

# Facilitation of Serotonergic Activity and Amnesia in Rats Caused by Intravenous Anesthetics

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**Background:** Midazolam and propofol often provoke retrograde amnesia after recovery from anesthesia in humans. Because an increase in central serotonergic activity impairs learning and memory, the authors examined the relation between changes in the serotonergic activity caused by intravenous anesthetics and memory.

**Methods:** Changes in extracellular concentrations of monoamines and their metabolites were investigated in rat striatum by a microdialysis procedure, and the effects of intraperitoneal injections of midazolam (5 mg/kg), propofol (60 mg/kg), and pentobarbital (15 mg/kg) were then examined. To evaluate the behavioral alteration with these agents, the authors used a step-through passive avoidance test.

**Results:** Midazolam and propofol slightly increased the extracellular concentration of 5-hydroxytryptamine in the striatum, although pentobarbital did not produce any changes. Midazolam and propofol increased the extracellular concentration of 5-hydroxyindoleacetic acid, a metabolite of 5-hydroxytryptamine, with the peak values each 138% and 138% of that in saline-injected animals, respectively. However, pentobarbital decreased the 5-hydroxyindoleacetic acid concentration to 61% of that in the saline group. Administration of midazolam or propofol immediately after the completing the passive avoidance learning reduced step-through latencies after 24 h, although pentobarbital-injected animals maintained a consistent performance. The effects of midazolam and propofol on step-through latencies were completely antagonized by intracerebroventricular administration of spiroxatrine (5 µg), a 5-hydroxytryptamine 1A antagonist, 30 min before training.

**Conclusions:** Midazolam and propofol increased central serotonergic activity and provoked retrograde amnesia. Because amnesia was completely diminished by a 5-hydroxytryptamine antagonist, facilitation of the serotonergic system may be involved in retrograde amnesia caused by these agents.

MIDAZOLAM (a benzodiazepine), propofol (a diisopropylphenol), and pentobarbital (a barbiturate) are extensively used as hypnotics and anesthetics in clinical situations. Midazolam and propofol have been reported to impair memory retention at sedative doses in human studies, and retrograde amnesia after recovery from anesthesia with anesthetic doses of midazolam and propofol is often observed in humans, whereas thiopental does not provoke amnesia.<sup>1,2</sup>

5-Hydroxytryptamine (5-HT) and dopamine are biogenic amines that are involved in a number of physio-

logic and pathophysiologic processes. Increases in serotonergic activity in the brain have been shown to impair learning and memory.<sup>3-6</sup> Administration of 8-hydroxy-2-(di-n-propylamino) tetralin, a specific agonist of 5-HT<sub>1A</sub> receptors, has been shown to impair memory processes in rats,<sup>7-10</sup> with this impairment reversed by 5-HT<sub>1A</sub> antagonists.<sup>11-13</sup> Hence, it seems that 5-HT<sub>1A</sub> receptors have an important role in learning and memory. With respect to central dopamine, facilitation of dopaminergic activity has been shown to enhance memory retrieval.<sup>14-16</sup> In contrast, suppression of dopaminergic neurotransmission by dopamine receptor antagonists, such as chlorpromazine or haloperidol, impairs memory performance.<sup>17-21</sup>

The goal of this study was to examine changes in monoaminergic activity by intravenous anesthetics and to elucidate the relation between neurochemical changes and behavioral alterations.

## Materials and Methods

This study was approved by the Committee on Animal Experimentation at Ehime University School of Medicine, Ehime, Japan. Male Wistar rats weighing approximately 320 g (Charles River, Yokohama, Japan) were housed in a temperature-controlled room at 23 ± 2°C and maintained under an alternating 12-h light and 12-h dark cycle (lights on at 6:00 AM). Food and water were provided *ad libitum*. In experiment 1, 27 rats were used in the evaluation of monoamine release in the striatum by a microdialysis procedure. In experiment 2, 80 rats were used to examine the tissue contents of monoamines and their metabolites in the striatum, hippocampus, and cerebral cortex. In experiment 3, 32 rats were used with the passive avoidance test to examine memory retention. In experiment 4, 64 rats were used to assess the relation between serotonergic activity and behavioral alteration.

### Experiment 1

This experiment was designed to determine changes in the extracellular concentrations of monoamines and their metabolites in the striatum caused by midazolam, propofol, and pentobarbital. Rats were anesthetized with 2% halothane in a gas mixture of 50% oxygen and 50% nitrous oxide, and they breathed spontaneously. After the animal was placed in a stereotaxic apparatus (Narishige Scientific, Tokyo, Japan) in the prone position, the skull was exposed and a small burr hole was drilled in the right hemisphere (0.3 mm posterior and 4.0 mm lateral to the bregma) for insertion of a micro-

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dialysis probe. An I-shaped microdialysis probe (A-I-8-01; Eicom, Kyoto, Japan) was inserted into the right striatum through the burr hole, and its tip was positioned 7.0 mm below the skull surface. The probe was fixed to the skull with dental cement and glue using a stainless-steel screw. Then, polyethylene tubing was placed in the intraperitoneal space, through which drugs were administered. The animal was allowed to recover from anesthesia.

