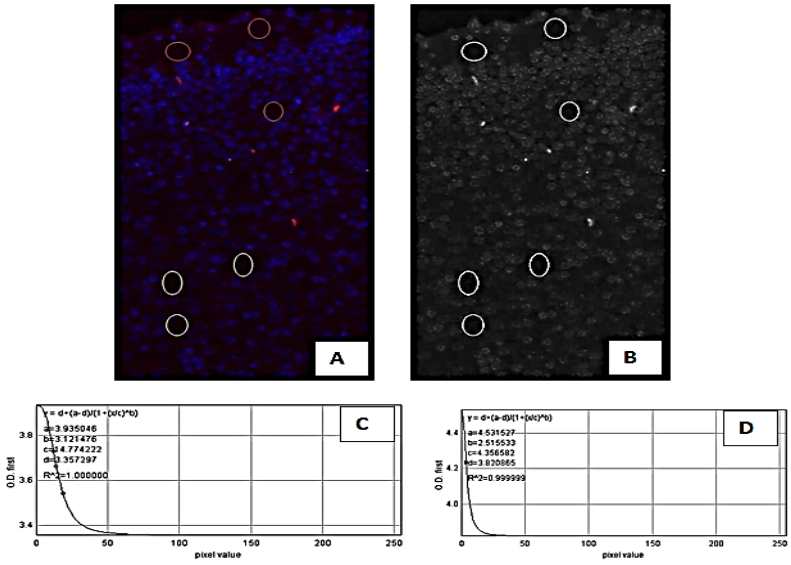


Figure 5: Image J analysis of optical density of control group of GFAP marker. A: colored figure of control group. B: Gray scale. C: Calibration curve of superficial half of the prefrontal cortex of mice neonate in the control group. D: Calibration curve of deep half of the prefrontal cortex of mice neonate in the control group

**Statistical analysis for the result of Immunohistochemical Staining with Anti-ZO-1:**

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The analysis of the counted mean value of optical density for the anti-GFAP fluorescent immunohistochemical reaction by image J analysis in prefrontal cortex of the mice neonates of the experimental groups revealed statistically significant variability compared to the related control group, (table 1).

Table 1: Measured mean value of the optical densities by image J analysis of the GFAP fluorescent activity in the prefrontal cortex of the mice neonates of the three experimental groups and statistical analysis by *t*-test compared with the related control group. (*p* value ≤ 0.05 considered statistically significant).

| Variables | | Mean (±SD) | Max. | Min. | P value |
|---|---|---|---|---|---|
| Week 1 | Control | 4.22(±0.02) | 4.26 | 4.05 | 0.001* |
| | EXP. | 4.05(±0.01) | 4.12 | 3.88 | |
| Week 2 | Control | 4.21(±0.02) | 4.25 | 4.18 | 0.0001* |
| | EXP. | 3.86(±0.03) | 3.95 | 3.55 | |
| Week 3 | Control | 4.23(±0.01) | 4.26 | 3.78 | 0.0001* |
| | EXP. | 3.64(±0.03) | 3.87 | 3.46 | |

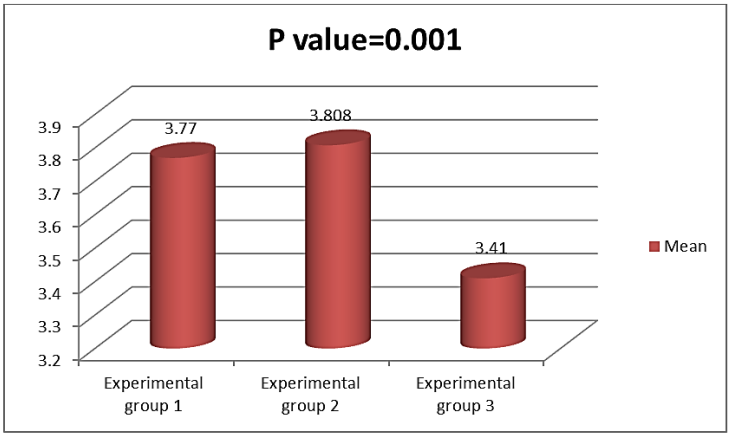


Figure 6: BAR chart for the mean difference in the optical densities of fluorescent activity of Anti-GFAP marker measured by image J software in the superficial half of the prefrontal cortex of mice neonates in the three experimental groups

