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Effect of Long-Term Oral Administration of Different Dosages of Caffeine on Anesthetic Emerging Time

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ABSTRACT

This paper aims to investigate the effect of prolonged oral ingestion of different doses of caffeine on anesthetic emergence time. In our experiment, eighteen male mice were selected and randomly divided into three groups of six mice, each according to the concentration of caffeine administered: a high-concentration caffeine group, a low-concentration caffeine group, and a blank control group. Mice were anesthetized with isoflurane and Zoletil 50, and the time of emergence was recorded for both anesthesia and the time of entry into anesthesia was recorded for Zoletil 50 anesthesia. Twenty-four hours after the anesthesia experiments, the mice were subjected to Morris water maze experiments, visual station experiments, hidden station experiments, and spatial exploration experiments. The results showed that both high-dose and low-dose coffee ig accelerated isoflurane and Zoletil 50 anesthesia emerging, with the high-dose being statistically more significant for the acceleration of anesthesia emerging. Prolonged oral coffee administration did not affect the time to enter anesthesia. Long-term oral coffee administration enhanced spatial exploration and spatial learning memory after anesthesia.

Introduction

Anesthesia is a generally drug-mediated reversible suppression of nervous system function widely used in modern surgical operations because it manifests as a loss of sensation, especially pain. Along with advances in surgical techniques in recent decades, there has been a dramatic development in anesthesia and the technology of life detection during anesthesia, which is more commonly used in medical treatments [1]. This has been accompanied by an increase in the number of vulnerable people requiring surgery who are sensitive to anesthesia, such as the elderly and young. Long recovery times and cardiovascular depression after anesthesia need to be avoided. Targeted anesthesia, where the dose of anesthesia is customized to the patient, is a common demand nowadays [1].

There are no clinical drugs that can reverse the coma-like state mediated by general anesthesia [2]. There have been many experimental attempts to find drugs that can shorten the duration of anesthesia or cause emerging from anesthesia, and some of these studies have shown that some drugs, such as trichothecenes and theophylline, are capable of emerging rodents from anesthesia [3]. Some of these drugs are administered intracranially by microinjection or intravenously, and most have significant side effects. The brains of young populations are still developing. Previous studies have shown that isoflurane, a common type of anesthesia, works by blocking the release of neurotransmitters from PC12 cells [3], and frequent general anesthesia has been shown to affect behavioral changes in young children, including decreased executive and reading abilities [4]. Getting children out of anesthesia as quickly as possible, waking them up from anesthesia, and reducing the time needed for recovery from anesthesia are significant issues that must be addressed today. The search for drugs that reduce the time to awaken from anesthesia and allow the patient to awaken from anesthesia more quickly is urgent.

Caffeine, an alkaloid that can be widely ingested from everyday life, has been shown in many studies to accelerate the time to emerge from anesthesia by increasing intracellular cAMP through inhibition of phosphodiesterase. In all instances, the rate of emerging from anesthesia was significantly accelerated after termination of anesthesia [5,6]. Caffeine does indeed play a role in accelerating the emergence of anesthesia. Although much research demonstrates the association between caffeine and a shorter emerging time from anesthesia, most experiments have used intravenous caffeine ingestion. Caffeine injections are invasive, and the young child population has unremarkable veins and is hyperactive, adding to the difficulties of intravenous administration. This study hopes to add consideration to the variable of long-term oral caffeine intake based on the confirmation that caffeine can have an accelerated emerging effect on anesthesia for the anesthetized population of young children. As a common drug in daily life, caffeine has a higher safety profile compared to other drugs. In common instant coffee, each eight fluid ounces contains 63 mg of caffeine [1]. Its mild medicinal properties make it safer for vulnerable groups.

