

Isoflurane Induces Transient Impairment of Retention of Spatial Working Memory in Rats

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Postoperative cognitive dysfunction (POCD) occurs in nearly one-third of patients after non-cardiac surgery. Many animal behavior studies have investigated the effect of general anesthesia on cognitive function. However, there have been no studies examining the effects on working memory specifically, with a focus on the retention of working memory. We demonstrate here that isoflurane anesthesia induces deficits in the retention of spatial working memory in rats, as revealed by an increase in isoflurane-induced across-phase errors in the delayed spatial win-shift (SWSH) task with a 30-min delay in an 8-arm radial arm maze on post-anesthesia days (PADs) 1, 2, 4, and 10. A post-hoc analysis revealed a significant increase in across-phase errors on PAD 1 and recovery on PAD 10 in the isoflurane group. In contrast, within-phase errors independent of the retention of working memory were unaffected by isoflurane. These results demonstrate that isoflurane anesthesia transiently impairs the retention of spatial working memory in rats.

Key words: postoperative cognitive dysfunction, isoflurane, spatial working memory, retention, delayed spatial win-shift task

Postoperative cognitive dysfunction (POCD) refers to problems in memory and thought processes after surgery [1]. Several human clinical studies have reported POCD as one of the major complications after surgery [2-4]. More than 30% of patients suffer from POCD within several days after a major noncardiac surgery, although most patients recover within a few months [4]. Anesthesia is suspected to be one of the main causes of POCD; however, it is difficult in clinical studies to differentiate between the effects of

general anesthesia and those of surgery and hospitalization [5]. It is important to establish animal models for evaluating the effect of anesthetics on cognitive function. Culley *et al.* performed many studies that evaluated learning and memory after general anesthesia in rats [5-9]. However, none of these investigated the effect of general anesthesia on working memory specifically, with a focus on the retention of working memory.

It is generally thought that memory, including working memory, consists of 3 processes: encoding,

retention, and recall. The delayed spatial win-shift (SWSh) task has been used to evaluate each of these processes of working memory [10–13]. This task is characterized by 2 phases, a training phase and a test phase, which are separated by a delay. In the delayed SWSh task, rats acquire information on maze arms that were or were not visited during the training phase, retain that information during the delay period, and then recall it during the test phase [10] (Fig. 1). Nagai *et al.* analyzed across-phase error separately from within-phase error in the delayed SWSh task and reported that extracellular signal-regulated kinase 1/2 phosphorylation may play a role in the retention of working memory [11]. Therefore, the delayed SWSh task may be appropriate to assess the retention of spatial working memory in the radial arm maze [11].

In the present study, we tested the hypothesis that general anesthesia by inhalation induces a deficit in the

retention of working memory during the post-anesthesia period using the delayed SWSh task in an 8-arm radial arm maze in rats 10 days after isoflurane anesthesia.

Materials and Methods

Animals. All experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Animal Research Control Committee of Okayama University Medical School.

Male Wistar rats ($n=36$), 10 weeks old and weighing 230 to 250 g at the beginning of the experiments, were obtained from Japan SLC (Shizuoka, Japan). The animals were housed 3 per cage with free access to food and water and in a regulated environment ($23^{\circ}\text{C} \pm 1^{\circ}\text{C}$, $60\% \pm 15\%$ humidity) on a 12:12-h light/dark cycle (with lights on at 8:00 a.m.).

Apparatus. Behavioral training and testing were carried out on an elevated 8-arm, acrylic plastic radial maze (Bio Research Center, Nagoya, Japan) on a central platform in a sound-proof testing room illuminated at 35 lux. The floor of the central platform and the arms were gray in color, and each arm contained a food cup 5 mm deep and 3 cm in diameter. The cups were glued to the surfaces of the arms approximately 1 cm from the end of the arm. The entrance to the arms could be blocked by guillotine doors 24.5 cm high and 11 cm wide. The central platform was 30 cm in diameter, and the arms were 50 cm long and 11 cm wide, with a 24.5 cm high transparent wall around the apparatus. The maze was elevated 43 cm from the floor level. The apparatus was surrounded by various extra-maze cues including a screen, a brightly colored poster, and a clock in the testing room.

Delayed SWSh task. The delayed SWSh task is comprised of 3 processes: the training phase, delay period, and test phase. In this task, rats acquire information on arms that were or were not visited during the training phase, retain that information during the delay period, and recall it during the test phase (Fig. 1), which corresponds to the memory stages of encoding, retention, and recall, respectively. This task is therefore appropriate for identifying the specific memory stage that is impaired by drugs [10].

