Since 1983
EPITHEORESE KLINIKES FARMAKOLOGIAS KAI FARMAKOKINETIKES | 42(Sup1): 119-123 (2024)

Received: 13 February 2024 | **Accepted**: 16 May 2024 | **Published**: 27 December 2024

Open Access | **RESEARCH**

Nissl stain expression in the neonatal mice occipital cortex after prenatal ketamine exposure

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ABSTRACT

Background: The occipital cortex is positioned on the back of the brain, and it is responsible for processing visual information. Ketamine is a drug used as an anesthetic. Common anesthetics can cause neurotoxicity, with the occipital cortex being one of the most vulnerable areas. *Aim*: To estimate the histological alteration in the occipital cortices of newborn mice receiving ketamine injections at therapeutic doses during pregnancy. *Methodology*: This study involved 30 pregnant female mice (8–12 weeks old), who are split into two groups: the experimental group, given 50 mg/kg ketamine hydrochloride intraperitoneally, and the control group, given distal water intraperitoneally. The mice were then subjected to a paraffin wax embedding procedure, and their neural tissue was examined using a Cresyl violet stain. The results were analyzed using the Spss software and the independent *t*-test. *Results*: Significant variability was seen when the number of cells in the mice's occipital cerebral cortex after ketamine injection during pregnancy was compared. In the control group, the difference between the mean of the superficial layer and the deep layer is 85.4%, while in the experimental group, the difference between the two layers is 85.1%. In this study, there was significant variability in the number of cells between the control groups (Mean ± SD) is 1326±14.4 cells and the experimental group (Mean ± SD) is798.06 ±26.9 cells in the occipital cortex. In calculation, the experimental newborn mice's occipital cortex showed apoptotic alterations following a ketamine injection during pregnancy. *Conclusion*: The experimental newborn mice's occipital cortex showed apoptotic alterations following a ketamine injection during pregnancy. These results are in line with growing concerns regarding the neurotoxic effects of anesthetic drugs on the developing brain.

KEYWORDS

occipital cortex, nissl stain, newborn mice, ketamine injection, apoptosis

How to cite this article: Suhail Najm M.: Nissl stain expression in the neonatal mice occipital cortex after prenatal ketamine exposure. *Epitheorese Klin. Farmakol. Farmakokinet.* 42(Sup1): 119-123 (2024). DOI: 10.61873/RALN7719

Publisher note: PHARMAKON-Press stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

1. INTRODUCTION

The occipital cortex is the part of the brain situated on the back [1]. It includes many different areas and it is responsible for the processing of several types of visual information, including color, motion, and depth perception, and it is responsible by each area separately [2,3]. The mice primary and secondary visual cortices are positioned in the occipital cortex [2]. As in the human brain, the visual cortices are situated on the back of the brain [4]. The Afferents from the dorsal lateral geniculate nucleus, which receives afferents from the retina, the

optic nerve, and the optic tract, are directed toward the Primary Visual Cortex (V1) [5,6]. While the secondary visual cortex (V2) and the subfields that surround V1 have created a map of the mouse visual cortex's region using tracing and receptive field recordings [7,8], visual neurophysiologists have mainly neglected the mouse visual cortex because they assume that since mice are nighttime, they rely on Sense of touch and olfactory information and only use their visual system as an event detector. Since the invention of mouse transgenic technology, which supports to study the role of certain gene responsible to the development and function of the visual cortex, this development has been largely responsible for the rise in interest in mouse visual cortex over the past several years [9]. In both human and veterinary medicine, ketamine is predominantly utilized as anesthetic drug, working as an uncompetitive antagonist at the (NMDA) receptor [10]. Patients with depression, who receive a sub anesthetic dosage of ketamine intravenously get an immediate antidepressant-like effect [11]. Despite the fact that the Food and Drug Administration (FDA) authorized ketamine for the treatment of adult patients with treatment-resistant depression [12], it has also been suggested as a therapy for chronic pain, anxiety, and bipolar illness [13,14]. However, ketamine has reinforcing effects that cause self-administration and conditioned location preference in rats, which is consistent with its potential for abuse in humans [15]. The United Nations Office on Drug Control classified ketamine in its 2019 World Drug Report as a novel psychoactive substance (NPS) that is not governed by international drug conventions and may be harmful to public health [16,17]. Ketamine affects neurocircuit structure and function in both preclinical and clinical studies [16]. The effects of ketamine appear to be primarily mediated by NMDA receptor blocking [18]. Glutamate receptors of the N-methyl-D-aspartate type play an important role in cerebral cortical functioning [19], being involved in developmental processes, sensory information transmission, synaptic plasticity, and there is indication that any changes in N-methyl-D-aspartate receptor function have an effect on neurotoxicity and a range of neurological and psychiatric illnesses [20,21]. Evidence that common anesthetics can cause neurodegeneration in neonatal animals. The occipital cortex is one of the brain areas that is most susceptible to the influences of ketamine [22]. Animal trials (rats, mice) have revealed that anesthetic drug exposure during formative periods can result in neuronal apoptosis or neuronal degeneration [21].

