

Original Article

Higher intravenous anesthesia dose protects memory in young rats and is associated with decreased release of corticotropin-releasing factor

Hui Wen^{1,2}, Ruowen Chen¹, Jianxiong An^{1,2}, Kun Niu¹, Rui Zhang¹, Lina Sun¹, John P Williams³

¹Department of Anesthesiology, Weifang Medical University, Weifang, Shandong Province, China; ²Department of Anesthesiology, Pain Medicine & Critical Care Medicine, Aviation General Hospital of China Medical University & Beijing Institute of Translational Medicine, Chinese Academy of Sciences, Beijing, China; ³Department of Anesthesiology, University of Pittsburgh School of Medicine, Pittsburgh 15213, USA

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Abstract: Postoperative cognitive decline (POCD) is a common complication following major surgery and general anesthesia. The mechanism of POCD is still unclear. Clinical research examining whether depth of anesthesia can affect POCD has recorded disparate results. We designed a standard surgery utilizing different depths of intravenous anesthesia on young rats with the goal to assess the relationship between depth of anesthesia and POCD (if any); further, we investigated a possible mechanism by assessing the effect of corticotropin-releasing factor (CRF) and its type-1 receptor (CRFR-1). Cognitive function tests were assessed on the day of operation and postoperative days 3 and 7. Biomarkers CRF and CRFR-1 were also measured on the 3rd and 7th post-operative days. The higher dosing of intravenous anesthetics was related to POCD, with the lower dosage of intravenous anesthesia group displaying persistent cognitive dysfunction on the 3rd and 7th day post-operatively; whereas, the higher dose of intravenous anesthesia group demonstrated transient cognitive decline with later recovery. Analysis of brain hippocampal tissue revealed a persistently high levels of CRF in lighter group; however, the relationship to CRFR-1 was not as consistent. These results suggest that variable dosing of intravenous anesthetics is related to the occurrence of POCD in young rats; as higher dosing of intravenous anesthetics seems to offer a protective effect on cognitive function. The persistently high levels of CRF in brain tissue may be one (but no means the only) mechanism responsible for POCD.

Keywords: Post-operative cognitive decline (POCD), deeper anesthesia, corticotropin-releasing factor (CRF), corticotropin-releasing factor receptor-1 (CRFR-1), surgical stress

Introduction

Following major surgery with general anesthesia, most patients demonstrate variable degrees of cognitive impairment that primarily affect learning, memory and perception, which is called postoperative cognitive decline or dysfunction (POCD) [1-3]. POCD affects both the patients' prognosis and quality of life; furthermore, it may also lead to dementia [1]. Although this syndrome was initially noted as a complication following cardiac surgery, POCD can also occur following non-cardiac surgery [2]. The mechanism of POCD is still unclear, possible pathogenic mechanisms include: patient-related factors (e.g. genetic susceptibility), surgical factors (e.g. hypoperfusion or hypoxia of brain),

and anesthetic factors (e.g. pain, depth of anesthesia) [3].

In 1987, Prys-Roberts defined anesthesia as "a state of unconsciousness in which the surgical patient neither perceives nor recalls noxious stimulation" [4]. Therefore, the depth of anesthesia can suppress to a significant degree the clinical responses to surgical stimuli. Previous work in humans found that deeper anesthesia can reduce early postoperative cognitive dysfunction [5, 6]. However other investigators have found that depth of anesthesia has no effect on POCD [7].

It has been confirmed that surgical stress activates the hypothalamic-pituitary-adrenal (HPA)

axis for prolonged periods [8, 9] and the activity of the HPA axis is associated with cognitive dysfunction [10, 11]. Previous work [10] has shown that one of the hormones of the HPA axis, corticotropin-releasing factor (CRF), plays an important role as a regulator of the endocrine stress response, in addition to having multiple effects on homeostatic balance during stress. Following a stress event, CRF is released by the endocrine cells of the paraventricular nucleus in the hypothalamus, and subsequently act on either type 1 or 2 receptors that are in multiple area throughout the brain [12]. CRF and CRF type 1 receptors (CRFR-1) are known to have an effect in a variety of stress-related diseases and [12-14] CRF acting on the CRFR-1 receptor are associated with in stress-induced cognitive decline [14].

The aim of the current study was to determine whether the depth of anesthesia (dosing of intravenous anesthetic agents used a surrogate measure of depth of anesthesia) can trigger a decline in cognitive function after surgery, and if higher dose of intravenous anesthetics can inhibit surgical stress and thereby decrease CRF secretion and CRFR1 activation.

