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Abstract: Although isoflurane, a non-water soluble agent, has been known to block Na+ currents, its spinal anesthetic effect was not exposed. The aim of this experiment was to evaluate to evaluate the local anesthetic effect of isoflurane in spinal anesthesia. After intrathecal injection of isoflurane on rats, the spinal anesthetic effect in motor function, proprioception and nociception were evaluated. Lidocaine, a common used local anesthetic, was used as control. Isoflurane acted like lidocaine and produced dose-related spinal blockades of motor function, proprioception and nociception. Although isoflurane [27.6 (25.4 - 30.0)] had less potency when compared with lidocaine [1.0 (0.9 - 1.1)] ($P < 0.001$) in spinal anesthesia, it caused a much longer duration of spinal blockades than lidocaine at equianesthetic doses ($P < 0.001$). Our results showed that when compared with lidocaine, isoflurane produced a less potency but much longer duration in spinal anesthesia.

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Dear Editors:

Enclosed please find an original manuscript entitled" Isoflurane for spinal anesthesia in the rat " by Drs. Hung, Chu, Chen, Chen, Hong, and Wang, which we wish to submit to you for consideration of publication in NEUROSCIENCE LETTERS.

The manuscript has been submitted solely to this journal and has not previously been published in any form in another publication of any type with the exception of preliminary reports in abstract form.

We look forward to receiving your correspondence in near future.

Sincerely yours,

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Highlights

> Intrathecal injection of isoflurane produced spinal blockades. > Isoflurane had less potency when compared with lidocaine. > Isoflurane caused a much longer duration than lidocaine at equianesthetic doses. > Isoflurane produced a longer duration of sensory blockade than the motor blockade.

Isoflurane for spinal anesthesia in the rat

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Abstract

Although isoflurane, a non-water soluble agent, has been known to block Na^+ currents, its spinal anesthetic effect was not exposed. The aim of this experiment was to evaluate to evaluate the local anesthetic effect of isoflurane in spinal anesthesia. After intrathecal injection of isoflurane on rats, the spinal anesthetic effect in motor function, proprioception and nociception were evaluated. Lidocaine, a common used local anesthetic, was used as control. Isoflurane acted like lidocaine and produced dose-related spinal blockades of motor function, proprioception and nociception. Although isoflurane [27.6 (25.4 – 30.0)] had less potency when compared with lidocaine [1.0 (0.9 – 1.1)] ($P < 0.001$) in spinal anesthesia, it caused a much longer duration of spinal blockades than lidocaine at equianesthetic doses ($P < 0.001$). Our results showed that when compared with lidocaine, isoflurane produced a less potency but much longer duration in spinal anesthesia.

Key Words: Intrathecal Injection; Isoflurane; Lidocaine; Spinal Anesthesia

Isoflurane, an inhaled anesthetic agent, is commonly used in clinical anesthesia, and its pharmacokinetic has been studied in healthy human volunteers and animals [7, 11, 15]. Important actions of inhaled anesthetics are associated with altered activity of neuronal ion channels, particularly the fast synaptic neurotransmitter receptors such as $GABA_A$, nicotinic acetylcholine, and glutamate receptors [1, 20]. There is also growing evidence that anesthetics affect neuronal ion channels by binding directly to protein sites [1, 10, 19]. For instance, isoflurane at concentrations that occur during clinical anesthesia inhibited both tetrodotoxin-resistant (TTX-r) Nav1.8 and tetrodotoxin-sensitive (TTX-s) Nav [10]. Blockade of Na^+ currents, which is one of the major mechanisms of local anesthesia, produces spinal anesthesia, cutaneous analgesia, and sciatic nerve block [5, 16].

Recently, it has been shown that subcutaneous injection of the three inhaled anesthetics (halothane, isoflurane, and enflurane), like local anesthetics (lidocaine and procaine), elicited a concentration-dependent, cutaneous analgesic effect on rat skin [6]. However, to the best of our knowledge, no study of isoflurane in spinal anesthesia has been reported to date. Spinal anesthesia is a relatively easy practice, which produces adequate surgical conditions via injecting a small dose of local anesthetics, giving a wide popularity to this practice. Dr. August Bier in 1899 first described intrathecal injection of cocaine to make large part of the body insensitive to pain for

surgical goal [12]. The aim of this study was to investigate whether isoflurane produced spinal blockades of motor, proprioception, and nociception, as well as the spinal block effect of lidocaine. Lidocaine, a commonly used local anesthesia, was used as a control.

