Doi: 10.5152/TJAR.2020.128

Original Article
Paediatric Anaesthesia

Turkish Journal of Anaesthesiology & Reanimation

Effect of Isoflurane Exposure with Administration of Polyunsaturated Fatty Acids on Cognition in Developing Rats

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Cite this article as: Aldemir Şensoy D, Demirgan S, Akyol O, Gümüş Özcan F, Selcan A. Effect of Isoflurane Exposure with Administration of Polyunsaturated Fatty Acids on Cognition in Developing Rats. Turk J Anaesthesiol Reanim 2020; 48(6): 477-83.

Abstract

Objective: The developing brain is vulnerable to the negative effects of anaesthetics. We aimed to investigate the effect of isoflurane and polyunsaturated fatty acids (PUFAs) on cognition.
Methods: A total of 64, ten days old rats were randomly divided into 4 groups: group O2 (oxygen group), group Iso (isoflurane group), group Iso-S (isoflurane+saline) and group Iso-PUFAs (isoflurane+intraperitoneal [IP] PUFAs emulsion). Rats in groups Iso, Iso-S and Iso-PUFAs were exposed to 1.5% isoflurane in 50% oxygen for 6 hours. Rats in group O2 breathed only 50% oxygen. Before anaesthesia, rats in group Iso-S were administered 0.5 mL isotonic and rats in group Iso-PUFAs were administered 5 mL kg^{-1} PUFAs emulsion by IP injection. The Morris water maze (MWM) test was performed on postnatal 28-33 days. Histological evaluation and immune histochemical staining (Bcl-2 antibody) were performed on postnatal day 11 on rat brains.
Results: As demonstrated by the reduction in the escape latency on days 3, 4 and 5 compared with day 1, all rats learned the task during the acquisition period. In contrast to others, rats in group Iso spent significantly lower time to find the platform on day 2 than on day 1 ($p=0.034$). No significant difference was found among the groups in terms of time spent in finding the platform. There were no significant differences in probe trials, histological features and Bcl-2 immunoreactivity among the groups.
Conclusion: Isoflurane did not cause cognitive dysfunction and neuronal death, and a single dose of PUFAs emulsion had no effect on cognition either.
Keywords: Cognition, infant rat, isoflurane, learning and memory, polyunsaturated fatty acids

Introduction

Isoflurane is still rarely used as an anaesthetic agent for infants. Although there have been studies on experimental animals showing that isoflurane anaesthesia leads to cognitive dysfunction (1, 2), other studies indicate no effect (3) or even positive effects on cognition (4). Mechanisms of cognitive dysfunction owing to neuronal degeneration are cytotoxicity, oxidative stress, mitochondrial integrity, neuroinflammation and neuroapoptosis (5-9). In recent years, the effect of polyunsaturated fatty acids (PUFAs), which constitute a substantial part of the structural lipids in the cell membrane, particularly the plasma membranes of the central nervous system cells, on learning and memory has been studied. Omega-3 fatty acids play important roles in regulation and maintenance of cell membrane homeostasis, stability of membrane composition, membrane receptor regulation and neuronal plasticity (10). It has been previously demonstrated that diet containing different concentrations of omega-3 fatty acids has positive effects on memory and learning in 3-week-old rats (11).

This study aimed to investigate the effect of prolonged isoflurane anaesthesia on cognition and neuronal death by intraperitoneal (IP) administration of PUFA emulsion in 10-day-old rat pups. This is the first study that evaluated the effect of high-dose PUFA emulsion administration before isoflurane anaesthesia on cognitive function in infant rats.

Methods

Animals

Our study was carried out in the experimental research and skills development centre of our hospital after the approval of Bağcılar Training and Research Hospital Animal Ethics Committee (protocol number: 2016/112, 09 August, 2016).

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Received: 22.11.2019
Accepted: 17.08.2020

The study included 64 postnatal day 10 (P10) infant Wistar Hannover rats weighing between 16 and 21 g, because it has been previously reported that they are vulnerable to anaesthesia-induced neurodegeneration (12). A total of 3 rats that died in the isoflurane-exposed groups were replaced with new ones. All efforts were made to minimise the number of animals used and the pain they suffered from. The rats were housed under standard laboratory conditions with 12-hour dark/light cycle at 20°C-22°C with 50%-60% humidity.