Material and methods

Animals

The protocols for all these studies were approved by Weifang Medical University. 42 young (4 weeks old) Sprague-Dawley albino rats (weighing 140-185 g) provided by the Laboratory Animal Center of the Academy of Military Medical Sciences were used in this research. The animals were housed with food and water available ad libitum, and kept under a light/dark cycle (12 hours/12 hours) under controlled temperature conditions (23°C-25°C). After the training of Morris Water Maze, 6 rats exclude for this research, because the cognitive test is abnormal. The other rats were randomly assigned to three groups, lower dose (Group A, n = 12), higher dose (Group B, n = 12) and control (Group C, n = 12), because all the rats need the similar preoperative cognitive function.

All rats received training in the Morris Water Maze using the acquisition trials per day for four consecutive days, on the day following the fourth acquisition trial and one day prior to the surgical procedure, the first probe trial was per-

formed. We excluded 6 abnormal rats by the performance of Morris Water Maze, because they could not find the escape platform before and on the probe trial. All the rats received a second probe trial three days following surgery; after that, 6 rats in each group were selected at random and sacrificed for the hippocampal tissue assessment. On the 7th day after surgery, the remaining 6 rats in each group underwent the third and final probe trial following which they were sacrificed for a final hippocampal tissue assessment.

Anesthesia and surgery

Venous catheterization was performed using vena caudalis access prior to anesthesia. Group A (n = 12) received 3.125 mg/kg (the human equivalent dose of 0.5 mg/kg) of intravenous propofol administered by pump at the rate of 312.5 mcg/kg-min for 10 minutes, followed by a continuous infusion of 625 mcg/kg-min (the human equivalent dose of 100 mcg/kg-min) for 15 minutes. This period was followed by an exploratory peritoneotomy that lasted five minutes.

Group B (n = 12) were induced using a dose of 12.5 mg/kg (the human equivalent dose of 2 mg/kg) intravenous propofol with the pump 1250 mcg/kg-min for 10 minutes, followed by the same continuous 625 mcg/kg-min for 15 minutes and received the same surgical procedure for 5 minutes.

The peritoneotomy surgical procedure was chosen because it could be controlled as a standardized tissue injury with the wound controlled as a 1 cm incision on the abdomen and time controlled for 5 minutes. Hepato-lobectomy, splenectomy or other animal model for POCD was not chosen because the surgical time was not easily controlled and might affect the mobility of animal, thus affecting the Morris Water Maze test. Both of the anesthetic groups received the same duration of intravenous anesthesia (10 minutes of induction and 15 minutes for maintenance). During last 10 minutes of the maintenance, all animals received the surgical procedure. The end of the anesthesia was on the terminal infusion of 15 minutes maintenance. The control group received neither anesthesia nor surgical procedure.

As the definition of the depth of anesthesia is constantly evolving, initially the definition of

Memory protection by higher dose of intravenous anesthesia

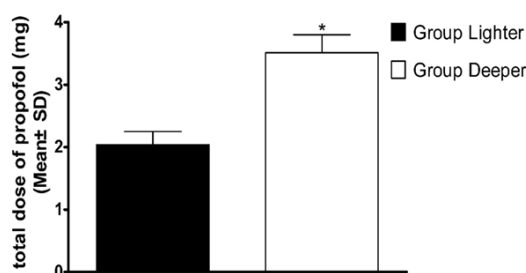


Figure 1. Assessment of the depth of anesthesia by dose of intravenous anesthesia drugs. Group lighter had a fewer drug dose (* $P = 0.001$).

anesthesia was, “a state of drug-induced unconsciousness in which the patient neither perceives nor recalls noxious stimulation”. Low concentrations of inhaled or intravenously administered anesthetics can eliminate pain, but they do not impede motor responses. Eger et al. [15] defined minimum alveolar concentration (MAC) as the drug concentration that blocked movement in response to surgical stimulation in 50% of subjects. So, we also used the total dose of propofol as a surrogate measure for the depth of anesthesia.

Morris watermaze for memory test

The Morris water maze generally consists of a black circular pool (180 cm diameter, 60 cm high) filled with water (30 cm depth) at 19–22°C and virtually divided into 4 equivalent quadrants. An escape platform (10 cm diameter) submerged 2 cm beneath the water surface was placed in the middle of one of the quadrants. The platform was painted black as was the pool wall and floor to make it invisible in the water. The pool was surrounded by several distal visual cues on the walls.