Twelve hours after surgery, the microdialysis probe was perfused with Ringer's solution (147 mM Na<sup>+</sup>, 4 mM K<sup>+</sup>, 2 mM Ca<sup>2+</sup>, 155 mM Cl<sup>-</sup>) at a rate of 2  $\mu$ l/min. During the experimental period, the rat was allowed to move freely in a box. After a 30-min stabilization period, brain perfusates were collected every 20 min into microtubes in ice and stored at -80°C until analysis. After two control samples were collected, animals were intraperitoneally injected with saline (0.7 ml/kg), midazolam (5 mg/kg), propofol (60 mg/kg), or pentobarbital (15 mg/kg) through the polyethylene tubing. Two minutes after the injection, an initial sample was taken, followed by nine later samples taken every 20 min until 200 min after injection. The duration between loss of the righting reflex and its recovery was defined as the duration of anesthesia. During anesthesia, the rectal temperature was maintained at 37.5  $\pm$  0.2°C with a heating lamp.

The concentrations of dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-HT, and 5-hydroxyindoleacetic acid (5-HIAA) in dialysates were determined using a high-performance liquid chromatography system with electrochemical detection (Eicom) according to a method produced by Magnusson *et al.*,<sup>22</sup> with slight modification. The detection limits of the system on DOPAC, dopamine, 5-HIAA, HVA, and 5-HT were 4, 2, 6, 5, and 5 pg, respectively.

### Experiment 2

This experiment was designed to examine changes in tissue contents of monoamines and their metabolites caused by midazolam, propofol, and pentobarbital in the striatum, hippocampus, and cerebral cortex. The animals were intraperitoneally injected with midazolam (0.5, 2.5, or 5 mg/kg), propofol (6, 30, or 60 mg/kg), or pentobarbital (1.5, 7.5, or 15 mg/kg). Thirty minutes after the drug injection, the rat was decapitated and the brain was rapidly removed, rinsed in saline, placed on ice, and dissected into the following three regions: hippocampus, striatum, and cerebral cortex. First, the whole hippocampus and striatum on both sides were dissected. Then, the brain was cut along the coronal planes at the optic chiasma and the caudal edge of the mammillary body. The dorsal portions of the cerebral cortex on both sides were cut from the sulcus rhinalis between these cut planes.

Each tissue sample was homogenized in 1 ml perchloric acid, 0.4 M, containing 0.1% L-cysteine. After being centrifuged, the supernatant was applied to a high-per-

formance liquid chromatography system with electrochemical detection (Eicom) to determine concentrations of dopamine, DOPAC, HVA, 5-HT, and 5-HIAA.

### Experiment 3

We used a step-through passive avoidance test according to methods by Jarvik and Kopp,<sup>23</sup> with slight modification, to determine effects of midazolam, propofol, and pentobarbital on memory retention. The step-through passive avoidance chamber, made of gray acrylic board, was separated into bright and dark compartments by a guillotine door. The dimensions of the bright box were 15  $\times$  20  $\times$  30 cm, and those of the dark box were 30  $\times$  30  $\times$  30 cm. The light box was illuminated by an overhead lamp with a 100-W white light bulb. Electric shocks could be provided to the grid floor in the dark box. To initiate training, the rat was placed in the bright box, and 60 s later, the guillotine door was opened. Almost all rats usually enter the dark box, because rats are nocturnal. The rat's latency to enter the dark box was measured. When the rat stepped through, defined as all four paws beyond the door, the guillotine door was closed, and three foot shocks (3 s, 0.5 mA, 10-s intervals) were provided. Immediately after the training, animals were intraperitoneally injected with saline (0.7 ml/kg), midazolam (5 mg/kg), propofol (60 mg/kg), or pentobarbital (15 mg/kg). On the test day, 24 h after training, the rat was again placed in the bright box, and the door was raised after 60 s. The rat's latency to enter the dark compartment was measured. When the rat did not step through in 300 s, the test was terminated. When memory of foot shock is not retained, the animal steps through as on the training day.

### Experiment 4

In this experiment, the effects of spiroxatrine, a specific antagonist of 5-HT<sub>1A</sub> receptors, on memory retention were examined in animals subjected to anesthesia with each agent. First, rats were anesthetized with 2% sevoflurane in a gas mixture of 50% oxygen and 50% nitrous oxide, with spontaneous breathing. After the animal was placed in a stereotaxic apparatus (Narishige Scientific) in the prone position, the skull was exposed, and a burr hole was drilled for drug administration (0.8 mm posterior and 1.5 mm lateral to the bregma). Either spiroxatrine (5  $\mu$ g) dissolved in 20  $\mu$ l dimethyl sulfoxide, 2%, or the vehicle (2% dimethyl sulfoxide) was administered into the lateral ventricle through the burr hole *via* a 27-gauge needle at a depth of 5 mm below the brain surface. Then, the surgical incision was sutured. Ten minutes after the drug injection, the animal was allowed to recover from anesthesia. Thirty minutes after cessation of sevoflurane inhalation, the training of passive avoidance test was performed in a manner identical to that in experiment 3. After the training, saline, midazolam (5 mg/kg), propofol (60 mg/kg), or pentobarbital

(15 mg/kg) was injected intraperitoneally. The rat's latency to enter the dark compartment was measured after 24 h.