Male Sprague-Dawley rats (300 ± 25 g) were obtained from the National Laboratory Animal Centre, Taipei, Taiwan, and then they were housed in groups of three, with food and water freely available until the time of testing. The climate-controlled room was maintained at 22 ˚C with approximately 50% relative humidity on a 12-h light/dark cycle (6:00 AM – 6:00 PM). The experimental protocol was approved according to the Institutional Animal Care and Use Committee of China Medical University, Taiwan, and conformed to the recommendations and policies of the International Association for the Study of Pain (IASP).

AERRANE (Isoflurane, USP) were purchased from Baxter Healthcare of Puerto Rico (Guayama, PR 00784, USA). Lidocaine base and sesame oil were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Isoflurane and lidocaine were freshly prepared in sesame oil as solution before intrathecal injections.

Three specific experiments were performed. In experiment 1, the time courses of isoflurane (60, 40, 30, 20, and 10 %), vehicle (sesame oil), and lidocaine (2.98, 2.17, 1.08, 0.81, and 0.54 %) in spinal anesthesia were evaluated (n=8 rats for each dose of

each drug) in Figs. 2 and 3. In experiment 2, at equianesthetic doses, the block effect of 60% isoflurane in spinal anesthesia was compared with 2.98% lidocaine (n=8 rats for each dose of each drug) in Table 1. In experiment 3, on equipotent doses (ED_{25}, ED_{50} and ED_{75}), the block duration of isoflurane was compared with that of lidocaine (n=8 rats for each dose of each drug) in Fig. 4.

Before intrathecal injections and behavioral tests, animals were handled to minimize stress-induced analgesia and to be familiarized with the experiments. The agents were intrathecally injected into conscious rats as previously described [3, 14]. In brief, a 27-gauge needle attached to a 50-μL syringe (Hamilton, Reno, Nevada) was inserted into the midline of the lumbar 4-5 (L4-5) intervertebral space and 25-μL of drugs was injected. Rats were then observed for paralysis of two hind limbs, meaning for spinal blockades. Rats that displayed unilateral blockades were excluded from the experiment and sacrificed by using an overdose of isoflurane. All animals were injected intrathecally one time in this study. After the experiment, rats were sacrificed by using an overdose of isoflurane.

For consistency, one experimenter who was blinded to the drugs and doses used, was responsible for handling all the rats and behavioral evaluations. Motor function, proprioception, and nociception were assessed as previously described [2, 12]. In brief, the motor function was evaluated via measuring 'the extensor postural thrust' of the

right hind limb of each rat on a digital scale. A force less than 20 g [4] was interpreted as a 100% motor block or 100% maximal possible effect (MPE), and the pre-injection control value was considered a 0% motor block or 0% MPE.

The % possible effect (PE) is calculated via the equation:

$$\% \text{ PE} = 100\% \times (\text{Gm}–\text{Gt}) \div (\text{Gm}–20)$$

where Gm is the peak muscle force (g) of each rat before drug injections and Gt is the peak muscle force (g) of each rat after drug injections. The maximum value of % PE is the %MPE.

The nociception was graded as 4 (normal or 0% MPE), 3 (25% MPE), 2 (50% MPE), 1 (75% MPE), and 0 (absent or 100% MPE) according to the withdrawal reflex or vocalization elicited via pinching the lateral metatarsus of the two hind limbs, the dorsal part of the mid-tail, and a skin fold on each rat's back at 1 cm from the proximal part of the tail. Proprioceptive evaluation was based on the postural reactions and resting posture (‘tactile placing’ and ‘hopping’). A predominantly proprioceptive block causes a delayed hopping followed by greater lateral hops to prevent the animal from falling. In the case of full blockade, there would be no hopping maneuvers. The functional deficit was graded as 3 (normal or 0% MPE), 2 (slightly impaired or 33% MPE), 1 (severely impaired, 67% MPE), and 0 (completely

impaired or 100% MPE).

After animals were intrathecal injected with different doses of isoflurane and lidocaine (n = 8 for each dose of each drug), the % MPE of each dose of each drug were obtained. The % MPE of each dose of each drug was then fitted by using SAS Nonlinear (NLIN) Procedures (version 9.1, SAS Institute, Cary, NC), and the value of ED_{50}, defined as the dose that elicited 50% spinal blockades, were gotten [12, 17]. The ED_{25} and ED_{75} of drugs were obtained via the same curve-fitting (SAS NLIN Procedures) that was used to derive the ED_{50} [17]. Drug potencies were compared via the ED_{50}, constructed from the % MPE of each dose of each drug.