Rats received the acquisition trials per day for four consecutive days. Each day, a trial was initiated by placing each rat into the water facing the pool wall from one of the 4 pre-determined positions. If animals failed to find the platform within 90 seconds, they were picked up and placed directly on it for 30 seconds before a new trial (inter-trial interval 30 seconds) commenced. The platform location remained in the same position throughout all trials and days, but the starting direction of the rat differed with each trial, each day. Swim speeds and latency to reach platform were video recorded.

On the fifth day (24 hours following the last hidden platform trial), a probe trial was conducted

in which the escape platform was removed from the pool to measure spatial bias for the previous platform location. We placed each rat in the pool in the quadrant opposite to the training platform location and tracked the rat for 60 seconds, measuring the percentage of time spent in the previous target quadrant as well as the number of crossings over the previous platform location. The swim speeds were also video recorded.

The time spent in the target quadrant was the standard as a vague spatial memory of location of the hidden platform, while the number of crossings above the location of the previously hidden platform was the standard for a clear spatial memory. The swim speeds represent the mobility of animals. The first probe trial was performed at one day prior to the surgery. Another 2 probe trials were performed on post-operative days 3 and 7. All procedures were carried out using exactly the same protocols.

Elisa Kit for CRF and Western bolt analysis for CRFR-1 in hippocampus

We randomly chose 6 rats to collect tissue supernatants on postoperative days 3 and 7. These samples were then stored at -80°C until analysis. One side of the rat's hippocampus was used for CRF by ELISA kit (Elabscience Biotechnology, Wuhan, China) according to the procedures provided by the manufacturer. The opposite side was used for testing the expression of CRFR-1 by western blot. Hippocampal cells were washed once with phosphate buffered saline (PBS) and then lysed. Equal amounts of protein were electrophoresed on 10% polyacrylamide gels and then transferred to a polyvinylidene fluoride membrane. After blocking with 5% bovine serum albumin (BSA), membranes were probed with antibody against CRFR-1 (Abcam, Cambridge, Mass Catalogue No. ab59023) at 1:4,000 dilution. GAPDH was used as an internal control (Santa Cruz Biotechnology, Dallas, Texas, U.S.A.) at 1:4,000 dilution. For detection, the membranes were incubated with the appropriate secondary antibody and the blots were visualized with enhanced chemoluminescence.

Statistical analysis

Statistical analyses were performed using SPSS version 22.0 (SPSS Inc., Chicago, IL, USA). Data were expressed as mean ± standard devi-