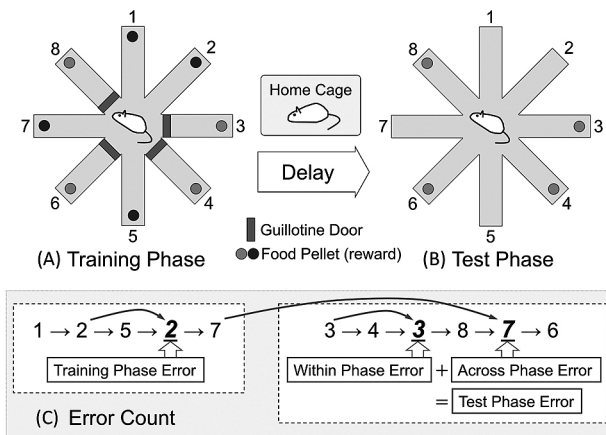


Fig. 1 Schema of the delayed SWSh task. *Task preparation:* A pellet was placed in each arm of the maze. Guillotine doors were inserted into 4 of the 8 arms (nos. 3, 4, 6, and 8) chosen at random. *Training phase (A):* The rat was allowed to enter 4 arms (nos. 1, 2, 5, and 7) and eat the pellets. The phase ended when the rat had consumed all of the pellets or 5 min had elapsed. *Delay period* (learning period: 5 min; assessment period: 30 min): The rat was removed from the maze and returned to its home cage. The 4 guillotine doors were removed. *Test phase (B):* The rat was free to enter all 8 arms, but had to consume all 4 pellets (arm nos. 3, 4, 6, and 8). The phase ended when the rat had consumed the 4 pellets or 5 min has elapsed. *Error count (C):* Entry into an arm without a pellet was counted as an error [training-phase errors; e.g., 1 → 2 → 5 → 2 → 7 (left)]. In the test phase, a within-phase error was defined as re-entry into an arm that had been previously visited in that phase, and an across-phase error was defined as entry into the four arms that were open during the training phase [e.g., 3 → 4 → 3 → 8 → 7 → 6 (right)].

The procedure for the delayed SWSH task in the present study has been previously described (Fig. 1) [11,14]. In the training phase, 45 mg of Precision Pellets (Bioserv, Frenchtown, NJ, USA) were placed in the food cups of all 8 arms, and 4 of the 8 arms were randomly blocked by guillotine doors. This setup was changed for each training phase. At the start of each phase, the rat was placed on the central platform and allowed to explore the maze to retrieve the pellets from the 4 open arms or until 5 min had elapsed, whichever was sooner. After the training phase, the rat was returned to its home cage for the delay period. In the test phase, all arms were opened, but food pellets were placed only in the arms that were blocked during the training phase. The rat was required to choose 4 of the 8 available arms to find a reward in each of the four arms that it had not previously visited during the training phase. The test phase ended when each rat had eaten the 4 food pellets or 5 min had elapsed.

An arm entry was recorded once the rat had entered the arm. An entry to any arm that contained no food pellet was regarded as an error (Fig. 1). The number of errors made during the training and test phases (training- and test-phase errors, respectively) and the response time (defined as time taken to complete the training phase/number of choices made in the training phase) were recorded. Test-phase errors were further divided into across- and within-phase errors. The former was defined as an entry into the 4 open arms during the training phase, and the latter was defined as re-entry into any arm that had been previously visited in the test phase.

Preparation of animals (learning of delayed SWSH task).

The experimental time line is shown in Fig. 2. In this experiment, we evaluated working memory after anesthesia only in rats that met the acquisition criterion for the delayed SWSH task through the learning period. For 7 days before the start of training on the maze, rats were subjected to a food restriction regime that reduced the body weight to approximately 85% of the initial value and then were maintained on a restricted diet during the experiment. Water was provided ad libitum. During the learning period, rats were allowed 2 trials of the 5-min delayed SWSH task per day for the first 5 days and one trial per day thereafter. The task continued until the rat met the acquisition criterion or had undergone a maximum of 55 trials. The criterion was a test-phase error score of 1 or less per trial for 5 consecutive trials, which allowed rats to master the task to standardize their performance level before anesthesia.