Experimental groups

The schematic representation of the experimental dosing is shown in Figure 1. P10 rats were randomly divided into 4 groups with 16 rats in each group and received the following treatments:

Group O_2 (oxygen): Rats were exposed to only 50% oxygen for 6 hours.

Group Iso (isoflurane): Rats were exposed to 1.5% isoflurane in 50% oxygen for 6 hours.

Group Iso-S (isoflurane+IP saline): Rats were exposed to 1.5% isoflurane in 50% oxygen for 6 hours, and each rat received IP 0.5 mL saline injection shortly before isoflurane exposure.

Group Iso-PUFAs (isoflurane+IP PUFAs emulsion): Rats were exposed to 1.5% isoflurane in 50% oxygen for 6 hours, and each rat received IP 5 mL kg^{-1} PUFA emulsion (Omegaven®, Fresenius Kabi, Turkey), the volume of which was made to 0.5 mL with saline, shortly before isoflurane exposure.

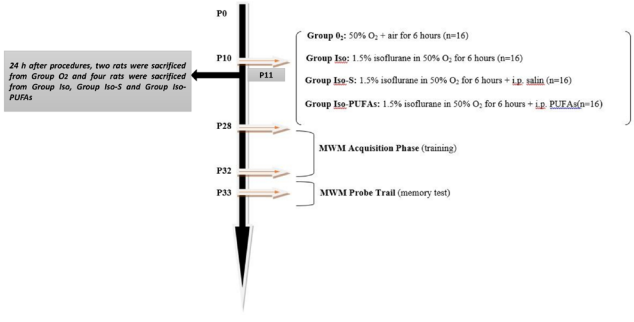

Figure 1. Schematic representation of the experimental design

Main Points:

- Isoflurane anaesthesia applying for a one time does not cause cognitive dysfunction and neuronal death in P10 rats.
- Polyunsaturated fatty acids administered just before isoflurane anaesthesia had no effect on learning and memory.
- Effect of long-term administration of polyunsaturated fatty acids on cognition is still uncertain.

Omegaven formula

The Omegaven parenteral PUFA emulsion used for the study was a 10% formulation. The emulsion included various species of PUFAs, especially eicosapentaenoic acid and docosahexaenoic acid. The recommended daily dose is 1 mL kg^{-1}. We preferred to use a higher dose (5 mL kg^{-1}) for IP administration because we administered it as a single dose before anaesthesia (13).

Anaesthesia administration

The P10 rat pups were randomly divided into 4 groups as follows: group O_2 (oxygen, n=16), group Iso (isoflurane, n=16), group Iso-S (isoflurane+IP saline, n=16) and group Iso-PUFAs (isoflurane+IP PUFA emulsion, n=16). Induction of anaesthesia was achieved with 1.5% isoflurane (Forane, Baxter International Inc., Deerfield, Illinois, USA) in 50% oxygen for 6 hours in group Iso, whereas the rats in group O_2 were exposed only to 50% oxygen for 6 hours in a temperature-controlled plexiglass chamber with the dimensions of 13×13×20 cm^3; a group of 8 pups were placed in the chamber at a time during the experimental procedure. Continuous monitoring of the levels of isoflurane, oxygen and carbon dioxide in the chamber was performed using infrared absorbance of the exhaled gas (Datex-Ohmeda S/5 Avance, General Electric, Boston, MA, USA). The total gas flow rate was adjusted between 5 and 7 L min^{-1} to maintain the end-tidal carbon dioxide levels sustained at 1-2 mmHg pressure. Heating pads were used to sustain normothermia (37°C±1°C). The respiratory frequency, depth and skin colour of the pups were observed during anaesthesia.

Morris water maze test

The Morris water maze (MWM) test was conducted with minor modifications by blinded investigators to evaluate the isoflurane-induced changes in learning and memory (14). P28 rats in all groups were trained in the MWM for 5 consecutive days, which included 2 sessions on each day (acquisition phase), and then subjected to the probe trial on day 6. A square escape platform (10×10 cm^2) was submerged 1 cm under the water surface (24°C±1°C) in a circular pool (diameter: 150 cm, depth: 60 cm). Opaqueness of the water was achieved using white tempera paint. The escape latency and time spent in the target quadrant were recorded by a digital chronometer and camera system. All the pups were acclimated to the experimental environment for 10 minutes before the test. The quadrant where the platform was located and the point of entry (initial quadrant) of each rat were randomised and counterbalanced. At each trial, the rats were given 60 seconds to find the hidden platform; if they failed, they were led to the platform and allowed to remain on it for 30 seconds. The platform was re-