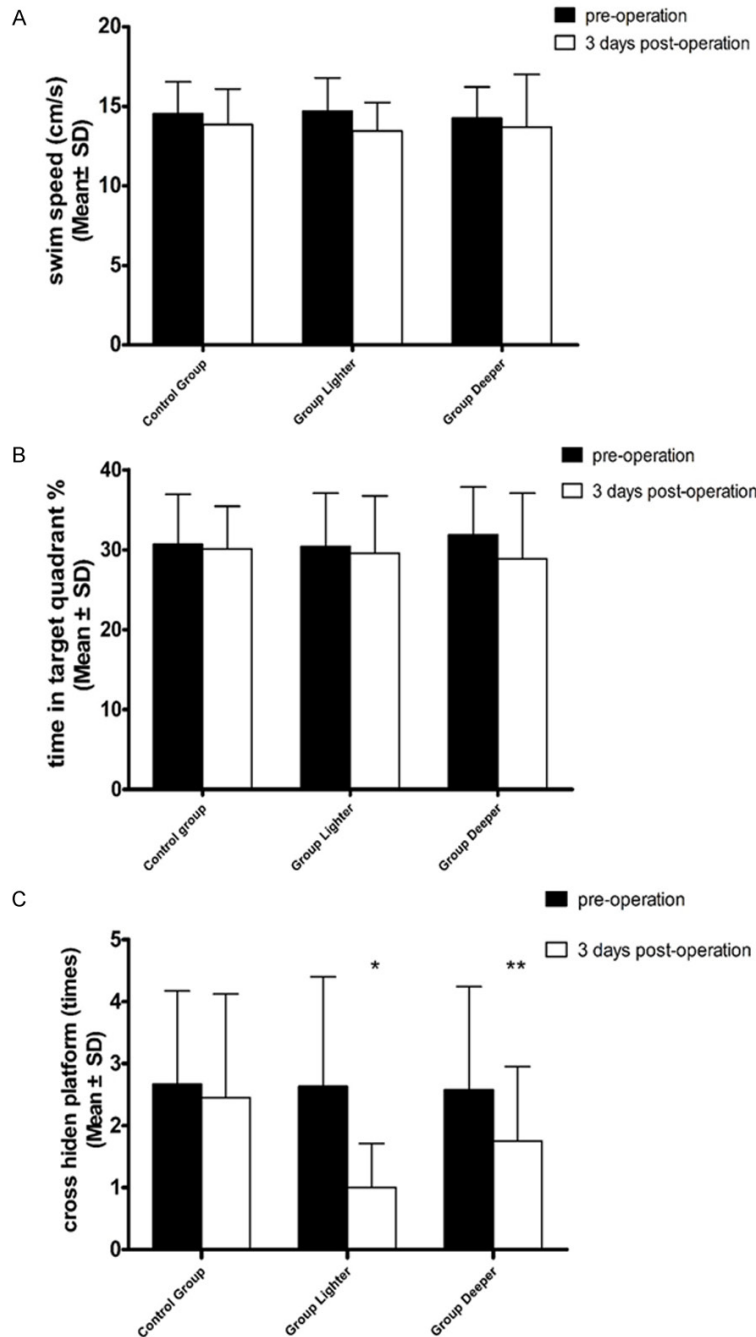


Figure 2. Morris Water Maze test pre-operation and 3 days post-operation. Before the operation and at the 3rd day post-operation in each group, the swim speed and time in target quadrant did not differ from each other ($P > 0.05$). Both two operative groups had a decline on the cross hidden platform than their pre-operation ($n = 12$ in each group, Group lighter $*P = 0.006$, Group deeper $**P = 0.034$). There is no significant difference between two operative groups ($F = 0.982$, $P = 0.332$).

ation. Repeated measures analyses of variance were used to analyze the difference of the Morris Water Maze test. The data, levels of CRF

and the expression of CRFR-1 in hippocampus tissue, was subjected to a one-way analysis of variance followed by Student's t-test to determine statistical differences between the experiment groups. $P < 0.05$ were considered statistically significant.

Results

The depth of anesthesia

The dosage of the anesthetic drugs used in intravenous anesthesia was determined by weight of the animals. The three groups of rats have a similar weight. The total dosage of propofol was used as a surrogate measure for depth of anesthesia. For depth of anesthesia, Group A has less total dose of propofol ($P = 0.001$) (**Figure 1**).

Morris water maze for memory test

The swim speed was used as a surrogate marker for mobility of the animals. As shown in **Figure 2A**, all rats ($n = 12$) exhibited similar performance with regard to swimming speed to find the hidden platform both prior to and three days following operation ($P = 0.889$). The number of crossing times of the hidden platform and time in the target quadrant both contributed to the memory test. As shown in **Figure 2B**, there were no significant differences between the three groups with regard to time in the target quadrant ($P = 0.448$). However, as noted in **Figure 2C**, on the third

day after operation, those rats that received anesthesia and surgery, scored significantly lower on crossing times for the hidden platform