### Statistical Analysis

The data from the microdialysis experiments were analyzed using repeated-measures two-way analysis of variance to detect differences among groups. When differences were found, the Bonferroni test was used *post hoc* to compare each fractional value with that of each corresponding time point. The data on brain monoamines and metabolites were evaluated with use of the Bonferroni test. The data obtained from behavioral experiments were evaluated with the Kruskal-Wallis test. When differences were found, the Mann-Whitney test was used *post hoc* to compare differences between two groups.

## Results

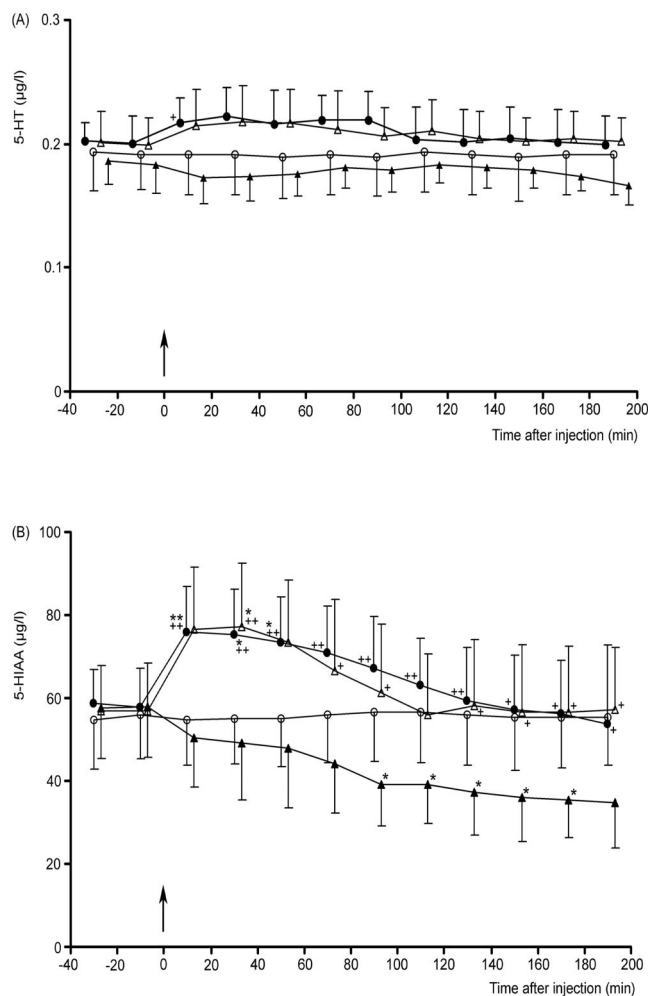
### Experiment 1

The righting reflex disappeared after  $9.4 \pm 3.5$  min (mean  $\pm$  SD,  $n = 9$ ),  $10.5 \pm 3.2$  min ( $n = 9$ ), and  $8.6 \pm 3.2$  min ( $n = 9$ ) after administration of midazolam, propofol, and pentobarbital, respectively. There was no difference among the groups. The durations of anesthesia were similar among the midazolam, propofol, and pentobarbital groups, with the values being  $25.2 \pm 2.5$ ,  $23.4 \pm 7.3$ , and  $23.4 \pm 3.8$  min, respectively.

The concentrations of monoamines and their metabolites in dialysates did not differ among the groups before drug administration. These concentrations in the saline group did not change during the experimental period.

Administration of pentobarbital did not alter the 5-HT concentration in dialysates (fig. 1A). In the midazolam and propofol groups, slight increases in the 5-HT concentration were observed immediately after administration, although there was no significant difference in the concentration between the saline and midazolam groups. The injections of midazolam and propofol produced marked increases in the 5-HIAA concentration immediately after administration (fig. 1B). The peak values of 5-HIAA in the midazolam and propofol groups were each 138% of that in the saline group. These 5-HIAA concentrations began to decrease 60 min after administration. The injection of pentobarbital markedly decreased the 5-HIAA concentration 80 min after administration, and the value in the pentobarbital group was lower than that in the saline group thereafter.