The objectives of this study aimed to estimate the histological alteration in the occipital cortices of newborn mice fallowing ketamine injections at therapeutic doses during pregnancy.

2. METHODOLOGY

2.1. Animal and experimental procedure

The Laboratory Animal House was where the animals used in this investigation were housed. In this investigation, 30 pregnant female *Mus musculus* were divided into two groups: 15 mice in the experimental group and 15 in the control group. The age of mice was between 8 and 12 weeks and weighed between 20 and 40 g. Pregnancy was confirmed the morning after mating by the presence of a vaginal plug, which was considered day 0 of gestation.

2.2. Ethical approval

All animals were handled in accordance with Ethical Guidelines approved by the National Institutes of Health (NIH). This study utilized animals from the Laboratory Animal House at the College of Medicine, Al-Nahrain University.

2.3. Experimental group and control group treatment

The mice in the experimental group were given 50 mg/kg ketamine hydrochloride intraperitoneally in a single injection on the fifth, tenth, fifteenth, and twentieth days of pregnancy. During the same gestational days and volume, the control group was injected with just distal water intraperitoneally.

2.4. Tissue preparation, staining, and image analysis

Mice neonatal brains were fixed in 10% formalin before being subjected to the standard paraffin wax embedding procedure. Sagittal sections of the paraffin blocks were prepared at a thickness of 5 cm. Cresyl violet (Nissl) stain was utilized to examine neural tissue, and images were recorded by a genex camera (5 mega pixels) incorporated within the microscope. Then open the image in the imageJ program, and split the image into squares, then count the number of cells by putting dot labelling above the cell. The ratio of the average number of cells in the cerebral cortex's deep layer divided by the mean number of superficial layer of cells.

2.5 Statistical analysis

The Statistical Package for the Social Sciences (Spss) was the software application used for

statistical evaluation, and the independent *t*-test was used for analysis of the result by taking the mean number of cells in Nissl stain and the p-value statistically calculated by taking the mean ± standard deviation of the rustle.

3. RESULTS

3.1. Nissl stain result in the control group of occipital cortices

The superficial layer (first, second, and third) average number of cells in occipital cortices of control group is 715±13.9 cells, and the average number of cells in deep layer (fourth, fifth and sixth) is 611±5.56 cells (Figure 1). The percentage of deep layer to superficial layer is 85.4%.

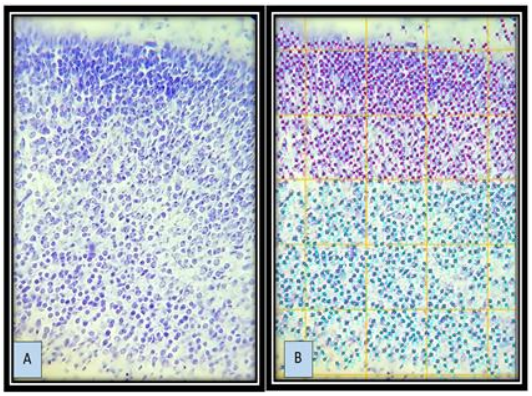

Figure 1. (A) Nissl stain of control group of occipital cortices the image seeing shape of cells. X200. (B) Screenshot: the image was divided into squares using the ImageJ program, which then counted the number of cells in the deep and superficial layers.

3.2. Nissl stain result in the experimental group of occipital cortices

In the experimental group mean number of cells in superficial layers of occipital cortices is 431±8.27 cells, whereas the mean number of cells in deep layers is 367±22.6 cells (Figure 2). The percentage of deep layer to superficial layer is 85.1%.