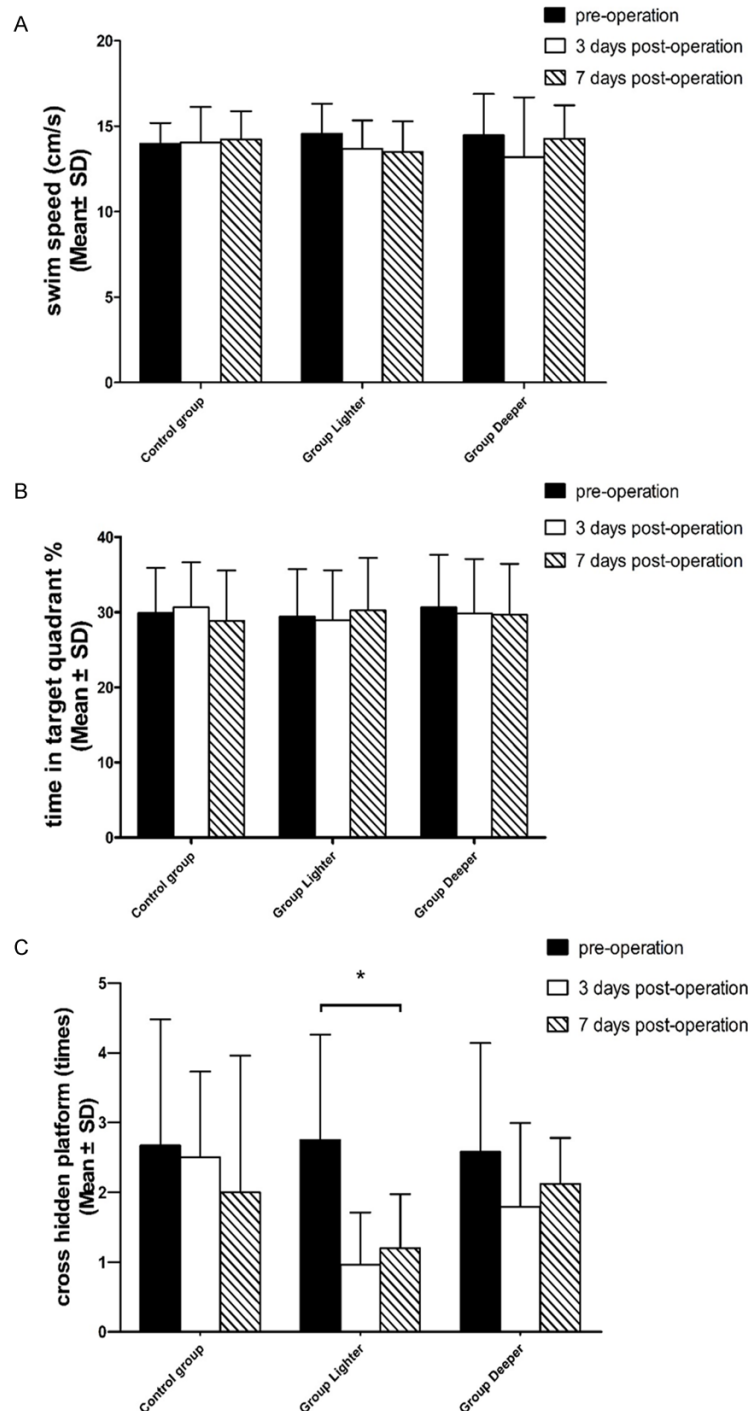


Figure 3. Morris Water Maze test pre-operation and 3, 7 days post-operation. In each group, the swim speed and time in target quadrant did not have differences from each other ($P > 0.05$). Group lighter had a significant difference on the cross hidden platform with group deeper ($F = 5.492$, $P = 0.041$) and control group ($F = 4.440$, $P = 0.047$) ($n = 6$ in each group).

compared to their preoperative performance (Group A, $P = 0.006$; Group B, $P = 0.034$). There were no significant differences between the

two operative groups ($P = 0.332$). On Day 7 after surgery, no significant differences between three groups ($n = 6$) were found in swim speed ($P = 0.255$) or time in the target quadrant on the memory test ($P = 0.426$) (Figure 3A, 3B). In the times for crossing the hidden platform, Group A demonstrated a significant difference compared to Group B ($P = 0.041$), who also had a significant difference with control group ($P = 0.047$). Group B was similar to the control group (Figure 3C).

The results of the Day 3 examination imply that with no difference in mobility, both operative groups suffered a decline in clear spatial memory, while vague spatial memory demonstrated no discernable difference; however, Group A suffered a greater decline in performance compared to Group B. On the seventh day postoperatively, Group B showed an improvement in clear spatial memory, but Group A did not.

The levels of CRF and expression of CRFR-1

CRF is widely known as one of the principal hypothalamic peptides to control pituitary-adrenal responses to stress [10]. CRF is distributed throughout the brain and is implicated in complementary autonomic and behavioral adjustments to stress. CRF levels in Group A and Group B were elevated compared to the control group on the third postoperative day. ($P < 0.001$). Group A was still elevated at the final sampling period (seventh postoperative

day postoperatively) ($P < 0.001$). However, both operative groups demonstrated a significant decline in CRF levels from the third to the sev-