Table 1. Escape latency of the animals (seconds)

	Group O_2 (mean±SD)	Group Iso (mean±SD)	Group Iso-S (mean±SD)	Group Iso-PUFAs (mean±SD)	p
Day 1	44.38±11.93	52.9±11.27	48.93±13.64	53.23±10.64	0.123
Day 2	40.97±15.6$^{\chi\chi}$	42.23±15.65*$^{\chi}$	41.57±16.03 $^{\chi}$	44.38±16.39 $^{\chi}$	0.960
Day 3	30.34±17.49*$^{\chi}$	32.77±13.21*$^{\#\chi}$	30.14±16.93* $^{\chi}$	29.38±17.42*$^{\#\chi}$	0.921
Day 4	18.81±10.86**$^{\#\#}$	25.3±15.56**$^{\#}$	21.07±16.59*$^{\#}$	25.77±20.1*$^{\#}$	0.654
Day 5	13.81±9.43**$^{\#\#}$	16.77±16.88**$^{\#\#}$	15.46±15.16*$^{\#\#}$	15.88±7.74**$^{\#\#}$	0.398

*p<0.05; **p<0.001 compared with day 1, $^{\#}$p<0.05; $^{\#\#}$p<0.001 compared with day 2, $^{\chi}$p<0.05; $^{\chi\chi}$p<0.001 compared with day 5, O_2: oxygen; Iso: isoflurane; Iso-S: isoflurane+saline; Iso-PUFAs: isoflurane + polyunsaturated fatty acids; SD: standard deviation

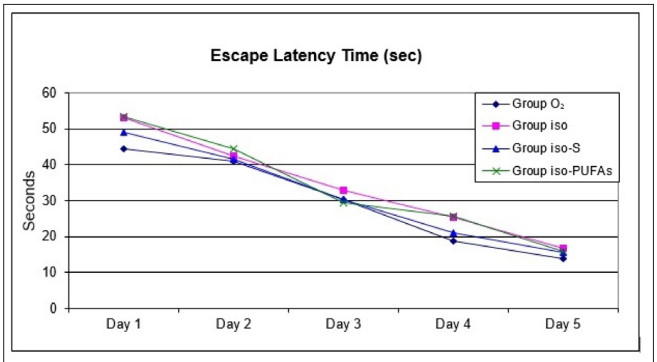

Figure 2. Latency of rats to find the hidden platform in the Morris water maze test at postnatal day 28-32, group O_2 (50% oxygen for 6 hours), group Iso (1.5% isoflurane for 6 hours), group Iso-S (1.5% isoflurane for 6 hours+intraperitoneal [IP] saline) and group Iso-PUFAs (1.5% isoflurane for 6 hours+IP polyunsaturated fatty acids [PUFAs] emulsion). The data are presented as mean±standard deviation of the 2 trials for each day (n=16 per group) (day 1, p=0.123; day 2, p=0.960; day 3, p=0.921; day 4, p=0.654 and day 5, p=0.398)

moved during the probe trials, and the rats were placed in the opposite quadrant of the initial quadrant.

Acquisition phase (training)

During training, the ability of the rats to understand the spatial relationship between the cues and escape platform (no cue rod) was evaluated. The platform was located in 1 quadrant, and its location remained stable during the trials. The point of entry was randomised for each rat, and the escape latency was recorded using a digital chronometer and camera system.

Probe trial (memory test)

Memory of the rats was assessed by probe trials conducted on P33 rats. The platform was removed from the target quadrant, and the rats were placed individually in the opposite quadrant of the initial quadrant and allowed to swim for 60 seconds. The time spent in each quadrant searching for the platform was recorded. The data are presented as the percentage of time spent by the animals in each of the quadrants.

Histopathology and immunohistochemical staining

The brain tissue samples from all the killed animals (4 rats were killed from groups Iso, Iso-S and Iso-PUFAs each and 2 rats from group O_2) were initially fixed in 10% buffered formalin, routinely processed, embedded in paraffin, sectioned in 5-µm thickness with a rotary microtome (Leica RM2255) and finally stained with haematoxylin and eosin and evaluated under a light microscope (Olympus BX50) for neuronal death.

Immunolabelling was assessed by light microscopy to assess the distribution and intensity of the immunoreaction with Bcl-2, an anti-apoptotic protein of the intrinsic pathway.