Anesthesia. The 14 of 36 rats that met the acquisition criterion of the 5-min delayed SWSH task were assigned to either the isoflurane or control group ($n=7$ each) (Fig. 2). Rats in the isoflurane group were placed in an anesthetic chamber and primed with 50% oxygen-nitrogen containing 3% isoflurane for 5 min. Anesthesia was subsequently maintained with 1.5% isoflurane. The vehicle gas flow was 4 l/min. Gas composition within the anesthetic chamber was measured using a calibrated Datex Capnomac Ultima instrument (Datex Instrumentarium Corp., Helsinki, Finland). During anesthesia, a pulse oximetry sensor (MLT324 Tail Wrap; ADInstruments, Dunedin, New

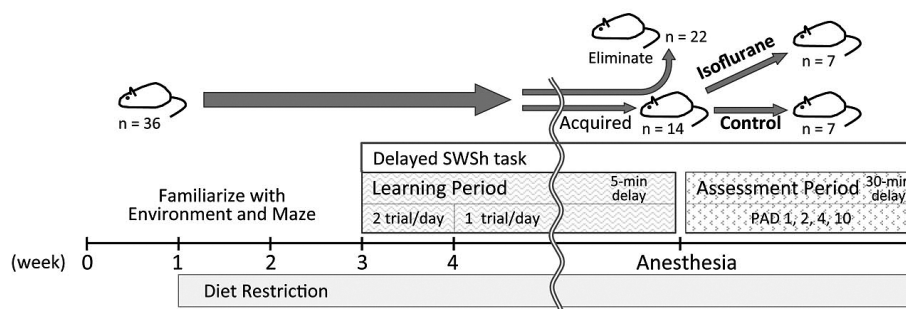


Fig. 2 Experimental timeline for the delayed SWSH task. Rats were subjected to dietary restriction for 1 week prior to and throughout the duration of the experiment. After adapting to the environment and maze, rats were trained for the 5-min delayed SWSH task until they met the acquisition criterion (cutoff: 55 trials; learning period). Only rats that met this criterion were used and were assigned to the isoflurane or control (vehicle gas) group. Rats were subjected to a 30-min delayed SWSH task 1, 2, 4, and 10 days after anesthesia (assessment period).

Zealand) was attached to the tail and a custom-made temperature probe was inserted into the rectum. These were connected to a data acquisition system (PowerLab, ADInstruments, Dunedin, New Zealand) in order to monitor the rats during anesthesia. Normothermia ($37.0^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$) was maintained using warming pads under the chamber and with heating lamps. After 4 h of isoflurane exposure, rats were allowed to recover for 30 min in a chamber with identical vehicle gas flow containing no anesthesia before they were returned to their home cage. Rats in the control group were placed in the anesthesia chamber with identical vehicle gas flow but without the anesthetic for the same time duration (4 h and 30 min).

Assessment of working memory with the delayed SWSH task. Rats in the isoflurane and control groups were subjected to the 30-min delayed SWSH task on post-anesthesia days (PADs) 1, 2, 4, and 10 (assessment period) (Fig. 2).

Working memory was evaluated using the training-, within-, and across-phase errors (Fig. 1).

Motor function and decision speed after anesthesia were assessed by calculating the response time (RT) ratio, obtained by dividing RT by the average of five RTs until the acquisition criterion.

Rats were handled by the same individual to maintain consistency in animal handling and procedures throughout this study.

Statistical analysis. All values are expressed as the mean \pm standard error (SEM). Parameters for the learning period of the delayed SWSH task were analyzed using the Student's *t*-test. Parameters for the assessment period of the delayed SWSH task were analyzed by repeated measures two-way analysis of variance with a Bonferroni post-hoc multiple-comparison correction. Statistical testing was carried out with GraphPad Prism v. 6.0 (Graph Pad Software, San Diego, CA, USA). In all statistical tests, a *p* value < 0.05 was considered significant.

Results

Learning period followed by anesthesia. Of the 36 rats, 14 met the acquisition criterion of the 5-min delayed SWSH task through the learning period with 34.9 ± 5.1 trials. These rats were assigned to the isoflurane or control group ($n = 7$ each). Rats in the isoflurane group were 20 ± 0.9 weeks old and those in

the control group were 19 ± 1.2 weeks old when they were administered anesthesia. There were no differences between the 2 groups with respect to age, body weight, and number of trials during the learning period (data not shown).

Spatial working memory after anesthesia.