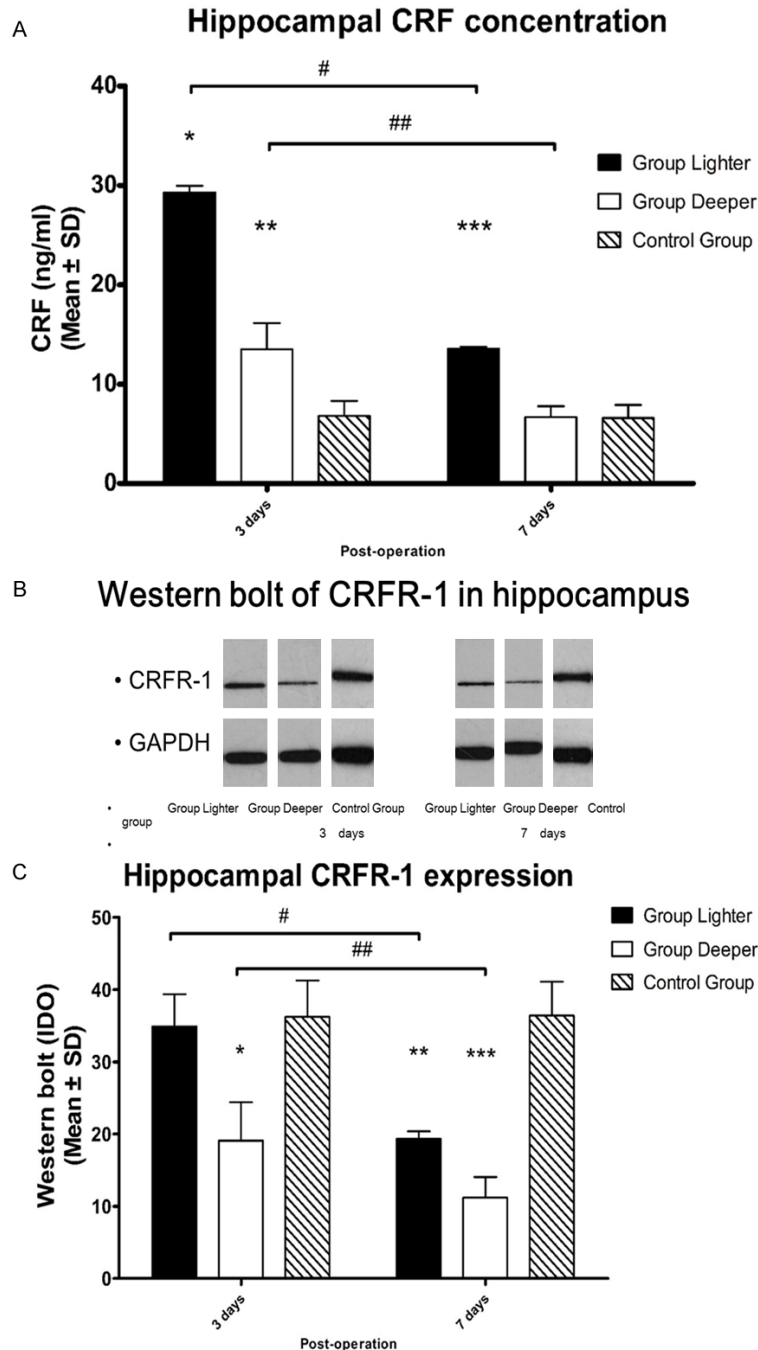


Figure 4. Elisa for CRF concentration and western blot for CRFR-1 in hippocampus. In A, CRF levels in group lighter and group deeper were higher than control group at the 3rd day post-operation (* $t = 35.319$, $P < 0.001$, ** $t = 4.789$, $P < 0.001$). In group lighter CRF was still high at the 7th day post-operation than control group (** $t = 12.182$, $P < 0.001$). Both two operative group had a significant decline in CRF levels from 3 day after operation to 7 day (# $t = 46.242$, $P < 0.001$, ## $t = 6.243$, $P < 0.001$). As shown in B and C, the expression of CRFR-1 by western bolt, group deeper had a decrease at the 3rd day post-operation (* $t = 4.552$, $P = 0.004$). After 7 days of operation, both two operative group had less expression of CRFR-1 than control group (** $t = 6.292$, $P < 0.001$, *** $t = 8.956$, $P < 0.001$). Also, there was a decline in two operative groups than their 3 days post-operation (# $t = 6.811$, $P < 0.001$, ## $t = 2.619$, $P = 0.04$).

enth day following operation ($P < 0.001$) (Figure 4A). On the third day following operation, the expression of CRFR-1 in Group B was lower than both the control group and Group A ($P = 0.004$). On the seventh postoperative day, CRFR-1 expression in both operative groups was significantly lower than at day three postoperatively ($P < 0.001$) and significantly different compared to the control group ($P < 0.001$) (Figure 4B and 4C).