Statistical analysis

Statistical analyses of the data obtained were conducted using the Number Cruncher Statistical Software 2007 statistical software (Number Cruncher Statistical System, Utah, USA) package program. The distribution of data was tested using the Kolmogorov-Smirnov test and determined as non-normally distributed. Therefore, the parameters were analysed using non-parametric tests. Besides the descriptive statistical methods (mean and standard deviation), repeated measures of the data of multiple groups were tested by the Friedman test, whereas the Kruskal-Wallis test followed by the Dunn's multiple comparison test was conducted for the analyses between multiple groups. A p value less than 0.05 was accepted as statistically significant.

Results

There were no significant differences between the experimental groups in terms of sex distribution (p=0.796).

Effect of exposure to isoflurane for 6 hours on cognition in P10 rats

The time spent by the rats to find the platform after being placed in the pool during the trial period (acquisition [training] phase) was defined as escape latency, and this period was recorded. As demonstrated by the reduction in the escape latency on days 3, 4 and 5 compared with that on day 1, all the rats were success-

ful in learning the task during the acquisition (training) period (5 days) in the MWM test (Figure 2 and Table 1). In contrast to other groups, group Iso rats spent significantly lesser time to find the platform on day 2 than on day 1 (p=0.034). This can be interpreted as the group exposed to isoflurane learnt earlier than the other groups. No significant difference was found between the groups in terms of time spent in finding the platform on the training days (day 1, p=0.123; day 2, p=0.960; day 3, p=0.921; day 4, p=0.654 and day 5, p=0.398) (Table 1).

In the MWM test, the probe trials were performed 24 hours after the last acquisition (training) phase, and there were no statistical differences between the groups (Figure 3; p=0.561).

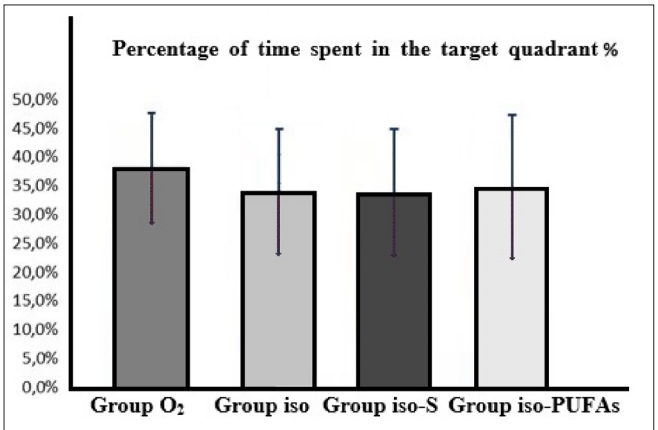

Figure 3. Effect of isoflurane and polyunsaturated fatty acid emulsion on spatial memory in the Morris water maze (MWM) test. Percentage of time spent in the target quadrant relative to all quadrants of the MWM for postnatal day 33 rats of all groups. There are no statistical differences between the groups. The data are presented as mean±standard deviation (n=16/group) (p=0.561)

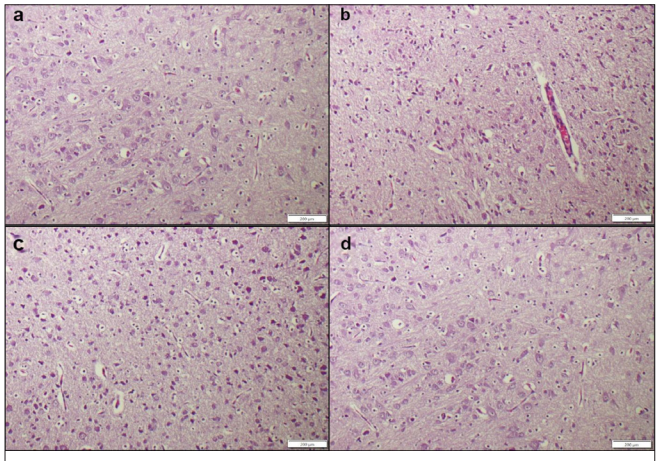

Figure 4. a-d. Histological features of the postnatal day 11 rat brains of each group; (a) group O_2, (b) group Iso, (c) group Iso-S and (d) group Iso-PUFAs (polyunsaturated fatty acids). There are no marked changes in pathology detected in the rat brains among the groups

Isoflurane anaesthesia does not cause neuronal death in the developing rat brain

Histological features of the P11 rat brains of each group are shown in Figure 4. There were no marked pathological changes detected in the brains of the rats from any group, and the neurons were clear and of moderate size with normal ultrastructure.

