Short Communication

Propofol Anesthesia Impairs Learning and Memory Function of Offspring of Late Pregnant Rats by Inhibiting BDNF/Trk B Pathway

SHAOPING WANG, Y. ZHANG AND HUIJUAN WANG*

Department of Anesthesiology, Shanghai General Hospital, Songjiang, Shanghai 201600, China

To investigate the effects of propofol anesthesia on learning and memory of offspring of late pregnant rats by inhibiting brain-derived neuro-affecting factor/tyrosine kinase B pathway. A total of 22 pregnant female rats were identified. Female rats fed for 3 w after pregnancy were randomly divided into a control group and a research group, with 11 rats in each group. The rats in the control group were fed normally, and the rats in the study group were given propofol 20 mg/kg with a concentration of 1 % by tail vein injection at a speed of 20 mg kg/h. The offspring of each group were randomly divided into the tyrosine kinase B excitation group and the non-tyrosine kinase B excitation group, with 25 offspring in each group. The expression of brain-derived neuro-affecting factor and tyrosine kinase B in hippocampus of rats in each group was observed by immunohistochemically staining. There was no significant difference in the blood gas index of each vein between the control group and the study group ($p>0.05$). Additionally, the count of passages across the initial platform location and the duration of stay in the third zone exhibited a substantial increase in comparison to the control group ($p<0.05$). Compared with the control group, the expression of brain-derived neuro-affecting factor and tyrosine kinase B in the hippocampus of the tyrosine kinase B-activated group decreased significantly ($p<0.05$), while the expression of brain-derived neuro-affecting factor and tyrosine kinase B in the hippocampus of the non-tyrosine kinase B-activated group decreased significantly ($p<0.05$). Maternal propofol anesthesia in late pregnancy can impair the learning and memory function of offspring rats, which may be achieved by inhibiting brain-derived neuro-affecting factor/tyrosine kinase B pathway.

Key words: Propofol, brain-derived neuro-affecting factor/tyrosine kinase B pathway, late pregnancy, anesthetia, cognitive impairment

Learning refers to the process of acquiring new knowledge, while memory involves retaining the learned information. The neuronal basis of learning and memory is synaptic plasticity, which encompasses synaptic development plasticity, synaptic transmission plasticity, and synaptic morphological plasticity. Among these, extended-duration enhancement (Long-Term Potentiation (LTP)) and extended-duration decrease (Long-Term Disability (LTD)) stand out as significant expressions of synaptic adaptability[1]. The prenatal period is a crucial phase of fetal growth and development, during which proliferation, differentiation, and synaptic growth of relevant brain neurons are highly sensitive to external stimuli. Research indicates that late gestation is a pivotal period for the structural and functional development of the nervous system and the maturation of learning and memory functions in the brain. During this time, the nervous system is susceptible to influences from both internal and external environments[2]. With the clinical application of general anesthesia techniques, increasing attention has been paid to the effects of general anesthetics on learning and memory functions. Studies have shown that general