The blockade duration, defined as the interval from drug injection to full recovery, caused by each drug (n = 8 rats for each dose of each drug) was evaluated at equipotent doses (ED_{25}, ED_{50}, and ED_{75}). In this study, we also evaluated the %MPE, complete blockade time, time to full recovery, area under curves (AUCs) of motor, proprioception and nociception for 60% isoflurane and 2.98% lidocaine. The AUCs of spinal blockades of drugs were obtained via Kinetica v 2.0.1 (MicroPharm International, USA).

Data were presented as mean±S.E.M. or ED_{50} value with 95% confidence interval (95% CI) and were analyzed by the Student’s t-test. The differences in duration (Table 2) were evaluated by using 2-way ANOVA followed by pairwise Tukey's

HSD test. SPSS for Windows (version 17.0) was used for all statistical analyses. Statistical significance was set at $P < 0.05$.

The structures of isoflurane and lidocaine are shown in Figure 1. Intrathecal isoflurane, as well as lidocaine produced spinal blockades of motor function, proprioception, and nociception in rats (Figs. 2 and 3). Isoflurane (60%) caused 100% spinal blockades (% MPE) of motor function, proprioception, and nociception with durations of actions of 53.8±4.2, 55.0±4.9, and 60.6±4.8 min, respectively (Fig. 2 and Table 1). Lidocaine (2.98%) elicited 100% spinal blockades of motor function, proprioception, and nociception with durations of actions of 26.3±3.6, 32.5±3.1, and 35.0±1.9 min, respectively (Fig. 3 and Table 1). To rule out the effect of vehicle, intrathecal injections of sesame oil produced no spinal anesthetic effects (Figs. 2 and 3). There were no significant differences in efficacy between 60% isoflurane and 2.98% lidocaine in spinal blockades of motor function, proprioception, and nociception (Figs. 2 and 3). However, complete block time, time to full recovery, and AUC of spinal blockade of 60% isoflurane are significantly greater than those of 2.98% lidocaine in motor function, proprioception, and nociception (Table 1).

After intrathecal injections (5 doses in each group), the time courses of motor function, proprioception, and nociception of isoflurane and lidocaine were constructed (Figs. 2 and 3). The ED_{50}s of drugs, which were constructed form Figs 2 and 3 by

using SAS Nonlinear (NLIN) Procedures, are shown in Table 2. On the ED_{50} basis, lidocaine was more potent than isoflurane in spinal anesthesia (Table 2; $P < 0.001$). On equianesthetic basis (ED_{25}, ED_{50}, and ED_{75}), the block duration in motor function, proprioception, and nociception caused by isoflurane ($P < 0.001$) was longer than that caused by lidocaine (Fig. 4). The nociceptive block potency (26.6 [24.4– 28.8]) by isoflurane was found to be greater than the motor one (31.4 [29.2 – 34.0]) in Table 2. All rats recovered completely after intrathecal injections of drugs or vehicles.

In this report we showed that intrathecal isoflurane produces a spinal anesthetic effect. Isoflurane has a weak potency but much longer duration when compares with lidocaine in spinal anesthesia in rats.

Local anesthetics are well-known to produce spinal anesthesia through their Na^+ channel blocking activities on the central nervous system [8, 16]. In this report, we found that the inhaled isoflurane produced dose-dependent, spinal anesthesia, similar to that of the local anesthetic lidocaine. Inhaled anesthetics are also known to have Na^+ channel blocking activities, not only on the peripheral nervous system [10], but also on the central nervous system [21]. Accordingly, it is possible that inhaled isoflurane may exert their spinal anesthetic effect through similar Na^+ channel blocking activities on the central nervous system, although more studies are needed to confirm this speculation.

Long-acting local anesthetics and analgesics currently used for surgery and postoperative pain in clinical practice [13, 16]. The nociceptive blockade (AUC) of isoflurane was approximately 1.7-folds greater than that of lidocaine at equivalent doses. Furthermore, the block duration in motor, proprioception, and nociception caused by isoflurane was longer than that caused by lidocaine at equianesthetic doses (Fig. 4). Although 60% isoflurane displayed completely spinal anesthetic effects, it is still higher than 2.98% lidocaine. Because isoflurane produced spinal anesthesia through a local mechanism after intrathecal injection, this mechanism might also play a role on the analgesic effect of inhaled anesthetics during general anesthesia.