3.3. Comparison between mean evaluations of both groups in the occipital cortices

The statistical result of the number of cells in the occipital cortex of control and experimental groups show significant variability, and the (*p*-values= 0.032), and the average number of total cells in the occipital cortex of control group is 1326±14.4, whereas in the experimental groups is 798.06 ±26.9 (Table 1).

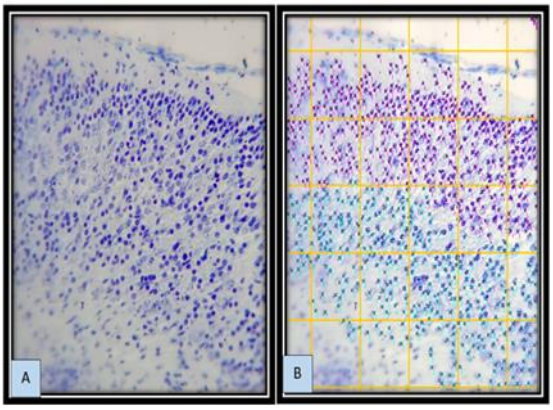

Figure 2. (A) Nissl stain experimental group of occipital cortices the image show shape of cells. X200. (B) Screenshot: the image was divided into squares using the ImageJ program, which then counted the number of cells in the deep and superficial layers

Table 1. Statistical analysis number of cells in both control and experimental groups of occipital cortices of newborn mice.

Group	Mean ± SD	*p*-values
Control group	1326±14.4	0.032 *(significant)
Experimental group	798.06 ±26.9	

*p-value ≤ 0.05 is considered statically significant.

4. DISCUSSION

In this study, we evaluated the morphological changes in the occipital cortex in mice after prenatal exposure to ketamine. We compared the mean occipital cortex cell number between the experimental and control groups. It has been observed that quantitative neuroanatomical investigations, particularly in the primate brain, lack precise cytological explanations of glial cell and neurons types [23]. The assessment of cellular concentration neurons and glial cells in the occipital cortices of the animals utilized in this research to explain the histological changes and quantitative alteration in neuroanatomical structure and caused by ketamine injection during pregnancy. This traditional Nissl's staining approach was adopted in this investigation because it marks all brain cells in various ways [23]. The procedure for comparing the ratio of cell number in the deep layer (4^{th}, 5^{th}, 6^{th}) with that in the superficial layer (1^{st}, 2^{nd}, 3^{rd}) was submitted based on the assumptions that:

First, this step is to determine the structure of the lamina in the cerebral cortex during early development, showing that the deeper cortical layers

form before the more superficial layers when post-mitotic cells move to generate the cortical plate in an inside-out pattern [24] [25].

Second, during the peak of synaptogenesis, ketamine caused significant apoptotic changes and the statistical investigation of the number of cells in the occipital cortex of control and experimental groups show significant variability [26,27]. The steady findings that neurons regulating their migration showed an earlier onset of synaptogenesis in deeper layers compared to superficial layers. This fact was also taken into consideration when evaluating the ratio of cells in deep and superficial layers [28] [29]. These findings support the hypothesis that apoptosis occurs more frequently in the occipital cortex following prenatal ketamine exposure. In both the control and experimental groups of the cerebral cortex investigated in this study, the percentage of cellular density in the deep/superior lamina revealed non-significant fluctuation. These findings corroborate with previous findings that cellular concentration in the brain correlates with neurological illnesses in addition to changes in the brain linked with pharmaceutical treatment [30]. Nevertheless, other methods have been employed in the past to quantify the number of neurons in the cerebral cortex [31].

5. CONCLUSION

This study demonstrates that prenatal exposure to ketamine causes significant apoptotic alterations in the occipital cortex of neonatal mice, as shown by altered neuronal construction, which increases DNA fragmentation. According to the research, ketamine, a common anesthetic and painkiller, may be harmful to fetal brain development if used during pregnancy, especially at crucial stages. Long-term functional impairments may result from ketamine-induced apoptosis in the occipital cortex, a region essential for visual processing. These findings highlight the necessity for a thorough risk-benefit analysis when using ketamine in pregnant patients and align with growing concerns regarding the neurotoxic effects of anesthetic drugs on the developing brain.

ACKNOWLEDGMENTS

The author would like to thank Professor Dr Hayder J. Mubarak from the College of Medicine at Al-Nahrain University for his support with the statistical analysis.

CONFLICT OF INTEREST STATEMENT

The author declares no conflicts of interest.

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