***Address for correspondence**
E-mail: Wanghuijuan198703@163.com

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anesthetics can have detrimental effects on neuronal structure and function during the peak period of brain development, leading to neuronal apoptosis and cognitive impairment in patients[3]. Propofol is a commonly used intravenous anesthetic in clinical practice, known for its rapid onset and quick recovery[4]. Tian *et al.*[5] suggested that propofol induces hippocampal neuronal death in young mice, impairing their learning and memory functions. Brain-Derived Neurotrophic Factor/Tyrosine Kinase B (BDNF/Trk B) is involved in LTP, which forms the foundation of animal learning and memory[6]. This study focuses on late gestation rats and aims to analyze the mechanism through which propofol anesthesia impairs learning and memory functions in the offspring. Healthy adult female Sprague-Dawley (SD) rats and adult male SD rats (provided by Nanjing Junke Biological Engineering Co., Ltd., production license SCXK [Ning] 2017-0001), weighing (206±14) g. Acclimation nourishment was implemented over a span of 7 d within an environment maintained at (23°±3°), 52 %±7 % humidity, and a light-dark cycle alternating every 12 h, ensuring unrestricted availability of nourishment and water. High-pressure sterilizer (Nanjing Xiaoxiao Instrument Equipment Co., Ltd., specifications: YX600W-300 l); low-temperature refrigerator (Jinan Chuangxiang Biological Technology Co., Ltd., model: MDF-193); electronic balance; low-temperature high-speed centrifuge (Guangzhou Jidi Instrument Co., Ltd., model: JIDI-16R); Morris water maze (Shanghai Yuyan Scientific Instrument Co., Ltd.); oven (Shanghai Kuntian Experimental Instrument Co., Ltd., model: 101-1AB); animal electrocardiograph (Youcheng Biological Technology Co., Ltd., model: SurgiVet V3404+); animal anesthesia box (Germany Drager Company, model: Fabius GS); slicer (Henan Zhengzhou Nanbei Instrument Equipment Co., Ltd., model: YD-202); blood gas analyzer (United Statas of America (USA) MEDICA Corporation, model: EasyBloodGas); BDNF primary antibody (Xinbosheng Biological Technology Co., Ltd., specifications: 0.5 mg); Trk B primary antibody (Shanghai Xishi Biological Technology Co., Ltd., specifications: 0.5 mg) and propofol injection (Fresenius Kabi AB, batch number: 20170023, specifications: 20 ml: 0.2 g). Female rats were co-housed with male rats at a ratio of 2:1 to facilitate mating and pregnancy. Pregnant female rats were separated from male rats upon confirmation of pregnancy and housed individually. A total of 22 pregnant female rats were included. Establishment of animal models and grouping including the randomized allocation was employed to segregate gravid female rats into two cohorts; a control cohort and a study cohort, encompassing 11 rats within each assemblage. Rats in the control category were housed under standard circumstances, whereas rats in the study category were subjected to a tail vein infusion of 20 mg/kg propofol with a 1 % solution. After anesthesia and upon regaining consciousness, the rats were returned to their original cages for continued feeding until parturition. 1 mo after birth, the offspring were separated from their mothers and housed individually. The offspring from each mother rat were randomly divided into Trk B stimulation group and non-Trk B stimulation group using a random number table, with 25 offspring in each group. A blood gas analyzer was used to assess the effects of propofol anesthesia on venous blood gas indices (K^+, Na^+, pH, Bicarbonate (HCO_3^-), pressure of Carbon dioxide (pCO_2), pressure of Oxygen (pO_2), and Ca^+) in late gestation rats. The cognitive performance and memory capacity of the offspring rodents were evaluated using the Morris aquatic labyrinth examination. The aquatic maze was partitioned into four sectors, each delineated by labeled ingress points. A submerged circular pedestal was positioned within the third sector. In the orientation task, conducted twice daily for three consecutive days, rats were immersed into the water from a randomized entry point, and the duration required to locate the pedestal (termed escape latency) within a 110 s interval was chronologically documented. Subsequently, on the 4th d, the pedestal was removed, and a spatial exploration assessment ensued. Rats were introduced into the water at the original pedestal position, and the time dedicated to investigating the initial pedestal region within 110 s was meticulously logged. Should a rat be unsuccessful in locating the pedestal within 110 s, it was guided to the designated point. After a lapse of 48 h, the experiments were reiterated to measure escape latency, the tally of passages across the primary pedestal region, and the temporal interval spent in the third sector. The offspring rats were euthanized, and their brain tissues were collected.