These results imply that the lower dose group maintained an elevated CRF concentration as well as a decrease in the expression of CRFR-1 during the postoperative period. The higher dose group had a transitory elevated CRF concentration, and had a lasting decrease on CRFR-1 expression.

Discussion

We confirmed that a surrogate marker for depth of anesthesia (dose of intravenous anesthetics) was associated with alterations in cognitive function following surgery, and that both groups who underwent anesthesia and surgery suffered a lack of memory retention compared to their preoperative performance. The higher dose anesthesia group performed better than the lower dose group when measured three days postoperatively. In a longer-term assessment at seven days postoperatively, the higher dose anesthesia group had better memory retention compared to both the lower dose group and the control group (no anesthesia and surgery).

In 1987, Prys-Roberts believed that the noxious stimula-

tion (mainly pain) of surgery induces a variety of reflex responses that may be independently modulated [4]. Thus, he defined general anesthesia as a state of drug-induced unconsciousness in which the patient neither perceives nor recalls noxious stimulation [4]. Under general anesthesia unconsciousness was induced at different degrees to inhibit the variety of surgical responses. So it was believed that variable depth of anesthesia can only be have under general anesthesia. However, in regional anesthesia, patients should have no loss of consciousness, and therefore there should be no or less cognitive decline after surgery, if POCD was only caused by general anesthesia. In fact, many investigators have [16-19] reported that the incidence of POCD is similar and affects postoperative surgical patients irrespective of the anesthetic technique [20]. This implies that drug-induced unconsciousness, or general anesthesia, has no relationship to POCD, and the depth of anesthesia related to POCD needs to be studied.

As the definition of the depth of anesthesia has continued to evolve, the crux of difficulty in defining anesthetic depth was that the unconsciousness could not be evaluated directly. Therefore, the measurement of autonomic response to surgical stimulus under different dosages of anesthesia might be the best method. Under general anesthesia, to control an adequate autonomic response in the normal physiological status, the more intense the surgical stimulus the greater the depth of anesthesia needs to be. Thus, if the surgical stimulus were the same between operations, the varieties of anesthetic depth might be different degrees of autonomic responses. Recently, two clinical investigations [5, 6] suggest that deeper general anesthesia may reduce the incidence of early postoperative cognitive dysfunction. So we designed the same surgical stimulus operation and the same procedures of anesthesia in this animal research. The standard surgery was utilized by a 1 cm wound with a controlled duration of exposure (five minutes of surgery) and 25 minutes total anesthesia time.

Although POCD is more prevalent in elderly patients, the incidence might, to a large degree, depend on the tests used to detect cognitive function [21]. In fact, because an appropriate

control group (e.g. patients who were subjected to surgery but no general anesthesia, which were suggested by another researcher [22]) has not been prospectively compared with regards to POCD, clinical investigations [23] are frequently questioned. The appropriate control group in this research might be a blank control.

It may also be that POCD is simply a misinterpretation of the data [24], as aging alone can contribute to cognitive decline. However, younger adults can also demonstrate a decline in cognition after surgery [25]. Therefore, we used younger rats to avoid problems related strictly to cognitive decline in the aged. Our results indicate that the operative groups both suffered memory impairment on third postoperative day: a finding that we similar to other investigators work [21]. The lower dose group demonstrated persistent memory impairment up to seven days postoperatively; however, the higher dose group demonstrated recovery in memory function. It is inferred that lower dose anesthesia leads to persistent memory impairment and higher dose anesthesia may protect against this impairment. These results corroborate prior clinical investigations [5, 6].

It is widely appreciated that psychological stress can influence cognitive function (e.g. stress-related disease). Additionally, the role of corticosterone may also be an important component in stress-induced memory changes; this suggests an important role for corticotropin-releasing factor (CRF) [26, 27]. CRF is one of the principal initiators of hypothalamus-pituitary-adrenal (HPA) axis response to stress. As previously mentioned, under the same surgical stimulus during operations, the depth of anesthesia might cause different degrees of autonomic responses. Within the brain, the autonomic nervous system is regulated by the hypothalamus. Thus, CRF was chosen for this POCD animal research to find the possible mechanism. As the results shown, CRF in both Group lower dose and Group higher dose was higher than the control group at day three following operation. Also, CRF in Group lower dose was higher than Group higher dose. This implies that the surgical and anesthetic procedures induced stress, and dosage of anesthetic (depth of anesthesia) was related to CRF concentration in the hippocampus.