Immunolabelling with monoclonal anti-Bcl2 of the P11 rat brains from each group is shown in Figure 5. There was no significant alteration of Bcl-2 immunoreactivity among the groups.

Discussion

The number of neonates and infants who are administered anaesthesia are increasing worldwide. The fact that neuronal formation and synaptogenesis are considerably higher in the developing brain than in the adult brain, the central nervous system may lead to more pronounced effects of anaesthetic agents in infants (15). Therefore, concerns about safety in paediatric anaesthesia have led to increased interest in research on this subject. There are controversial results about the effect of isoflurane on cognition in the literature (1-4). These variations in effects are thought to be owing to the concentration of isoflurane, duration of exposure, postnatal day of administration and whether it is administered in a single or repetitive manner. In this study, we found that 1.5% isoflurane anaesthesia for 6 hours does not cause cognitive dysfunction and neuronal death in P10 rats.

There are many studies in the literature reporting that inhalation anaesthetics, especially isoflurane, cause cognitive dys-

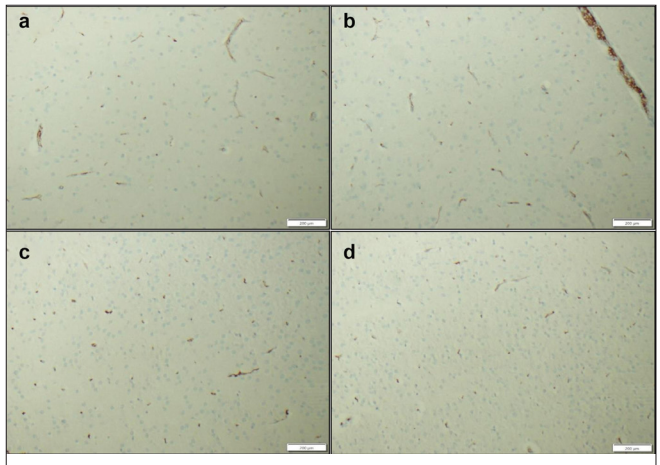

Figure 5. a-d. Immunolabelling with monoclonal anti-bcl2 of the postnatal day11 rat brains of the groups; (a) group O_2, (b) group Iso, (c) group Iso-S and (d) group Iso-PUFAs (polyunsaturated fatty acids). There is no significant alteration of Bcl-2 immunoreactivity among the 4 different groups

function in rats. This negative effect of isoflurane is thought to arise during the synaptogenesis stage. Synaptogenesis occurs between P4 and P14 in the rat brain. P7 is the day when synaptogenesis peaks and is thought to be the most susceptible period to the effects of inhalational anaesthetics in rats (16). Therefore, these studies were mainly conducted on P7 rodents (16, 17). Nevertheless, synaptic and neuronal dysfunctions caused by isoflurane exposure P14 have been reported at by some studies (2). However, cellular degeneration owing to apoptosis has been shown to increase on prolonged (6 hours) exposure to 1.5% isoflurane in P7 mice; adult neuronal density and spatial learning or memory function were not affected significantly (18). These different results indicate that the postnatal day on which the animals are exposed to isoflurane is not the only determinant factor, and although cellular degeneration develops, spatial learning or memory may not necessarily be impaired.

In the literature, P10 rats are considered as older neonates or infants (19, 20). To investigate the effect of isoflurane anaesthesia in infant rats, we used P10 rats in our study and concluded that single-dose prolonged isoflurane anaesthesia did not cause cognitive dysfunction. We believe that the reason for not detecting cognitive dysfunction may be the day of postnatal administration, but this may not be the only reason. Murphy et al. (4) had observed cognitive dysfunction in rats administered repetitively with 1.8% isoflurane on P7, P10 and P13 for 2 hours in their study, whereas single administration of isoflurane did not cause cognitive decline in P7 rats. Similar results were obtained in another study in P7 mice. Liu et al. (21) exposed P7 mice for 3 consecutive days for 2 hours to 1.5% isoflurane anaesthesia in their study, and they identified cognitive dysfunction in the MWM test conducted on P30. Although the experimental groups that developed cognitive dysfunction were exposed to isoflurane at a concentration and duration (1.5% and 1.8% iso 2 hours x 3 times) in these 2 studies similar to those in our study (1.5% Iso 6 hours), the reason why we obtained different results is that we exposed the animals to isoflurane continuously at a single time.