A controlled anesthesia experiment was conducted using mice cultured ahead of time for long-term caffeine ingestion at different doses to investigate the change in anesthesia emerging time under short-term anesthesia, such as isoflurane used in this experiment, versus prolonged anesthesia, such as Zoletil 50 used in this experiment. A pre-test was added to the study to investigate the effect of long versus short-term coffee administration on the time to awaken from isoflurane anesthesia. The experiment used the Morris water maze, a behavioral research model, to assess the ability of mice to learn to remember spatial locations after emerging from anesthesia to assess the toxic effects of caffeine on the nociceptors of mice. This experiment is intended to demonstrate the association between caffeine and shortened emerging time from anesthesia and to contribute to the understanding of shortening anesthesia time and tailoring anesthesia dose to determine the safety and efficacy of caffeine in accelerating anesthesia emerging in a young adult population.

Materials and Methods

Caffeine: This Experiment used American Cold Brew Black Coffee, Caffeine Content 3.65g/100g, Yunnan Cold Extraction Technology Co.

Based on the conclusions drawn from previous experiments, the correlation between changes in dose and time to emerging from anesthesia was maximized when caffeine was injected at a dose of 0.9 mg/kg. In this experiment, instant coffee was used as a source of caffeine by gavage, and the dose was set at 1.8 mg/kg for the low-dose group, 50 mg/kg for the medium-dose group, and 100 mg/kg for the high-dose group due to the difference between oral administration and injection.

Mice Used in the Experiment

Mice: The mice used in this experiment were approved by the Institute of Cancer Research (USA) as 8-week males to establish an animal model suitable for this experiment and to comply with applicable guidelines. Every effort was made to minimize the suffering of the experimental animals and to reduce the number of animals used.

Exploring the Effect of Long-Term Versus Short-Term Coffee Administration on the Time to Awaken from Isoflurane Anesthesia

Nine male mice were selected and randomly divided into three groups: long-term coffee gavage, temporary coffee gavage, and blank control. The coffee long-term gavage group was fed five days of lyophilized caffeine

pure aqueous solution according to body weight at a concentration of 100mg/kg and 50mg/ml converted from body weight. Coffee, temporary gavage group dose was calculated similarly, fed one day of freeze-dried caffeine pure aqueous solution. The blank control group temporary gavage group was fed with an equal amount of pure water.

Isoflurane (produced by Hebei Qidafu Pharmaceutical Co., Ltd.) Anesthesia Mice were placed in a gas-tight anesthesia chamber, and the experiment began with isoflurane at a concentration of 3%, being administered at a flow rate of 3.5 ccs for 20 minutes while the mice fell into an anesthetized state after four minutes. The isoflurane concentration was then reduced to 2% and held for five minutes, after which the isoflurane concentration was reduced again to 1% and held for five minutes. Anesthesia input was then stopped, and the mice were removed from the anesthesia bellows and placed limb side up on a warming pad until the mice began to awaken from anesthesia, and the time of emergence of the mice was recorded.

Investigating The Effects of Long-Term Administration of Different Doses of Coffee On the Time to Awaken from Prolonged Versus Short Anesthesia

Eighteen male mice were selected and randomly divided into three groups of six mice, each according to the dose administered by gavage, namely, the high-dose caffeine group, the low-dose caffeine group, and the blank control group.

Isoflurane Anesthesia Mice were placed in a gas-tight anesthesia chamber for isoflurane anesthesia. isoflurane gas at a flow rate of 2 cc/min*100 (3%) was administered for ~4 min to induce anesthesia. During this time, mice were placed under general anesthesia and became unresponsive to external stimuli. The isoflurane dose was then adjusted to 2%, and the mice entered anesthesia for up to 20 min. After 20 minutes, the anesthetic gas was turned off. When the remaining isoflurane in the anesthesia bellows was expelled (~3 min), the mice were removed from the anesthesia bellows and placed limb-side up on a warming pad. The time of emergence started to be recorded.

Zoletil 50 (Vickers Ltd., France) Anesthesia 48 hours after the end of the last experiment, the same batch of mice were injected intramuscularly with Zoletil 50 diluted in buffer at a concentration of 10 mg/mL, 0.7 mL/10 g. The time from the time of injection to the time of loss of response to the outside world was recorded. The mice were placed sequentially limb-side up on an insulating pad and waited for the mice to awaken, and the time of emergence was recorded. The same mice were used for this experiment as for isoflurane anesthesia.