The hippocampal tissues were dissected and fixed in 4 % paraformaldehyde. After routine dehydration, paraffin embedding was performed, followed by slicing into 5 μm-thick sections using a microtome. The sections were then stained using immunohistochemistry. The expression of BDNF and Trk B in the hippocampus of each group was observed using immunohistochemically staining. Positive staining for BDNF and Trk B appeared brownish-yellow. Four fields of view were randomly selected under high magnification, and the average gray value of positive reaction substances was calculated after multiple measurements. Regarding continuous variables, a comparative analysis was conducted using an independent sample t-test. In the case of categorical variables, the Chi-square (χ^2) test was applied. The threshold for statistical significance was set at a $p<0.05$. All statistical computations were performed utilizing Statistical Package for the Social Sciences (SPSS) 18.0 software. No statistically notable contrast was observed in the venous blood gas metrics between the control group and the study group ($p>0.05$) as shown in Table 1. The research results showed that the escape latency of offspring in the Trk B excited group was significantly reduced compared to the non Trk B excited group, and the number of times they crossed the original platform and the residence time in the third region were significantly increased compared to the non Trk B excited group ($p<0.05$); Compared with the study group, the Trk B excited group mice in the control group had a significantly lower escape latency, a significantly higher number of times they crossed the original platform, and a significantly longer residence time in the third region ($p<0.05$); The non Trk B excited group mice in the control group had a significantly reduced escape latency compared to the non Trk B excited group mice in the study group, and the number of times they crossed the original platform and the residence time in the third region were significantly increased ($p<0.05$) as shown in Table 2. BDNF predominantly manifests its presence within the cytoplasmic and nuclear domains of the hippocampal tissue in the progeny rodents. In comparison to the non-Trk B-activated group of the control cohort, the BDNF expression within the hippocampal tissue of the Trk B-activated group within the study segment exhibited marked attenuation ($p<0.05$). Likewise, the BDNF expression in the hippocampal tissue of the non-Trk B-activated group within the study set showcased significant decline relative to the non-Trk B-activated group of the control cohort ($p<0.05$). Trk B, on the other hand, finds its primary occurrence within the cytoplasmic compartment of the hippocampal tissue in the offspring rats. Notably, the Trk B expression within the hippocampal tissue of the non-Trk B-activated group within the study set experienced notable reduction in comparison to the non-Trk B-activated group of the control segment ($p<0.05$). This Trk B expression within the hippocampal tissue of the non-Trk B-activated group within the study set was also discernibly diminished when compared to the non-Trk B-activated group of the control cohort ($p<0.05$) as shown in Table 3. Pregnancy is a critical period for fetal growth and development, during which the fetal nervous system undergoes rapid development. The proliferation, differentiation, and synaptic growth of brain neurons are highly susceptible to damage from external stimuli during this period[7]. Although it has been found that many general anesthetics can provide neuroprotection by reducing neuronal metabolism and minimizing ischemia-reperfusion injury, there are reports suggesting that general anesthetics can increase neuronal apoptosis during the peak period of brain development and cause long-term damage to neural behavior[8]. Li *et al.*[9] discovered in a study on mice that exposing neonatal mice to inhaled isoflurane during their 1st w of life could impair their long-term cognitive function. Propofol, with its rapid onset, quick recovery, and sedative effects, is a commonly used intravenous anesthetic in clinical practice. Research has indicated that propofol can induce neuronal apoptosis in the brain and subsequently impact cognitive development[10]. Behavioral testing of animals has been applied in various areas of neuroscience, especially in assessing animal models of cognitive impairment-related diseases and investigating physiological mechanisms[11]. In this study, the Morris water maze test was employed to assess the learning and memory function of rat offspring. The results revealed that propofol anesthesia in late gestation rats had a detrimental effect on the memory function of their offspring. However, the Trk B agonist partially ameliorated the impairments caused by propofol anesthesia in