Other factors affecting the changes in isoflurane-related cognition are concentration and duration of exposure. In a study conducted on adult mice, the animals were exposed to 3 different concentrations of isoflurane (1%, 1.5% and 2%) for equal time periods, and the group exposed to 1% isoflurane was found to exhibit worse learning performance than the control group and the groups exposed to higher isoflurane concentrations (22). In another study investigating the effects of isoflurane anaesthesia at different concentrations and for different time periods, it was shown that high concentration and long duration of exposure increased neuronal apoptosis and caused cognitive dysfunction. In the same study, it was reported that isoflurane exposure led to improvement in learning and memory performance at sub-anaesthetic doses (0.5 minimum alveolar concentration=0.7%) (23). In our study, when the differences in the duration of finding the platform in the MWM test on subsequent days was compared with that on day 1, a significant decrease was found on day 2 in group Iso, whereas a significant decrease in duration was observed on day 3 in other groups. This could mean that learning is faster and learning function is improved in group Iso. However, our results showing that there is no effect of single isoflurane exposure on memory function are in parallel with those in the literature (3, 24).

Inhalational anaesthetics, including isoflurane, are known to be neurotoxic for brain development. The mechanism of isoflurane-induced cognitive impairment is currently unclear, but the most suspected one is neuroapoptosis. Activation of apoptosis by isoflurane occurs mainly through the intrinsic mitochondrial pathway (25), which involves the downregulation of anti-apoptotic proteins from the Bcl-2 family (for example, Bcl-2 and Bcl-xL). In our study, we found that there was no significant alteration of Bcl-2 immunoreactivity among the 4 groups. Moreover, histological features of the groups were similar. These results explain why we could not detect differences in cognitive impairment between the groups.

PUFAs, especially omega-3 fatty acids, have crucial effects on the stability of cell-membrane content, membrane receptor regulation, neuronal plasticity and brain development (10, 26). PUFAs also have an important role in learning and memory functions (27). The positive effect of omega-3 fatty acids on cognition has been shown in human and animal studies (11, 28). Studies have reported that a diet poor in PUFAs reduces the amount of PUFAs in the brain and results in learning and memory deficits (29). Although there has been a large number of studies investigating the effects of omega-3 and omega-6 fatty acids on brain development, learning and memory and neurological and neuropsychiatric diseases, studies investigating the effects of omega-3 and omega-6 fatty acids on anaesthesia-related cognitive functions are limited. Therefore, we aimed to observe the efficacy of a single high-dose PUFA emulsion on cognition when used together with isoflurane. This is the first study investigating the effect of a combination of isoflurane and PUFA emulsion on cognition. In a study in which sevoflurane was used, rats were fed with a diet rich in PUFAs during the gestation and lactation period and their pups were exposed to 3% sevoflurane anaesthesia on P7 for 6 hours. The results demonstrated that sevoflurane increased neuroapoptosis in rat pups and caused cognitive dysfunction, whereas PUFAs decreased sevoflurane-induced apoptosis and cognitive dysfunction (30). Li et al. (31) administered the rats with different doses of arachidonic acid for 10 days before exposing the rats to 1.2% isoflurane anaesthesia and observed that arachidonic acid administered at high

doses prevented isoflurane-induced cognitive dysfunction. In our study, we observed that high-dose PUFAs administered just before isoflurane anaesthesia had no effect on cognition. We believe that this difference is mainly because the effect of PUFAs on cognition appears after long-term use; in our study, we administered the rats with a single dose.

In our study, we used only the MWM test to evaluate the cognitive functions. Therefore, according to our results, we can only speculate that 1.5% isoflurane anaesthesia for 6 hours improved learning abilities in P10 rats.

Conclusion

This study aimed to investigate the effect of prolonged isoflurane exposure on cognition of infant rats administered with PUFA emulsion. Our study was the first in the literature that evaluated the effect of a combination of isoflurane and PUFA emulsion on neurocognition. We demonstrated that isoflurane exposure for 6 hours in P10 rats does not impair acquisition learning or memory in the MWM test and does not lead to neuronal death. However, it was found that a single dose of PUFA emulsion administered just before isoflurane anaesthesia had no effect on learning and memory functions. Future studies should focus on the impact of isoflurane exposure with long-term administration of PUFA emulsion on cognition in different protocols.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Bağcılar Training and Research Hospital (protocol number: 2016/112, 09 August, 2016).