Observation of mice for signs of emerging the criterion used as a criterion for emerging in this experiment was the occurrence of a flip-flop response, i.e., the mice autonomously flipped from the limb-up state.

Morris Water Maze Experiment

Twenty-four hours after the anesthesia experiment, the mice were subjected to the Morris water maze experiment. A 0.8 m diameter water maze was filled with warm water and placed into an elevatable station. The water maze was divided into four quadrants, and the quadrant where the stations were located was defined as the southwest quadrant. Geometric image cues were placed on each station. A schematic diagram of the water maze is shown in Figure 1 below:

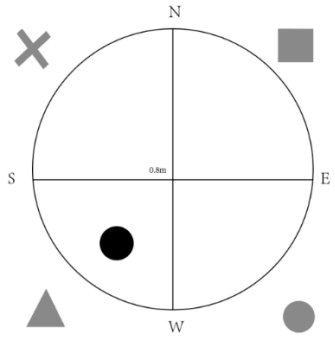

Figure 1. A schematic diagram of the water maze

Visual station test Mice were placed into the water from one of the four quadrants (Northeast, Northwest, Southeast, and Southwest) facing the wall of the pool, and the station was placed in the Southeast quadrant. Each time, the mice swam for a total of 70 s to find the visual station on the water surface. If the mouse succeeded in finding the platform, it was given a 15-s rest period on the platform; if it failed in finding the platform within 70 seconds, it was manually placed on the platform by the experimenter and given the same 15-s rest period. If the mouse fell off the platform, then the mouse was placed back on the platform and retimed so that the time interval reached 15 seconds to ensure that each mouse had equal time to observe and acquire spatial information after each experiment. This was repeated three times, and the entire video was recorded from an overhead vertical angle for analysis.

The hidden station test was performed four hours after the visual station experiment. Mice were placed in the water from one of the four quadrants (Northeast, Northwest, Southeast, and Southwest) facing the wall of the pool, and the station was placed in the Southeast quadrant. Each time, the mice swam for a total of 70 seconds to search for the hidden station. If the mouse succeeded in finding the platform, it was given a 15-s rest period on the platform; if the mouse failed to find the platform within 70 seconds, it was manually placed on the platform by the experimenter and given the same 15-s rest period. If the mouse fell off the platform, then the mouse was placed back on the platform and retimed so that the time interval reached 15 seconds to ensure that each mouse had equal time to observe and acquire spatial information after each experiment. This was repeated three times, and the entire video was recorded from an overhead vertical angle for analysis.

Space exploration test the station was removed 24 hours after the end of the concealed station test. Then, the rats were placed into the water at the same entry point, and their swimming path was recorded within 90 seconds. The original location of the station was marked with a circular ring. The residence time and the number of times the rats crossed the original station quadrant were recorded to observe the spatial localization ability of the rats and the changing pattern during the spatial exploration process.

All t-tests used in this experiment were unpaired t-tests.

Results

Exploring The Effect of Long-Term Versus Short-Term Coffee Administration On the Time to Awaken from Isoflurane Anesthesia

This experiment investigated the effects of long-term and short-term coffee consumption on the emerging time of anesthesia. The emerging times of mice are shown in the graph below (Figure 2). To ensure the rigor of the results, the emerging times of the temporary coffee gavage group and the long-term coffee gavage group were compared with those of the blank control. The difference between the long-term coffee gavage group and the

blank control group was significant, significantly lower than the blank control, which was statistically significant; the difference between the temporary coffee gavage group and the control group was not significant, which was not statistically significant. Therefore, it can be proved that long-term coffee intake reduces the time of anesthesia emerging.