## IV. Discussion

Ketamine could pass through the blood-placental barrier, its levels in the cord blood of newborns reach the levels in maternal venous blood as early as 1 minute and 37 seconds after intravenous injection to healthy mothers (Ellingson *et al.*, 1977). In addition, rapid transplacental passage of ketamine has been demonstrated in animals following intravenous administration of the drug to the dam (Musk *et al*., 2012).

The blood-brain barrier differentiated during fetal life (Daneman, 2012). The endothelial cells of the BBB, together with the pericytes, perivascular microglial cells, astrocytes and neurons form the so called neurovascular unit (Luissint *et al*., 2012). The neurotoxic outcome on the embryo brain after intra-peritoneal administration of ketamine to the mothers at variable gestational periods proved the inefficient function of the embryonic brain

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barriers against protection from ketamine neurotoxicity.

It was stated that "developmental tightening" of brain tissue barriers showed stage-specific expression of transmembrane and cytoplasmic tight junction proteins during development. The appraisal of this study was established for comparing the neurotoxicity of prenatal ketamine exposure in association with the progressive maturation of the barrier junctional complexes at the progressive gestational period. The conclusions obtained revealed that ketamine neurotoxicity is associated with inconstant pattern of neurohistological and histochemical variability at the different periods of pregnancy. The structures exerting the barrier functions overlap with the early vascularization of the growing CNS. It was documented that angiogenesis in the mice brain begins at day E9 of development (Vasudevan *et al*., 2008). Therefore, the prenatal ketamine exposure during the 2nd and 3rd week (group II & III) overlap with angiogenesis in the mice brain (beginning at day 9) which is associated with active barrier formation. However, it seems that these developing barriers in the cerebral cortex did not minimizing the neurotoxic effect of ketamine resulting in the exaggerated histological abnormality seen in the prefrontal cortex postnatal.

The results of this study showed maximum GFAP expression in the cortices of the newborn animals obtained from the pregnant mice of 1$^{st}$ group compared to both the 2$^{nd}$ & 3$^{rd}$ groups, group II showed more IHC reactivity compared to group III .

The GFAP massively increases in the prefrontal cortex of the newborn after prenatal ketamine exposure during the 1st week indicating the massive astrogliosis occurring during the next 2 weeks after ketamine exposure as a machinery to overcome the massive neurodegeneration of prenatal ketamine toxicity. This compensation seems to be satisfactorily effective that it results in preservation of the hexalaminar organization of the prefrontal cortex of the newborn mice obtained from group I .

On contrary, prenatal ketamine exposure during the 2nd week (group II) accompanied the period of early barrier formation that could not minimize the ketamine neurotoxicity resulting in massive neuro-degeneration compared to that seen in the prefrontal cortex of the animals from the 1st group. Therefore, the astrogliosis occurring during the next one week (i.e. during the 3rd week) after ketamine exposure during the 2nd week to compensate for neurodegeneration is reflected in the increased statistical values of GFAP immunohistochemical reactivity of the cortex of the newborn animal obtained from group II, this increased values are less than the increment of values obtained from the 1st group due to shorter period witnessing astrocytosis (for only one week, i.e. the 3rd week). The neurodegeneration resulting from prenatal ketamine exposure of the fetus during the 3rd week could not be compensated by a period of subsequent astrocytosis as the newborn mice were sacrificed immediately on the first postnatal day. Accordingly, the statistical values of GFAP immunohistochemical reactivity in the cortex of the newborn mice obtained from the pregnant mothers of group III was the least values compared to that of both group I &II.

The histological section of the prefrontal cerebral cortex of the newborn mice obtained from pregnant animals of both group II and III showed more massive distortion of the histological configuration compared to that of group I.