We compared training- and within-phase errors (in the test phase) in the delayed SWSH task with a 30-min delay between the isoflurane and control groups (Fig. 3A-C). Rats in both groups committed almost no training-phase errors ($F_{1,12} = 0.75$, $p = 0.40$; Fig. 3A) or within-phase errors ($F_{1,12} = 1.13$, $p = 0.31$; Fig. 3B) during the entire assessment period. In contrast, with respect to the number of across-phase errors, a two-way analysis of variance showed a significant effect of treatment (isoflurane vs. control) ($F_{1,12} = 6.90$, $p = 0.02$). However, there was no effect of PADs ($F_{3,36} = 0.40$, $p = 0.75$) and no significant interaction ($F_{3,36} = 1.98$, $p = 0.13$) (Fig. 3C). On PAD 1, rats in the isoflurane group committed 2.6 ± 0.4 errors, whereas those in the control group made 0.7 ± 0.4 errors; a post-hoc test showed a significant difference between the 2 groups ($p = 0.05$; Fig. 3C). On PADs 2 and 4, the number of errors in the isoflurane group was greater than that in the control group, although the difference was not significant. On PAD 10, rats in the 2 groups made almost the same number of errors (isoflurane: 1.1 ± 0.5 ; control: 1.3 ± 0.5 ; Fig. 3C).

In regard to motor and decision performance during the delayed SWSH task through the assessment period, the RT ratio was stable and the value was close to 1 over the entire test period in both groups ($F_{1,12} = 0.06$, $p = 0.81$; Fig. 3D). This indicates that isoflurane anesthesia did not affect motor function or decision speed.

Discussion

In the present study, we observed that isoflurane anesthesia significantly increased across-phase, but not training- or within-phase, errors in the delayed SWSH task. If the encoding and/or recall processes of working memory were impaired by isoflurane, then we would expect to find increased training-phase and/or within-phase errors. On the other hand, rats had to retain the information acquired in the training phase for 30 min during the delay in order to have a low