Informed Consent: N/A.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – S.D., D.A.Ş.; Design – S.D., D.A.Ş.; Supervision – A.S., F.G.Ö., S.D.; Resources – Ş.Ö., O.A.; Materials – O.A., Ş.Ö.; Data Collection and/or Processing – D.A.Ş., S.D.; Analysis and/or Interpretation – S.D., F.G.Ö.; Literature Search – D.A.Ş., O.A., S.D.; Writing Manuscript – S.D., D.A.Ş.; Critical Review – A.S., F.G.Ö., S.D.; Other – Ş.Ö., O.A.

Conflict of Interest: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

References

1. Sha HY, Zhao JB, Sha MX, Guo SM. Effects of Vitamin B12 on postoperative cognitive dysfunction induced by isoflurane anesthesia in rats. Eur Rev Med Pharmacol Sci 2017; 21: 1959-66.
2. Zhu C, Gao J, Karlsson N, Li Q, Zhang Y, Huang Z, et al. Isoflurane anesthesia induced persistent, progressive memory impairment, caused a loss of neural stem cells, and reduced neurogenesis in young, but not adult, rodents. J Cereb Blood Flow Metab 2010; 30: 1017-30. [CrossRef]
3. Demirgan S, Akyol O, Temel Z, Şengelen A, Pekmez M, Demirgan R, et al. Isoflurane exposure in infant rats acutely increases aquaporin 4 and does not cause neurocognitive impairment. Bosn J Basic Med Sci 2019; 19: 257-64. [CrossRef]
4. Murphy KL, Baxter MG. Long-term effects of neonatal single or multiple isoflurane exposures on spatial memory in rats. Front Neurol 2013; 4: 87. [CrossRef]
5. Lee JH, Zhang J, Wei L, Yu SP. Neurodevelopmental implications of the general anesthesia in neonate and infants. Exp Neurol 2015; 272: 50-60. [CrossRef]
6. Wu X, Lu Y, Dong Y, Zhang G, Zhang Y, Xu Z, et al. The inhalation anesthetic isoflurane increases levels of proinflammatory TNF-α, IL-6, and IL-1β. Neurobiol Aging 2012; 33: 1364-78. [CrossRef]
7. Sinner B, Becke K, Engelhard K. General anaesthetics and the developing brain: an overview. Anaesthesia 2014; 69: 1009-22. [CrossRef]
8. Boscolo A, Starr JA, Sanchez V, Lunardi N, DiGruccio MR, Ori C, et al. The abolishment of anesthesia-induced cognitive impairment by timely protection of mitochondria in the developing rat brain: the importance of free oxygen radicals and mitochondrial integrity. Neurobiol Dis 2012; 45: 1031-41. [CrossRef]
9. Jevtovic-Todorovic V, Hartman RE, Izumi Y, Benshoff ND, Dikranian K, Zorumski CF, et al. Early exposure to common anesthetic agents causes widespread neurodegeneration in the developing rat brain and persistent learning deficits. J Neurosci 2003; 23: 876-82. [CrossRef]
10. Gomez-Pinilla F, Tyagi E. Diet and cognition: interplay between cell metabolism and neuronal plasticity. Curr Opin Clin Nutr Metab Care 2013; 16: 726-33. [CrossRef]
11. Hajjar T, Meng GY, Rajion MA, Vidyadaran S, Othman F, Farjam AS, et al. Omega 3 polyunsaturated fatty acid improves spatial learning and hippocampal peroxisome proliferator activated receptors (PPARα and PPARγ) gene expression in rats. BMC Neurosci 2012; 13: 109. [CrossRef]
12. Hansen TG. Anesthesia-related neurotoxicity and the developing animal brain is not a significant problem in children. Paediatr Anaesth 2015; 25: 65-72. [CrossRef]
13. Gungor AN, Turkon H, Albayrak A, Ovali M, Islimye M, Gencer M, et al. Does Omegaven have beneficial effects on a rat model of ovarian ischemia/reperfusion? Eur J Obstet Gynecol Reprod Biol 2014; 181: 240-5. [CrossRef]
14. Vorhees CV, Williams MT. Morris water maze: procedures for assessing spatial and related forms of learning and memory. Nat Protoc 2006; 1: 848-58. [CrossRef]
15. Hudson AE, Hemmings HC Jr. Are anaesthetics toxic to the brain? Br J Anaesth 2011; 107: 30-7. [CrossRef]
16. Yon JH, Daniel-Johnson J, Carter LB, Jevtovic-Todorovic V. Anesthesia induces neuronal cell death in the developing rat brain via the intrinsic and extrinsic apoptotic pathways. Neuroscience 2005; 135: 815-27. [CrossRef]
17. Sen T, Sen N. Isoflurane-induced inactivation of CREB through histone deacetylase 4 is responsible for cognitive impairment in developing brain. Neurobiol Dis 2016; 96: 12-21. [CrossRef]