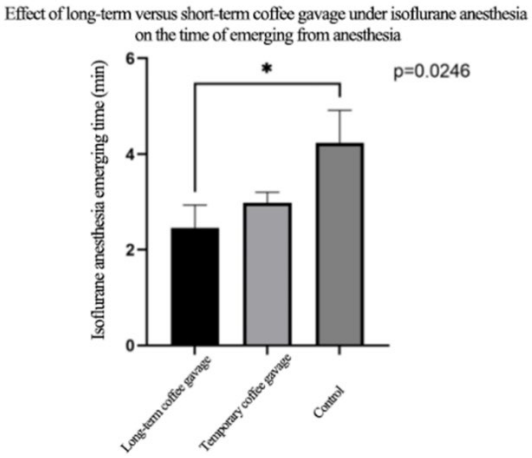

Figure 2. Difference in time to awaken from anesthesia between long-term recipients of 50 mg/kg of caffeine, short-term recipients of 50 mg/kg of caffeine, and blank control group.

Caffeine Accelerates the Time of Emerging from Isofluorane Anesthesia

In order to investigate the most effective and safe dose of caffeine, mice were divided into four groups and were gavaged with different doses of caffeine. The following graph (Figure 3) shows the time to emerge after isofluorine anesthesia for the four groups of mice: blank control, low dose, medium dose (50mg/kg), and high dose. There was a statistically significant difference in the emerging time of mice in the blank control group versus the high-dose group and the low-dose versus the high-dose group. Caffeine has been shown to accelerate the time of emerging from isofluorine anesthesia.

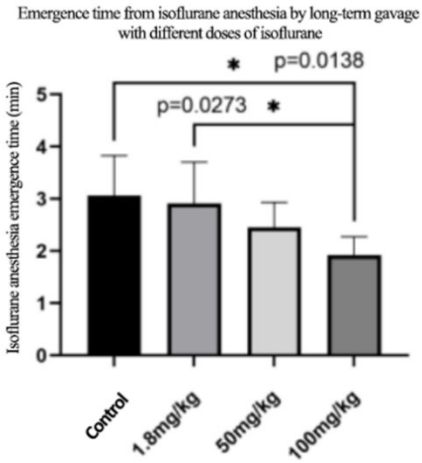

Figure 3. Time to emerging after the mice were removed from the anesthetized bellows

The graph below (Figure 4) shows the percentage change in mice's mean isofluorine anesthesia emerging time at different caffeine doses compared to the blank control group. The graph shows a statistically significant change in emerging time in the high-dose group compared to the blank control group. In the low dose group, the association of change between unit dose and anesthesia emerging time reached its maximum.

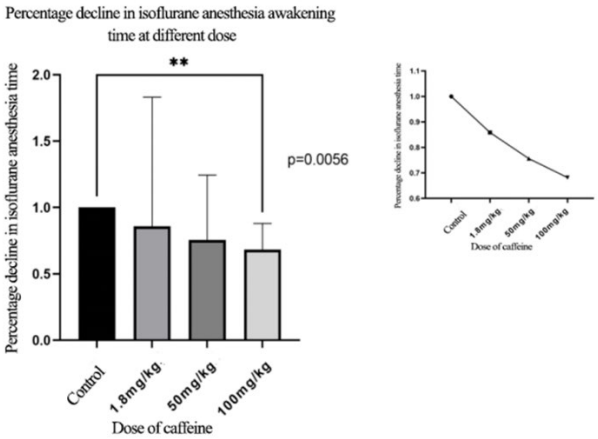

Figure 4. Comparison of the rate of change of isoflurane anesthesia emerging time in high dose, medium dose, and low dose mice.

Caffeine Accelerates the Time of Emerging from Zoletil 50 Anesthesia

To further validate the effect of caffeine on the emerging time of long-term anesthetics, mice were injected intramuscularly with the long-term anesthetic Zoletil 50. The graph below (Figure 5) shows the emerging time in Zoletil 50 for the control variable group, the low-dose group versus the high-dose group. There was a significant difference in the emerging time between the blank control and high-dose groups, and the results were statistically significant.