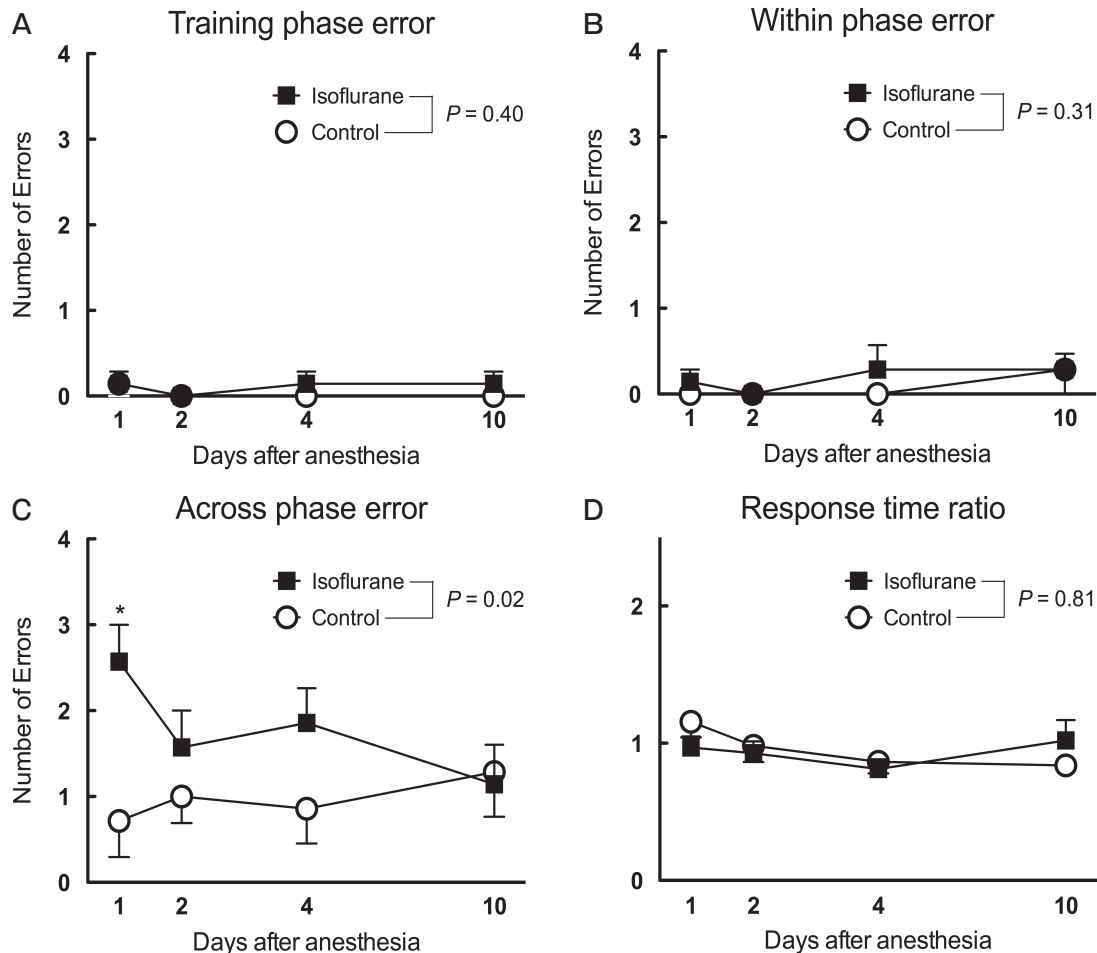


Fig. 3 Effect of isoflurane on memory performance in the delayed SWSH task. Rats were tested 1, 2, 4, and 10 days after anesthesia. Isoflurane = isoflurane (1.5%, 4 h; $n = 7$); control = vehicle gas (50% O₂, 50% N₂, 4 h; $n = 7$). **A**, Number of training-phase errors; **B**, Number of within-phase errors during the test phase; **C**, Number of across-phase errors in the test phase. Rats in the isoflurane group committed more errors than controls during the experimental period ($p = 0.02$, analysis of variance); the number of errors was significantly higher in the isoflurane than in the control group on PAD 1 ($*p = 0.05$, post-hoc comparison); **D**, The response time ratio was similar between groups during the training phase. Values represent the mean \pm SEM.

number of across-phase errors. Therefore, our results showing increased across-phase errors suggest that isoflurane impairs the retention of spatial working memory. In addition, the across-phase errors increased on PAD 1, and then gradually decreased over the next 10 days. Hence, we speculate that the working memory impairments induced by isoflurane are transient.

We used the delayed SWSH task in the present study, as this radial arm maze task is recommended for assessing the time-dependent spatial working memory [11]. This task, with its interposed delay, makes it possible to evaluate the errors committed across the 2 phases, which are separate from the errors commit-

ted within each phase. Prior studies conducted with this task showed that methamphetamine [13] and ketamine [15] do not affect the number of errors committed within a phase but do increase the number of across-phase errors. Culley *et al.* used a delay-interposed radial arm maze task to investigate the effects of general anesthesia on working memory [5]. However, when they analyzed the number of errors committed across the 2 phases, the number was not distinguishable from the number of within-phase errors. Culley *et al.* also published other studies on the effects of general anesthesia on spatial working memory by using the standard radial arm maze task [6–9]. In the

radial arm maze task, rodents, including rats, sometimes prefer to enter adjacent arms in order, which can lead to reduced errors. In such cases, working memory may not be evaluated appropriately. In the test phase of the delayed SWSH task used in the present study, this was not considered an acceptable way to reduce errors. Thus, we evaluated the effects of general anesthesia on working memory in more detail compared to previous studies. Importantly, our findings implied that time-dependent spatial working memory was impaired by isoflurane anesthesia.

In this study, we showed that a deficit in spatial working memory after isoflurane anesthesia was apparent on PAD 1, but improved by the end of the 10-day assessment period. Consistent with these results, Zurek *et al.* reported that recognition memory—that is, non-spatial memory—was impaired by isoflurane on PAD 1 but recovered on PAD 3, as shown by the results of a novel object recognition task in mice [16]. These findings strongly suggest that the impairment of memory induced by isoflurane is reversible.

There were some limitations to this study. First, 22 of the 36 rats initially entered into this study were subsequently excluded during the learning period. We set a strict criterion for performing the delayed SWSH task in order to evaluate the effects, which we assumed would be small, of isoflurane on working memory. Unfortunately, the 22 rats that did not reach the criterion had to be eliminated because the number of their test-phase errors did not decrease, although the training for learning the task was continued. Secondly, the anesthetic was administered under a single condition (1.5% isoflurane for 4 h). Other agents, doses, or durations of anesthesia may lead to different outcomes. Therefore, further investigation is required to evaluate the effect of general anesthesia on working memory.

In conclusion, we have demonstrated that 4 h of isoflurane exposure reversibly impairs the retention of spatial working memory in rats. Thus, impairment of memory retention may contribute to the reversible cognitive dysfunction induced by general anesthesia.

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