18. Loepke AW, Istaphanous GK, McAuliffe JJ, Miles L, Hughes EA, McCann JC, et al. The effects of neonatal isoflurane exposure in mice on brain cell viability, adult behavior, learning, and memory. Anesth Analg 2009; 108: 90-104. [CrossRef]
19. Chen H, Burris M, Fajilan A, Spagnoli F, Tang J, Zhang JH. Prolonged exposure to isoflurane ameliorates infarction severity in the rat pup model of neonatal hypoxia-ischemia. Transl Stroke Res 2011; 2: 382-90. [CrossRef]
20. Tsukamoto A, Konishi Y, Kawakami T, Koibuchi C, Sato R, Kanai E, et al. Pharmacological properties of various anesthetic protocols in 10-day-old neonatal rats. Exp Anim 2017; 66: 397-404. [CrossRef]
21. Liu J, Zhao Y, Yang J, Zhang X, Zhang W, Wang P. Neonatal Repeated Exposure to Isoflurane not Sevoflurane in Mice Reversibly Impaired Spatial Cognition at Juvenile-Age. Neurochem Res 2017; 42: 595-605. [CrossRef]
22. Valentim AM, Di Giminiani P, Ribeiro PO, Rodrigues P, Olsson IA, Antunes LM. Lower isoflurane concentration affects spatial learning and neurodegeneration in adult mice compared with higher concentrations. Anesthesiology 2010; 113: 1099-108. [CrossRef]
23. Liu J, Wang P, Zhang X, Zhang W, Gu G. Effects of different concentration and duration time of isoflurane on acute and long-term neurocognitive function of young adult C57BL/6 mouse. Int J Clin Exp Pathol 2014; 7: 5828-36.
24. Su D, Zhao Y, Wang B, Li W, Xiao J, Chen J, et al. Repeated but not single isoflurane exposure improved the spatial memory of young adult mice. Acta Anaesthesiol Scand 2011; 55: 468-73. [CrossRef]
25. Zhang Y, Dong Y, Wu X, Lu Y, Xu Z, Knapp A, et al. The Mitochondrial Pathway of Anesthetic Isoflurane-Induced Apoptosis. J Biol Chem 2010; 285: 4025-37. [CrossRef]
26. Carlson SJ, Fallon EM, Kalish BT, Gura KM, Puder M. The role of the ω-3 fatty acid DHA in the human life cycle. JPEN J Parenter Enteral Nutr 2013; 37: 15-22. [CrossRef]
27. Gamoh S, Hashimoto M, Hossain S, Masumura S. Chronic administration of docosahexaenoic acid improves the performance of radial arm maze task in aged rats. Clin Exp Pharmacol Physiol 2001; 28: 266-70. [CrossRef]
28. Bauer I, Hughes M, Rowsell R, Cockerell R, Pipingas A, Crewther S, et al. Omega-3 supplementation improves cognition and modifies brain activation in young adults. Hum Psychopharmacol 2014; 29: 133-44. [CrossRef]
29. Su HM. Mechanisms of n-3 fatty acid-mediated development and maintenance of learning memory performance. J Nutr Biochem 2010; 21: 364-73. [CrossRef]
30. Lei X, Zhang W, Liu T, Xiao H, Liang W, Xia W, et al. Perinatal supplementation with omega-3 polyunsaturated fatty acids improves sevoflurane-induced neurodegeneration and memory impairment in neonatal rats. PLoS One 2013;8:e70645. [CrossRef]
31. Li C, Wang Q, Li L, Liu Y, Diao H. Arachidonic acid attenuates learning and memory dysfunction induced by repeated isoflurane anesthesia in rats. Int J Clin Exp Med 2015; 8: 12365-73.