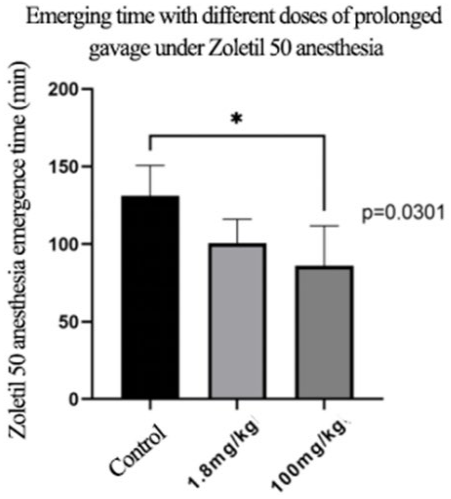

Figure 5. Time from entry into anesthesia to emerging in mice

Oral Caffeine Does Not Affect the Time to Enter Zoletil 50 Anesthesia

The clinical expectation of anesthesia is that the anesthetized person will enter anesthesia as soon as possible. An experiment to investigate whether long-term oral caffeine administration leads to an increase in time to anesthesia is also of clinical importance. The graph below (Figure 6) shows the monitoring of the time to enter anesthesia in this experiment for the blank control group, the low dose group, and the high dose group, and found that the difference in time to enter anesthesia with Zoletil 50 was not statistically significant between the groups.

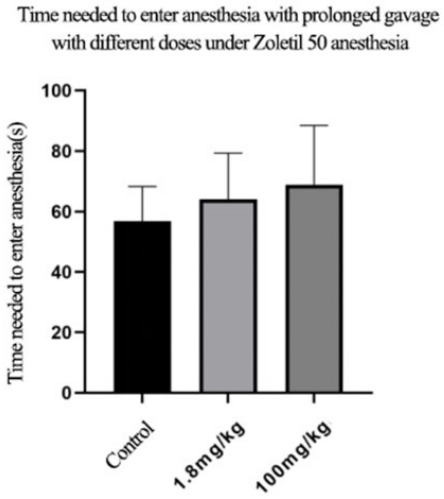

Figure 6. The time from when the mice were injected with anesthesia to when they entered anesthesia was recorded.

Oral Caffeine Accelerates Recovery of Spatial Exploration After Anesthesia

Along with the increasing use of anesthesia in surgery, accelerating the neurological recovery time after anesthesia is an important issue nowadays.

The graph below (Figure 7) shows the likelihood of finding a station in the visual station experiment at a fixed time after the mice in the blank control, low-dose, and high-dose groups were placed in the water maze 24 hours after emerging from anesthesia. There is a statistically significant difference in the likelihood of finding the station between the blank control group, the low-dose group, and the high-dose group in both the short and long term.

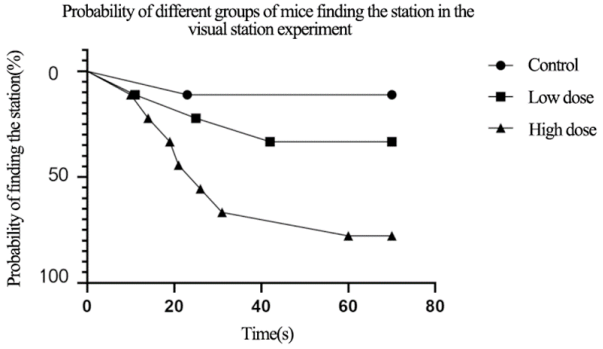

Figure 7. The time between entering the water maze and climbing onto the platform in the visual platform experiment and analyzes the likelihood of finding the platform at different points in time for each group of mice. The Morris water maze experiment utilizes the mice's instinct to search for resting platforms in order to measure the mice's spatial exploration and learning ability. The graph shows the likelihood of mice finding a rest stop during the first set of experiments, i.e., the visual rest stop experiment, which was shown to be statistically significant by the log-rank test ($p<0.01$).

The graph below (Figure 8) shows the likelihood of finding a station at a fixed time in the hidden station experiment between the blank control, low-dose, and high-dose groups of mice placed in the water maze 24 hours after emerging from anesthesia. There is a statistically significant difference in the likelihood of finding the station between the blank control group, the low-dose group, and the high-dose group in both the short and long term.