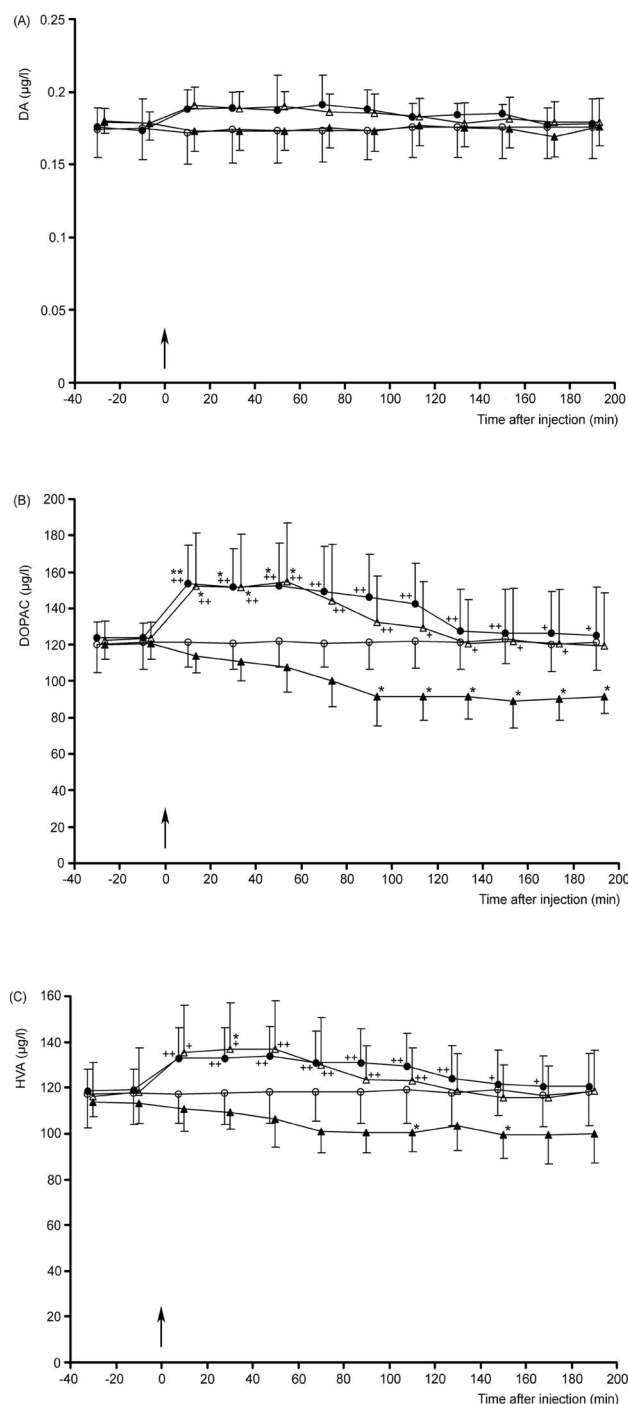
The concentration of dopamine in dialysates did not change in the saline and pentobarbital groups (fig. 2A). In the midazolam and propofol groups, slight increases in the dopamine concentration were observed immediately after administration, although these changes were



**Fig. 1.** Effects of intravenous anesthetics on the concentrations of 5-hydroxytryptamine (5-HT, A) and 5-hydroxyindoleacetic acid (5-HIAA, B) in dialysates from the striatum. Saline (0.7 ml/kg,  $\circ$ ), midazolam (5 mg/kg,  $\Delta$ ), propofol (60 mg/kg,  $\bullet$ ), and pentobarbital (15 mg/kg,  $\blacktriangle$ )-injected groups. Arrows represent administration of saline or intravenous anesthetics. Each value represents the mean  $\pm$  SD ( $n = 9$ ). Changes in 5-HIAA by intravenous anesthetics were significant:  $F = 4.682$ ,  $P = 0.008$  for the drug;  $F = 43.217$ ,  $P < 0.001$  for time;  $F = 14.136$ ,  $P < 0.001$  for the drug  $\times$  time interaction. \*  $P < 0.05$ , \*\*  $P < 0.01$  as compared with each corresponding value in the saline group. +  $P < 0.05$ , ++  $P < 0.01$  as compared with each corresponding value in the pentobarbital group.

not significant. The injections of midazolam and propofol produced increases in the DOPAC and HVA concentrations (figs. 2B and C). The values then returned to the basal concentrations 120 min after administration. The peak values of DOPAC concentrations in the midazolam and propofol groups were 126% and 127% of that in the saline group, respectively. The peak values of HVA concentrations were 113% and 117%, respectively. The injection of pentobarbital produced a marked decrease in the DOPAC and HVA concentrations, with the DOPAC concentration being significantly reduced 80 min after administration.





**Fig. 2.** Effects of intravenous anesthetics on the concentrations of dopamine (DA, *A*), 3,4-dihydroxyphenylacetic acid (DOPAC, *B*), and homovanillic acid (HVA, *C*) in dialysates from the striatum. Saline (0.7 ml/kg, ○), midazolam (5 mg/kg, Δ), propofol (60 mg/kg, ●), and pentobarbital (15 mg/kg, ▲)-injected groups. Arrows represent administration of saline or intravenous anesthetics. Each value represents the mean  $\pm$  SD ( $n = 9$ ). Changes in DOPAC by intravenous anesthetics were significant:  $F = 7.562$ ,  $P = 0.006$  for the drug;  $F = 29.416$ ,  $P < 0.001$  for time;  $F = 9.859$ ,  $P < 0.001$  for the drug  $\times$  time interaction. Changes in HVA by intravenous anesthetics were significant:  $F = 5.377$ ,  $P = 0.0041$  for the drug,  $F = 11.281$ ,  $P < 0.001$  for time,  $F = 4.6$ ,  $P < 0.001$  for the drug  $\times$  time interaction. \* $P < 0.05$ , \*\* $P < 0.01$  as compared with each corresponding value in the saline group. + $P < 0.05$ , ++ $P < 0.01$  as compared with each corresponding value in the pentobarbital group.