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It seems that shorter period of astrogliosis occurring in the cortex during the 3rd week in newborn animals of group II, and the absence of astrogliosis in the cortex of newborn animals of group III was manifested by the sever histological aberration and loss of the ultimate normal hexalaminar organization in the prefrontal cortex of the newborn obtained from these groups.

It was stated that ketamine transiently increase glutamate neurotransmission in the prefrontal cortex of rodents (Abdallah Chadi *et al*., 2018). The neuronal damage was found to increase glutamate release and subsequent excitotoxicity, mitochondrial dysfunction, excessive production of reactive oxygen species (ROS), and disruption of glucose metabolism/release (Vijayan and Reddy, 2016) eventually causing acute necrotic cell death and/or delayed apoptotic cell death (Nicole *et al*., 2001). Further damage can occur by prolonged neuro-inflammation that exacerbate the injury and may underlie long-term pathogenesis (Caso *et al*., 2007). The least GFAP IHC reactivity seen in prefrontal cortex of the animals from group III may indicate that the massive hazard of ketamine administration during the later periods of pregnancy is related to downfall of astrocytosis that could occur in association with the final stages of neural tissue maturation. The low astrocyte GFAP in animals of group III may lead to long term sequel including Alzheimer's disease as this disease is associated with excessive glutamate is released (Chamoun *et al.*, 2010). The long term follow up study should be done to discriminate the long term effect of administrating ketamine during the prenatal period and thus establishing the safety of using ketamine in late gestational periods.

In support to the conclusion of this study in consideration to the role of astrocytosis in preservation of the cortical organization and thus maintenance of functional mannerisms of brain, it was reported that the role of astrocytes been defined as the "brain glue", this definition was deliberated because the astrocytes modulating the synaptic transmissions in the neural tissue following injury or neurotoxicity. In addition, astrocytosis was reported to have a role in managing energy metabolism and homeostasis (Sofroniew and Vinters 2010). Similar reported supporting the conclusions made in this study had been stated that the astrocyte have essential roles in synaptic function and nervous system repair (Okada *et al.*, 2006), and that the astrocytes promote differentiation of neural stem cell into neurons and help in motor neuron neurite outgrowth (Thompson *et al.*, 2017).

The reported animal studies demonstrating that ketamine induces neuronal degeneration and disturbs neuronal development in the immature brains, were also concerned about evidences for the neuroprotective effects of ketamine for stresses as ischemia and traumatic brain injuries. It was concluded that the extracellular glutamate levels were elevated in these stressful conditions (Palmer *et al*., 1993) and thus contributing to the excessive NMDA glutamate receptor activation which in turn leads to excitotoxic neuronal cell death and eventually brain damage.

## REFERENCES

[1] Abdallah Chadi G., Henk M. De Feyter, Lynnette A. Averill, Lihong Jiang, Christopher L. Averill, Golam M. I. Chowdhury, Prerana Purohit, Robin A. de Graaf, Irina Esterlis, Christoph Juchem, Brian P. Pittman, John H. Krystal, Douglas L. Rothman, Gerard Sanacora and Graeme F. Mason (2018): The effects of ketamine on prefrontal glutamate neurotransmission in healthy and depressed subjects. Neuropsychopharmacology 43:2154–2160.

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[2] Abou-Madi N. (2006): Anesthesia and Analgesia of Small Mammals: Recent Advances in Veterinary Anesthesia and Analgesia: Companion Animals. In: Gleed RD, Ludders JW, eds. Ithaca NY: *International Veterinary Information Service* (www.ivis.org). pp. 1-9.

[3] Caso J. R., J. M. Pradillo, O. Hurtado, P. Lorenzo, M. A. Moro, and I. Lizasoain, (2007): "Toll-like receptor 4 is involved in brain damage and inflammation after experimental stroke," *Circulation*, vol. 115, no. 12, pp. 1599–1608.