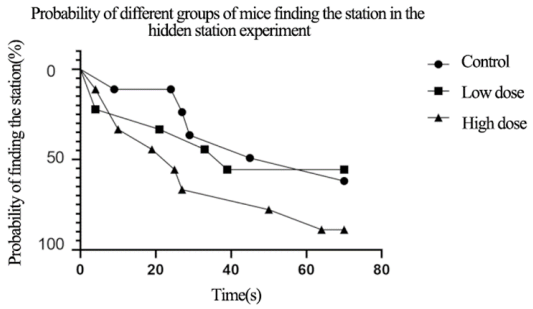

Figure 8. The time between mice entering the water maze and climbing onto the platform in the hidden platform experiment, analyzing the likelihood that each group of mice will find the platform at different points in time. The Morris water maze experiment utilizes the mice's instinct to search for resting platforms to measure their spatial exploration and learning ability. In contrast, the hidden platform experiment takes advantage of the fact that rodents do not usually find hidden platforms under the water's surface when they first swim—invisible stations. The graph shows the likelihood of finding a station when mice performed the first set of experiments, i.e., the visible station experiment, which was proved statistically significant ($p<0.05$) by the log-rank test.

The following graphs (Figures. 9a, 9b, 9c) show the swimming paths of mice in the visible station experiment versus the concealed station experiment. As the dose rises, the mice's path of action becomes simpler, with a gradual decrease in the distance traveled. In the blank control group, the mice found the station only once in 9 experiments, the mice in the low dose group could find the station 5 times in 9 experiments, and the high dose group found the station 7 times in 9 experiments.

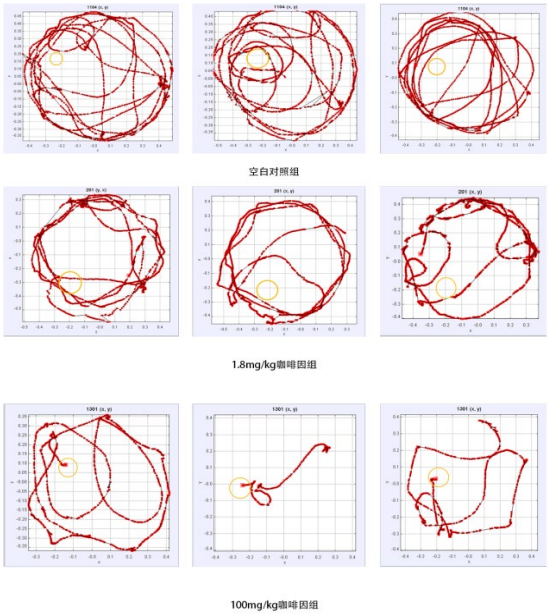

Figure 9a. Walking paths of each group of mice in the visual platform experiment (yellow circles are where the platforms are located)

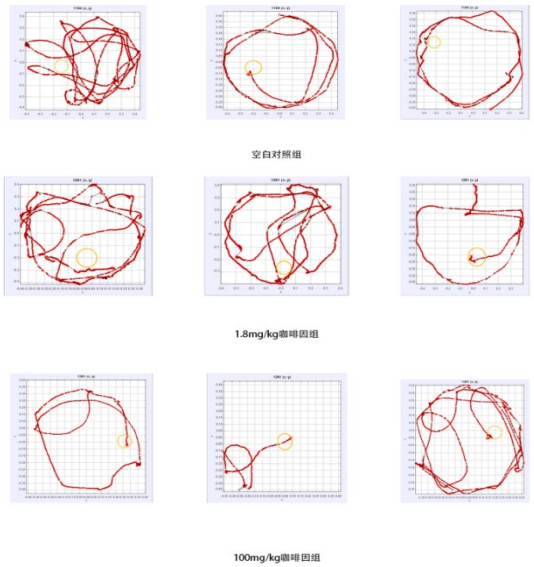

Figure 9b. Walking paths of mice in the hidden platform experiment (yellow circles are where the platforms are located).

Figure 9c. Mouse 1104 in a water maze with geometric markings for visual station experiments

Oral caffeine accelerates the recovery of spatial memory capacity after anesthesia.

This study conducted spatial exploration experiments for anesthetized mice, in which mice were placed in the Morris water maze. At the same time, the station was removed, and the paths of mice were observed in a fixed period to analyze their spatial localization ability and the pattern of change in the spatial exploration process.