**Table 1.** Effects of Intravenous Anesthetics on Brain 5-HT and Metabolite\*

	5-HT	5-HIAA	5-HIAA/5-HT
<b>Cerebral cortex</b>			
Sal	149 $\pm$ 11	246 $\pm$ 18	1.650 $\pm$ 0.114
Mid (0.5 mg/kg)	152 $\pm$ 11	248 $\pm$ 23	1.636 $\pm$ 0.142
Mid (2.5 mg/kg)	156 $\pm$ 13	244 $\pm$ 21	1.575 $\pm$ 0.172
Mid (5 mg/kg)	152 $\pm$ 12	304 $\pm$ 33†	2.016 $\pm$ 0.207†
Pro (6 mg/kg)	149 $\pm$ 12	251 $\pm$ 29	1.699 $\pm$ 0.300
Pro (30 mg/kg)	155 $\pm$ 24	248 $\pm$ 17	1.626 $\pm$ 0.240
Pro (60 mg/kg)	149 $\pm$ 61	300 $\pm$ 10†	2.021 $\pm$ 0.114†
Pen (1.5 mg/kg)	150 $\pm$ 22	243 $\pm$ 22	1.625 $\pm$ 0.131
Pen (7.5 mg/kg)	152 $\pm$ 79	235 $\pm$ 31	1.549 $\pm$ 0.205
Pen (15 mg/kg)	159 $\pm$ 83	210 $\pm$ 34	1.316 $\pm$ 0.167‡
<b>Striatum</b>			
Sal	313 $\pm$ 17	558 $\pm$ 20	1.784 $\pm$ 0.107
Mid (0.5 mg/kg)	311 $\pm$ 10	549 $\pm$ 23	1.767 $\pm$ 0.108
Mid (2.5 mg/kg)	311 $\pm$ 25	551 $\pm$ 27	1.779 $\pm$ 0.113
Mid (5 mg/kg)	317 $\pm$ 21	641 $\pm$ 58†	2.021 $\pm$ 0.116†
Pro (6 mg/kg)	303 $\pm$ 17	554 $\pm$ 15	1.834 $\pm$ 0.139
Pro (30 mg/kg)	300 $\pm$ 18	551 $\pm$ 25	1.846 $\pm$ 0.187
Pro (60 mg/kg)	314 $\pm$ 11	671 $\pm$ 52†	1.846 $\pm$ 0.187†
Pen (1.5 mg/kg)	305 $\pm$ 11	562 $\pm$ 16	1.845 $\pm$ 0.054
Pen (7.5 mg/kg)	315 $\pm$ 28	537 $\pm$ 28	1.709 $\pm$ 0.110
Pen (15 mg/kg)	424 $\pm$ 29	320 $\pm$ 20	1.586 $\pm$ 0.113
<b>Hippocampus</b>			
Sal	177 $\pm$ 27	305 $\pm$ 38	1.728 $\pm$ 0.107
Mid (0.5 mg/kg)	173 $\pm$ 10	308 $\pm$ 38	1.792 $\pm$ 0.228
Mid (2.5 mg/kg)	173 $\pm$ 11	305 $\pm$ 15	1.762 $\pm$ 0.099
Mid (5 mg/kg)	170 $\pm$ 11	361 $\pm$ 17†	2.132 $\pm$ 0.113†
Pro (6 mg/kg)	169 $\pm$ 10	305 $\pm$ 29	1.805 $\pm$ 0.120
Pro (30 mg/kg)	176 $\pm$ 12	304 $\pm$ 61	1.773 $\pm$ 0.140
Pro (60 mg/kg)	164 $\pm$ 18	377 $\pm$ 24†	2.320 $\pm$ 0.294†
Pen (1.5 mg/kg)	180 $\pm$ 19	304 $\pm$ 12	1.703 $\pm$ 0.128
Pen (7.5 mg/kg)	182 $\pm$ 13	295 $\pm$ 15	1.633 $\pm$ 0.156
Pen (15 mg/kg)	189 $\pm$ 19	263 $\pm$ 29	1.400 $\pm$ 0.157†

The brain contents of 5-HT and its metabolite were determined 30 min after administration of saline, propofol (6, 30, or 60 mg/kg), midazolam (0.5, 2.5, or 5 mg/kg), or pentobarbital (2.5, 7.5, or 15 mg/kg). Each value represents the mean  $\pm$  SD ( $n = 8$ ).

\* 5-HT and metabolite (ng/g) and ratios of metabolite to 5-HT. † $P < 0.01$ , ‡ $P < 0.05$  as compared with each corresponding value in the saline group. 5-HIAA = 5-hydroxyindoleacetic acid; 5-HT = 5-hydroxytryptamine; Mid = midazolam; Pen = pentobarbital; Pro = propofol; Sal = saline.

### Experiment 2

The righting reflex disappeared after administration of midazolam (5 mg/kg), propofol (60 mg/kg), and pentobarbital (15 mg/kg), although it was maintained with lower doses of any agents.

There were no differences among the groups in the concentration of 5-HT in each tissue region examined (table 1). Administration of midazolam (5 mg/kg) or propofol (60 mg/kg) increased the tissue concentration of 5-HIAA with all regions, although the administration of 15 mg/kg pentobarbital significantly decreased 5-HIAA concentrations. Therefore, the 5-HIAA/5-HT ratio was increased by anesthetic doses of midazolam and propofol and was decreased by an anesthetic dose of pentobarbital.