offspring learning function. The hippocampus is a brain region with special functions such as spatial learning and memory, playing a crucial role in various behaviors and cognitive functions. BDNF is a member of the neurotrophic factor family, primarily distributed in the cerebral cortex, hippocampus, brainstem, basal forebrain, and peripheral nervous system, with the hippocampus showing the broadest distribution[12]. Research has shown that BDNF can enhance cholinergic neuron synaptic acetylcholine release, influence synaptic plasticity, and increase N-Methyl-D-Aspartic Acid (NMDA) receptor activity to regulate hippocampal synaptic transmission and induce LTP[13]. Trk B is a high-affinity functional receptor for BDNF. Broad *et al.*[14] found that BDNF and Trk B play important roles in the formation of learning and memory, inducing long-lasting LTP in hippocampal granule cells. Trk B receptor agonists, newly discovered selective neurotrophic factors, effectively activate intracranial Trk B receptors, enhance learning and memory function, inhibit neuronal apoptosis, and improve the degree of neuronal damage. It has been reported that BDNF/Trk B has a positive effect on cholinergic neurons, increasing the synthesis and storage of acetylcholine in the forebrain and maintaining memory function. BDNF/Trk B plays a crucial role in maintaining learning and memory functions[15]. The findings of the study revealed that BDNF predominantly manifested its presence within the cytoplasmic and nuclear compartments of hippocampal tissue in the progeny rat. In comparison to the control group, the expression of BDNF witnessed marked reduction within the Trk B agonist subgroup of the study set ($p<0.05$). Furthermore, the BDNF expression within the non-Trk B agonist subgroup of the study group exhibited notable decrease when contrasted with the non-Trk B agonist subgroup of the control set ($p<0.05$). Trk B, in turn, was primarily detectable within the cytoplasmic domain of hippocampal tissue in the offspring rat. Relative to the non-Trk B agonist subgroup of the control cohort, the expression of Trk B within the non-Trk B agonist subgroup of the study segment underwent significant decline ($p<0.05$). Additionally, the expression of Trk B within the non-Trk B agonist subgroup of the study set was discernibly lower compared to the non-Trk B agonist subgroup of the control group ($p<0.05$). These findings suggest that propofol anesthesia administered during the late stages of rat gestation could exert an influence on the learning and memory capabilities of the offspring, potentially associated with the suppression of BDNF and Trk B expression. Importantly, Trk B agonists exhibited the capacity to elevate the expression of both BDNF and Trk B, thereby augmenting the learning and memory functionalities within the offspring. In summary, the learning and memory functions of rats display intimate links with the expression profiles of BDNF and Trk B. The utilization of propofol anesthesia during the latter stages of rat gestation appears to negatively affect the learning and memory abilities of their progeny. This phenomenon could be attributed to the downregulation of BDNF and Trk B expression, as well as the inhibition of the BDNF/Trk B signaling pathway, thereby influencing the learning and memory capacities of the rat offspring.

TABLE 1: THE EFFECT OF PROPOFOL ANESTHESIA ON VENOUS BLOOD GAS INDICATORS IN LATE PREGNANT RATS

Values	Group (n=22)		t	p
	Control	Trial		
K^+	4.52±0.65	4.81±0.51	1.164	0.258
Na^+	135.47±1.43	134.66±1.26	1.409	0.174
pH	7.39±0.05	7.37±0.26	0.251	0.805
HCO_3^-	30.64±3.76	28.46±2.69	1.564	0.134
PCO_2	51.26±3.43	50.65±5.88	0.297	0.769
PO_2	47.14±5.46	48.27±4.85	0.513	0.613
Ca^+	1.26±0.24	1.38±0.17	1.353	0.191

TABLE 2: COMPARISON OF LEARNING AND MEMORY FUNCTIONS OF OFFSPRING MICE IN EACH GROUP

Group		Escape latency(s)			Times of crossing the original platform (times)	Stay time in the third area (s)
		1 d	2 d	3 d		
Control	Trk B excitement	85.72±13.64	62.82±7.38	38.68±4.31	3.72±1.01	40.56±5.93
	Non-Trk B excitation	106.68±5.15[a]	76.75±3.83[a]	47.06±3.94[a]	3.08±0.95[a]	36.75±3.16[a]
Trial	Trk B excitement	111.42±3.17[b]	89.15±4.69[b]	63.89±2.21[b]	2.41±0.93[b]	31.29±4.08[b]
	Non-Trk B excitation	117.51±2.45[ac]	94.32±3.48[ac]	77.59±4.59[ac]	1.81±0.94[ac]	21.57±4.83[ac]

Note: [a]p<0.05 compared to the Trk B excited group within the same group, [b]p<0.05 compared to the Trk B excited group in the control group, and [c]p<0.05 compared to the non Trk B excited group in the control group

TABLE 3: EXPRESSION OF BDNF AND TRK B IN THE HIPPOCAMPAL TISSUE OF OFFSPRING RATS IN EACH GROUP

Group		BDNF	Trk B
Control	Trk B excitement	108.48±3.65	110.21±3.35
	Non-Trk B excitation	103.67±3.38[a]	104.29±2.79[a]
Trial	Trk B excitement	92.21±3.72[b]	90.49±3.28[b]
	Non-Trk B excitation	86.62±2.95[ac]	83.27±3.17[ac]

Note: [a]p<0.05 compared to the Trk B excited group within the same group, [b]p<0.05 compared to the Trk B excited group in the control group, and [c]p<0.05 compared to the non Trk B excited group in the control group

Conflict of interests:

The authors declared no conflict of interests.

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