[4] Chamoun R., D. Suki, S. P. Gopinath, J. C. Goodman, and C. Robertson, (2010): "Role of extracellular glutamate measured by cerebral microdialysis in severe traumatic brain injury," *Journal of Neurosurgery*, vol. 113, no. 3, pp. 564–570.

[5] Cheek, T. G. & Baird, E. (2009): Anesthesia for non-obstetric surgery: *maternal and fetal considerations. Clinical Obstetrics and Gynecology* 52, 535–545.

[6] Craven, R. Ketamine (2007). Anaesthesia 62 (S1), 48–53.

[7] Daneman, Richard (2012): The blood-brain barrier in health and disease. *Annals of Neurology* 72(5):648-72.

[8] Ellingson, A., Haram, K., and Solheim, E. (1977). Transplacental passage of ketamine after intravenous administration. *Acta. Anaesthesia. Scand.* 21, 41–44.

[9] Ferreira T. and Rasband W.: The Image J User Guide – V; 1.46. October 2012 .

[10] Hahn, N., Eisen, R.J., Eisen, L. & Lane, R.S. (2005). Ketamine-medetomidine anesthesia with atipamezole reversal: practical anesthesia for rodents under field conditions. *Lab. animal*, 34(2), p. 48-52 .

[11] Luissint A.C., Federici C., Guillonneau F., Chretien F., Camoin L., Glacial F. et al. (2012). Guanine nucleotide-binding protein Galphai2: a new partner of claudin-5 that regulates tight junction integrity in human brain endothelial cells. *Journal of Cerebral Blood Flow Metabolism.* 32, 860–873.

[12] Musk, G. C., Netto, J. D., Maker, G. L., and Trengove, R. D. (2012). Transplacental transfer of medetomidine and ketamine in pregnant ewes. *Lab. Animals.* 46, 46–50 .

[13] Nicole O., C. Ali, F. Docagne et al. (2001): "Neuroprotection mediated by glial cell line-derived neurotrophic factor: involvement of a reduction of NMDA-induced calcium influx by the mitogen-activated protein kinase pathway," *The Journal of Neuroscience*, vol. 21, no. 9, pp. 3024–3033.

[14] Okada S., M. Nakamura, H. Katoh et al., (2006): "Conditional ablation of Stat3 or Socs3 discloses a dual role for reactive astrocytes after spinal cord injury," *Nature Medicine*, vol. 12, no. 7, pp. 829–834 .

[15] Palmer, A. M., Marion D. W., Botscheller M.L., Swedlow P.E., Styren S.D., and DeKosky S.T. (1993): Traumatic brain injury-induced excitotoxicity assessed in a controlled cortical impact model. *Journal of Neurochemistry*. 61, 2015–2024.

[16] Sofroniew M.V., & Vinters, H.V. (2010). Astrocytes: biology and pathology. *Acta neuropathologica*, 119(1), 7-35.

[17] Taylor C.R., Shi S.R., Barr N.J., and Wu N. (2006): Techniques of immunohistochemistry. Principles, pitfalls and standardization. *Diagnostic Immunohistochemistry* 2: 3-44.

[18] Thompson R. E., A. Lake, P. Kenny et al., (2017): "Different mixed astrocyte populations derived from embryonic stem cells have variable neuronal growth support capacities," *Stem Cells and Development*, vol. 26, no. 22, pp. 1597–1611.

[19] Vasudevan A., Long J.E., Crandall J.E., Rubenstein J.L., and Bhide P.G. (2008): Compartment-specific transcription factors orchestrate angiogenesis gradients in the embryonic brain. Nature. *Neuroscience.* 11, 429–439.

[20] Vijayan M. and P. H. Reddy (2016): "Stroke, vascular dementia, and Alzheimer's disease: molecular links," *Journal of Alzheimer's Disease*, vol. 54, no. 2, pp. 427–443.

[21] Zheng, H. et al. (2013): Sevoflurane anesthesia in pregnant mice induces neurotoxicity in fetal and offspring mice. *Anesthesiology* 118, 516–526.

**DOI: 10.37200/IJPR/V24I4/PR201507**
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