There were no differences among the groups for the dopamine concentration in all regions examined (table

**Table 2. Effects of Intravenous Anesthetics on Brain DA and Metabolites\***

	DA	DOPAC	HVA	(DOPAC + HVA)/DA
<b>Striatum</b>				
Sal	8,845 ± 1,222	2,502 ± 160	467 ± 57	0.339 ± 0.033
Mid (0.5 mg/kg)	8,848 ± 523	2,531 ± 194	470 ± 40	0.340 ± 0.029
Mid (2.5 mg/kg)	8,780 ± 838	2,481 ± 192	463 ± 22	0.337 ± 0.021
Mid (5 mg/kg)	9,013 ± 476	2,935 ± 119†	522 ± 24	0.385 ± 0.026
Pro (6 mg/kg)	8,787 ± 362	2,501 ± 143	461 ± 29	0.337 ± 0.017
Pro (30 mg/kg)	8,779 ± 741	2,487 ± 328	456 ± 32	0.335 ± 0.025
Pro (60 mg/kg)	9,019 ± 976	3,022 ± 151†	523 ± 33	0.396 ± 0.036†
Pen (1.5 mg/kg)	8,786 ± 463	2,520 ± 239	476 ± 65	0.342 ± 0.024
Pen (7.5 mg/kg)	8,798 ± 645	2,342 ± 234	449 ± 30	0.319 ± 0.041
Pen (15 mg/kg)	8,971 ± 438	2,177 ± 222	424 ± 29	0.290 ± 0.024‡

The brain contents of dopamine and its metabolites were determined 30 min after administration of saline, propofol (6, 30, or 60 mg/kg), midazolam (0.5, 2.5, or 5 mg/kg), or pentobarbital (2.5, 7.5, or 15 mg/kg). Each value represents the mean ± SD (n = 8).

\* Dopamine and metabolites (ng/g) and ratios of metabolites to dopamine. †  $P < 0.01$ , ‡  $P < 0.05$  as compared with each corresponding value in the saline group.

DA = dopamine; DOPAC = 3,4-dihydroxyphenylacetic acid; HVA = homovanillic acid; Mid = midazolam; Pen = pentobarbital; Pro = propofol; Sal = saline.

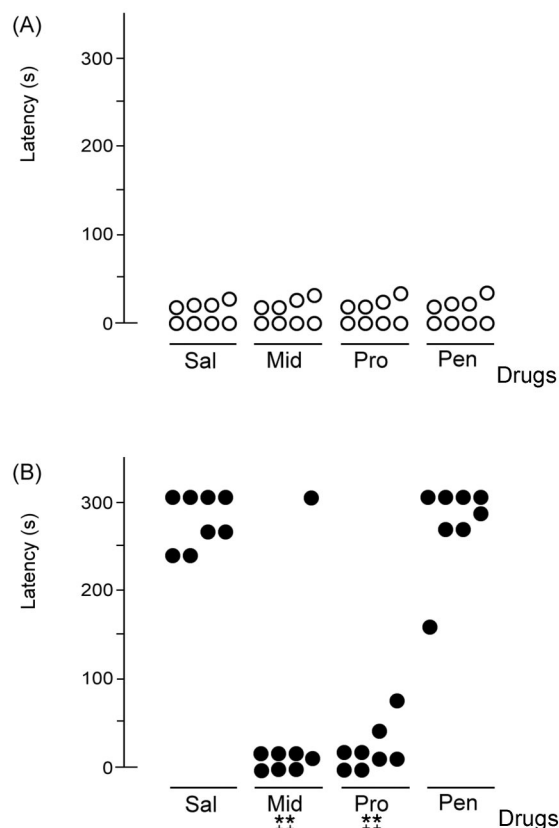
2). The concentrations of DOPAC and HVA increased in the striatum, hippocampus, and cortex in the midazolam (5 mg/kg) and propofol (60 mg/kg) groups. Administration of pentobarbital (15 mg/kg) tended to decrease the concentrations of metabolites, although the effects were not significant. The (DOPAC + HVA)/dopamine ratio increased with an anesthetic dose of propofol and decreased with an anesthetic dose of pentobarbital.

### Experiment 3

The step-through latencies to enter the dark compartment for the individual groups are shown in figure 3. On the training day, the step-through latencies demonstrated no difference among the midazolam, propofol, pentobarbital, and saline groups (fig. 3A). All animals entered the dark box within 30 s. On the test day, five of eight animals in the saline group and six of eight animals in the pentobarbital group did not enter the dark box within the allowed 300 s (fig. 3B). There was no difference in latency between the saline and pentobarbital groups. However, the step-through latencies 24 h after the training were significantly shorter in the midazolam and propofol groups than in the saline group. One of eight animals in the midazolam group did not enter the dark box within 300 s, and all of the eight animals in the propofol group entered the dark box within 300 s.