The following figures (Figures 10a, 10b, 10c) show the action paths of mice in each group in the water maze during the spatial exploration experiment. Compared with the blank control mice and the low-dose mice, the mice in the high-dose group focused their actions more on the area where the original platform was located. In contrast, the mice in the blank control and low-dose groups crossed the area where the platform was located three times on average, with stagnation (the blue circle area). The mice in the high-dose group crossed the area where the platform was located six times without stagnation.

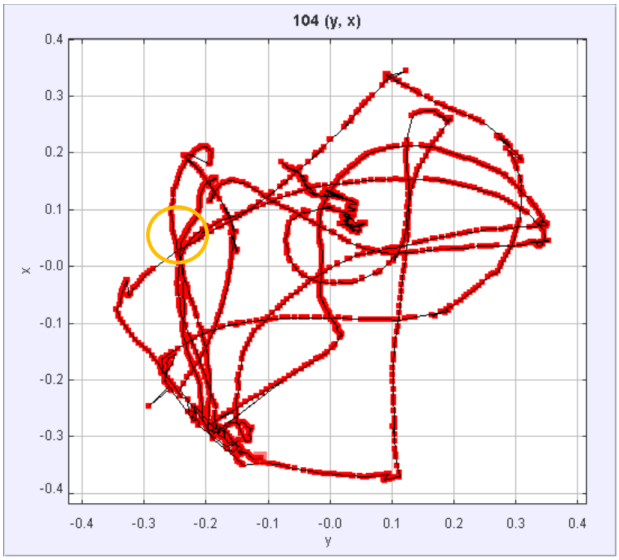

Figure 10a. Paths of blank control group mice in space exploration experiments (yellow circles show where

stations are located)

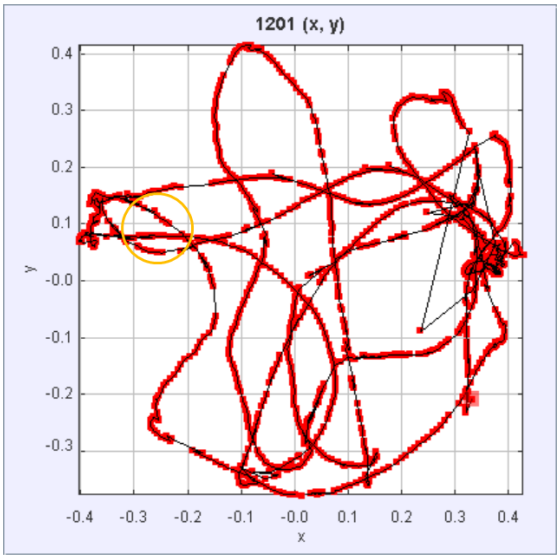

Figure 10b. Paths of mice in the low-dose group in the space exploration experiment (yellow circle shows where the station is located)

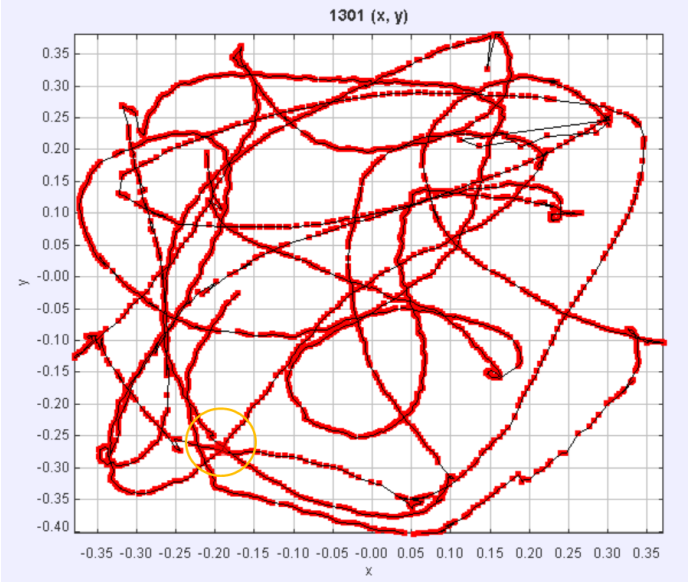

Figure 10c. Paths of mice in the high-dose group in the space exploration experiment (yellow circle is where the station is located)