In the central nervous system, the adaptation of internal or/and external environmental changes begin with the synthesis and secretion of CRF by the periventricular hypothalamic nucleus, which gathers information from higher centers and the periphery [28]. CRF is a stress-related neuropeptide that coordinates the physiological and behavioral responses to stress, partly through activation of the hippocampus to change memory formation and retention [10]. In stress-related syndromes in humans like posttraumatic stress disorders (PTSD), not only have high levels of CRF had been found in the cerebrospinal fluid [29], but it also correlates with the severity of PTSD symptoms [30]. In our study, we found CRF in Group lower dose was higher than Group higher dose at day three and seven following operation. This implies that the severity of POCD in Group lower dose was higher than Group higher dose. At day seven after operation, Group higher's CRF concentration recovered from this elevated level and was similar to the control group; however, the CRF concentration of Group lower dose was remained elevated. This suggests that there is an association of persistent memory impairment in the lower dose group with persistently elevated levels of CRF in the hypothalamus.

However, the activation of hippocampus by CRF through its receptor, CRFR-1, should be considered as the other main agent involved with cognitive alterations. We found that CRFR-1 expression in the hippocampus was inhibited by different dosages of anesthetics. At three days postoperatively, Group lower dose had a similar expression of CRFR-1 compared to the control group and Group higher dose demonstrated significant reduction. At seven days postoperatively, both surgical groups demonstrated lower expression of CRFR-1 than the control group.

A recent study [31], which aimed at accurately determining the presence of CRFR-1 in the brain, revealed that this receptor is present in glutamatergic neurons of the hippocampus. During anesthesia, glutamatergic neurons of the hippocampus are susceptible to induced cell death [32]. Therefore, CRFR-1 neurons may be involved in inducing cell death during anesthesia. The more drug usage might induce more CRFR-1 neurons death. We found that at 3 days following operation, CRFR-1 in Group higher dose was less expression than Group lower

dose. At 7 days post-operation, CRFR-1 was kept in decreasing expression at both Group higher dose and Group lower dose. Also, the persistence of CRFR-1 neuron has no effect on memory function impairment. The main reason we considered was how this receptors and their ligands act and how much this receptor can be activated by their ligands, and this part of research needs more investigations.

We infer that lower dose anesthesia control surgical noxious stimulation which causes more CRF release from HPA axis. This increase in release has a persistent effect on hippocampus could lead a persistent memory impairment. The higher dose anesthetic control surgical noxious stimulation which cause a transient CRF release from HPA axis. This increase leads to a transient cognitive decline. For CRFR-1 neuron, we did not find an appropriate explain to this phenomenon.

We found several limitations in our research. First, we did not use an electroencephalograph to evaluate the depth of anesthesia. We did not use this because invasive intracranial electrode implantation might cause inflammation in the brain which might then lead to cognitive dysfunction and this procedure could not easily be controlled as a second standard surgical noxious stimulation. Secondly, only male rats were used in this experimental study, and were observed and tested for only seven days after surgery. Thirdly, the animal model used in this study could not duplicate the clinical situation of POCD we could only infer this from the memory impairment. Finally, we did not use analgesic drugs postoperatively because these drugs can inhibit the HPA axis and confound the results.

The results of this study suggest that dosage of anesthetics (as a surrogate for depth of anesthesia), played an important role in postoperative cognitive impairment in young rats and that the persistently high level of CRF in the hippocampus was associated with this process. Further studies are needed to comprehensively examine the underlying mechanisms of CRFR-1 neurons and neuronal plasticity that follow changes in depth of anesthesia and surgical stress. Enhanced understanding of these processes may lead to potential therapeutic strategies for the treatment or prevention of POCD.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Jianxiong An, Department of Anesthesiology, Weifang Medical University, Weifang, Shandong Province, China; Department of Anesthesiology, Pain Medicine & Critical Care Medicine, Aviation General Hospital of China Medical University & Beijing Institute of Translational Medicine, Chinese Academy of Sciences, Beijing, China. E-mail: anjianxiong@yahoo.com

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