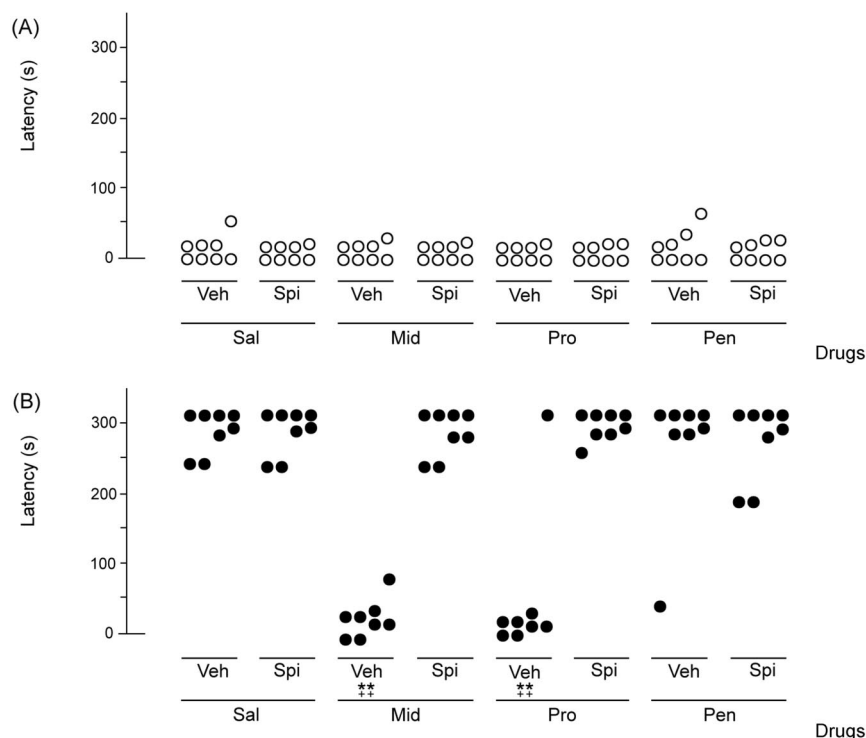
### Experiment 4

The step-through latencies to enter the dark compartment for the individual groups are shown in figure 4. On the training day, the step-through latencies showed no difference among the groups (fig. 4A). Spiroxatrine did not change the step-through latency in rats injected with saline (fig. 4B). Likewise, administration of the agent before pentobarbital treatment did not change the latency, and there was no difference in the latency be-



**Fig. 3.** Effects of intravenous anesthetics on passive avoidance performance. Training day (A, ○); testing day (B, ●). Saline (Sal), propofol (60 mg/kg, Pro), midazolam (5 mg/kg, Mid), or pentobarbital (15 mg/kg, Pen) was injected immediately after training. The step-through retention latencies were measured 24 h later. \*\*  $P < 0.01$  as compared with each corresponding value in the saline group (midazolam vs. saline:  $U = 6.5$ ,  $P = 0.0069$ ; propofol vs. saline:  $U = 0$ ,  $P = 0.0007$ ). ++  $P < 0.01$  as compared with each corresponding value in the pentobarbital group (midazolam vs. pentobarbital:  $U = 5.5$ ,  $P = 0.0042$ ; propofol vs. pentobarbital:  $U = 0$ ,  $P = 0.0006$ ).

**Fig. 4.** Effects of spiroxatrine on passive avoidance performance in rats injected with intravenous anesthetics. Training day (A, ○); testing day (B, ●). The vehicle (Veh) or spiroxatrine (5  $\mu$ g, Spi) was injected intracerebroventricularly 30 min before training, with saline (Sal), propofol (60 mg/kg, Pro), midazolam (5 mg/kg, Mid), or pentobarbital (15 mg/kg, Pen) injected intraperitoneally immediately after training. The step-through retention latencies were measured 24 h later. \*\*  $P < 0.01$  as compared with each corresponding value in the vehicle-with-saline group (midazolam *vs.* saline:  $U = 0$ ,  $P = 0.0006$ ; propofol *vs.* saline:  $U = 5.5$ ,  $P = 0.0043$ ). ++  $P < 0.01$  as compared with each corresponding spiroxatrine group. (Midazolam:  $U = 0$ ,  $P = 0.0007$ ; propofol:  $U = 5.5$ ,  $P = 0.0043$ .)



tween the saline and pentobarbital groups. However, spiroxatrine completely abolished the effects produced by either midazolam or propofol.

## Discussion

In the current study, anesthetic doses of midazolam and propofol increased both the extracellular concentration and the tissue content of 5-HIAA and disturbed memory retention during the passive avoidance test. The disturbance was completely inhibited by blockade of 5-HT<sub>1A</sub> receptors.

Released 5-HT is taken up into nerve endings by a specific uptake mechanism, a part of which is metabolized to 5-HIAA by monoamine oxidase and transported to the extracellular space. In the current study, the tissue content of 5-HIAA was increased by anesthetic doses of midazolam and propofol, although the 5-HT content did not change. As a result, the ratio of 5-HIAA/5-HT was increased by anesthetic doses of midazolam and propofol. These findings suggest increases in serotonergic metabolism by these anesthetics. In addition, increases in the extracellular concentrations of 5-HT and 5-HIAA by these agents were found in the microdialysis study. Taken together with these findings, anesthetic doses of midazolam and propofol may facilitate 5-HT release in the brain. The tissue content of 5-HT did not increase, despite increased serotonergic metabolism. The absence of such an increase may be caused by facilitation of 5-HT release. In contrast, the usage of pentobarbital decreased both the extracellular and tissue concentrations of

5-HIAA. Pentobarbital may thus reduce serotonergic activity at anesthetic doses.