Conclusion

Anesthesiology now supports patients throughout the preoperative preparation and postoperative period, in addition to during surgery. Improving the quality of recovery and avoiding neurocognitive side effects are becoming new goals for anesthesiologists. Among them, ensuring a controlled anesthetic emerging time or shortening the postoperative emerging time is a significant topic. With the development of surgical techniques, the targets of

surgical and anesthesia services have become more diversified, and the need for this technique is most obvious in young children who are vulnerable to anesthesia. Coffee is a common food ingredient that only produces a few side effects. International experiments have previously demonstrated that intravenous caffeine intake has the most significant per-unit change in anesthesia emerging time. Young children belong to a hyperactive group, and there are obvious limitations to this type of manipulation due to the fact that the veins are not visible at a young age. The present study is the first to investigate the effect of prolonged oral administration of coffee on anesthesia emerging time, avoiding the need for intravenous administration, to improve anesthesia emerging time and comfort in a group of young children.

This study investigated the effects of long-term oral intake of different doses of caffeine at the time of emerging from anesthesia and on the spatial exploration ability and spatial learning ability of mice after emerging. The anesthetics used in this study were isofluorine, a short-duration inhalational anesthetic, which is widely used in clinical practice as a common inhalational anesthetic, and sulphadoxine 50, a long-duration intramuscular anesthetic, which is also widely used in clinical practice and experimental practice. The study of these two anesthetics is of high clinical significance.

In this study, the effect of long-term and short-term caffeine intake on the duration of anesthesia was investigated. Long-term caffeine intake had a significant effect on the time to awaken from anesthesia. Caffeine, a widely available ingredient in modern popular beverages, should be considered in the targeted dosing of anesthetized patients. Asking anesthetized patients before anesthesia whether they have consumed coffee or other caffeinated beverages over a long period is beneficial to the anesthesiologist in selecting the most appropriate anesthetic dosage for the patient to enhance the anesthetic experience.

This study was designed to focus on a young child population, and therefore, oral administration was taken into consideration. It was demonstrated that even a low dose of long-term oral caffeine could significantly reduce the time to emerge from anesthesia, and the low-dose group was set at 1.8 mg/kg, which is a very safe dose even for young children. The low dose of caffeine also had a significant effect on the time to emerge from anesthesia. Other studies have also demonstrated that intravenous caffeine at 0.9 mg/kg can affect emerging from anesthesia [3]. This experiment focused on anesthesia in a group of young children. It yielded the result that oral administration of a low dose of caffeine could accelerate the time to awaken from anesthesia.

In the present study, the Morris water maze experiment was conducted 24 hours after the anesthesia experiment to assess mice's spatial exploration ability and spatial memory ability. It was demonstrated that long-term caffeine intake also reduced the time to recover spatial exploration and spatial memory after anesthesia.

The recovery period after emerging from anesthesia is equally essential, and ingesting caffeine prior to anesthesia and thereby reducing the duration of the recovery period from anesthesia is also clinically relevant.

Challenges and Prospects

Coffee is the world's most popular beverage and an essential stimulant for modern life. Internationally, several studies have demonstrated that caffeine can lead to shorter emerging time in general anesthesia. In the present study, we investigated the association between long-term coffee intake and the emerging time of general anesthesia. In future experiments, we expect to examine the blood oxygen concentration of mice emerging from anesthesia to understand further why caffeine leads to a shorter emerging time from anesthesia. Meanwhile, 48 hours had passed since the last gavage when the Morris water maze experiment was performed. The half-life of caffeine in the body is usually between 2.5 and 4.5 hours, whereas in newborns, the half-life can be up to 80 hours due to limited metabolism. At the same time, however, caffeine still affects recovery from anesthesia and the recovery time of spatial exploration and spatial memory. We want to observe the rate of caffeine metabolism in the blood as a way to gain more insight into why caffeine speeds up recovery time after anesthesia.

Acknowledgment

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