There are several reports that suggest the relation between intravenous anesthetics and monoaminergic activity. Diazepam has been reported to decrease serotonergic activity in the brain. Dissimilar to diazepam, alprazolam, a benzodiazepine, has been shown to increase central serotonergic activity,<sup>24</sup> although there is no report that studied the effects of anesthetic doses of midazolam on brain monoaminergic activity. On the other hand, anesthetic doses of propofol have been reported to increase both serotonergic and dopaminergic activity,<sup>25</sup> whereas pentobarbital decreases the turnover of brain serotonergic and dopaminergic systems.<sup>26,27</sup>

There are several reports that showed the impairment of memory retention by midazolam and propofol.<sup>28-30</sup> In our investigation using a step-through passive avoidance test, the administration of midazolam or propofol after training reduced retention latencies 24 h later, whereas the administration of pentobarbital did not reduce retention latencies. An infusion of 5-HT into the striatum has been reported to impair retention latencies during the passive avoidance test in rats.<sup>31</sup> In other studies using the passive avoidance test, administration of 8-hydroxy-2-(di-n-propylamino) tetralin, a specific agonist of 5-HT<sub>1A</sub> receptors, impaired rats' performances,<sup>7-10</sup> and the effects were reversed by a specific antagonist of 5-HT<sub>1A</sub> receptors.<sup>11-13</sup> These findings are consistent with our current results and well explain the relation between the increase in serotonergic activity and amnesia. The facilitation of serotonergic activity in animals injected with

midazolam or propofol may have reduced retention latencies during the passive avoidance test.

In both humans and experimental animals, the increase in 5-HT release induced by various kinds of drugs and pathophysiologic states on the impairment of memory has been reported.<sup>5,7,9,32-39</sup> Facilitation of the serotonergic system caused by sepsis is considered to have a role in the impairment of memory retention.<sup>3</sup> An excess release of 5-HT due to injections of *p*-chloroamphetamine impairs learning ability in rats.<sup>34,35,40,41</sup> Because the striatum, hippocampus, and cortex are closely related to learning and memory,<sup>42,43</sup> the increase in serotonergic activity by midazolam or propofol in these regions may induce amnesia. Furthermore, when spiroxatrine was administered in the lateral ventricle before training, memory impairment was completely diminished. Therefore, the increase of serotonergic activity by these agents may induce amnesia through 5-HT<sub>1A</sub> receptor stimulation.

In studies on conflict behavior and 5-HT release in rats, a punishment test has been shown to enhance the release of 5-HT in the dorsal hippocampus.<sup>44,45</sup> In these studies, either midazolam or propofol suppressed the enhancement of the 5-HT release. The suppression seems to be related to anxiolytic properties, and the findings seem to be inconsistent with our own results. This discrepancy may be explained by the different doses of the agents. Lower doses than anesthetic doses may suppress the increase in 5-HT release caused by the punishment test. However, because we administered anesthetic doses of the agents, by which the righting reflex of the animals disappeared, conflict may not have been involved in the behavioral alteration of the animals. Although conflict caused by the punishment test may increase the release of 5-HT, the anesthetic doses of individual agents may enhance 5-HT release.

There are many reports that analyzed the involvement of the central dopaminergic system on learning and memory. Several dopaminergic stimulants have been shown to facilitate memory retrieval.<sup>14-16</sup> In contrast, degradation of the central dopaminergic system by an injection of 6-hydroxydopamine results in deficits in learning and memory.<sup>17,18,21</sup> Likewise, the blockade of dopamine receptors by chlorpromazine or haloperidol impairs memory performance.<sup>19,20</sup> In the current study, however, anesthetic doses of midazolam and propofol increased both extracellular concentration and tissue contents of DOPAC and HVA, suggesting increases in dopaminergic activity. Our findings on dopaminergic activity seem to be inconsistent with those from previous studies. This may be caused by differences in the extent to which the roles of the serotonergic and dopaminergic systems affect memory. The effects of the enhanced serotonergic activity on memory retention may be superior to those produced by the dopaminergic systems, because the impairment of memory retention

by midazolam or propofol was diminished because of the blockade of 5-HT<sub>1A</sub> receptors.

Although the anesthetic dose of pentobarbital decreased brain dopaminergic activity in our investigation, the administration of pentobarbital after training did not reduce retention latencies 24 h later. Because the decrease in dopaminergic activity caused by the pentobarbital was gradual, the injection of pentobarbital may not have produced memory impairment.

In the current study, we did not evaluate the effects of dopamine receptor antagonists on amnesia induced by midazolam or propofol. Dopamine receptor antagonists, which are used in psychiatry and neuroleptanalgesia, are known to diminish spontaneous motor activity in both experimental animals and humans.<sup>46-48</sup> The agents induce characteristic cataleptic immobility that allows the animals to be placed in abnormal postures.<sup>49-51</sup> The animal seems to be indifferent to most stimuli, although it continues to withdraw from those that are noxious and painful. Therefore, animals that received dopamine antagonists may not enter the dark box, even when memory on the training day is not retained. For these reasons, it is difficult to evaluate the relation between changes in dopaminergic activity and amnesia in the current experimental procedures.

In conclusion, the increase in central serotonergic activity immediately after administration of midazolam and propofol may be a contributing factor in retrograde amnesia caused by these agents.

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