In this study, sesame oil was used as a vehicle for inhaled isoflurane. Before this study, several solvents (e.g., saline, intralipid, lecithin, sesame oil etc.) had been tested for their potential suitability as vehicles for inhaled anesthetics. Among these solvents, sesame oil showed the best solubility for inhaled isoflurane. Meanwhile, it remains unclear whether the high concentration isoflurance affects the function of spinal cord to modify the results of spinal anesthesia. However, all rats recovered completely after intrathecal injections.

Bupivacaine in resemblance to the clinical impression is the drug of choice when a more sensory-selective action over motor blockade [9, 18]. Intrathecal injection of isoflurane also produced a longer duration of sensory blockade than the motor

blockade (Figs. 2 and 4). Furthermore, we found that the potency (ED_{50}) of isoflurane in nociceptive blockade was more potent than that in motor blockade (Table 2). The sensory/nociceptive blockade in isoflurane was almost 1.2-folds higher potency (ED_{50}) than the motor blockade. Bupivacaine is rarely noted the sensory/motor potency in clinical practice because complete blockades are practiced. Further studies on sciatic nerve block and related neural and cardiovascular toxicities will be warranted.

In conclusion, this preclinical study demonstrated that isoflurane is shown to hold spinal (local) anesthetic properties. Although isoflurane is less potent to lidocaine in spinal anesthesia, its anesthetic action is much more long-lasting than that of lidocaine.

Acknowledgements

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Table 1. Percent of maximal possible effect (%MPE), duration of drug action, and area under curve (AUC) values for motor, proprioception, and nociception after intrathecal injection of 60% isoflurane or 2.98% lidocaine.

	%MPE	Duration (min)		AUC (%min)
		Complete blockade time	Time to full recovery	
Isoflurane				
Motor	100 ± 0	14.1 ± 2.2**	53.8 ± 4.2***	2776 ± 266***
Proprioception	100 ± 0	16.0 ± 3.1**	55.0 ± 4.9***	3217 ± 366***
Nociception	100 ± 0	16.4 ± 3.0**	60.6 ± 4.8***	3537 ± 300***
Lidocaine				
Motor	100 ± 0	8.1 ± 1.7	26.3 ± 3.6	1449 ± 219
Proprioception	100 ± 0	8.1 ± 1.7	32.5 ± 3.1	1763 ± 215
Nociception	100 ± 0	11.9 ± 2.1	35.0 ± 1.9	2119 ± 189

Values are mean±S.E.M.; n = 8, each group. Of note, all of the rats showed complete blockade (100%MPE) of any function tested. Symbols (***, **) indicate $P < 0.01$ and $P < 0.001$, respectively, when isoflurane compared with lidocaine.

Table 2. The 50% effective dose (ED_{50}) values of isoflurane and lidocaine with 95% confidence interval (95% CI) on spinal blockades of motor, proprioception, and nociception in rats.

	ED_{50} (95% CI)			Mean		
	Motor	Proprioception	Nociception	ED_{25}	ED_{50}	ED_{75}
Isoflurane	31.4 (29.2 – 34.0)	27.6 (25.4 – 30.0)	26.6 (24.4– 28.8)	21.8	28.5	37.4
Lidocaine	1.0 (0.9 – 1.1)***	1.0 (0.9 – 1.1)***	0.9 (0.8 – 1.0)***	0.7	1.0	1.3

The ED_{50}s of isoflurane and lidocaine (%) were obtained from Figs. 2 and 3 by SAS Nonlinear (NLIN) Procedures. CI = confidence interval. The symbol (***) indicates $P < 0.001$ when isoflurane compared with lidocaine.

Legends to figures

Fig. 1. The chemical structures of isoflurane (A) and lidocaine (B).

Fig. 2. Time courses of spinal blockade (% PE) by isoflurane (60-10%) and sesame oil in rats. Neurological evaluation was constructed after drug injection. Data are presented as mean±S.E.M.; each group, n=8.

Fig. 3. Time courses of spinal blockade (% PE) by lidocaine (0.54-2.98%) and sesame oil in rats. Neurological evaluation was constructed after drug injection. Data are presented as mean±S.E.M.; each group, n=8.

Fig. 4. Full recovery time of action of isoflurane and lidocaine on spinal blockades of motor, proprioception, and nociception at equipotent doses of ED_{25}, ED_{50}, and ED_{75} (n = 8 at each testing point). Values are expressed as mean±S.E.M. The differences in duration were evaluated by using 2-way ANOVA followed by pairwise Tukey's HSD test.

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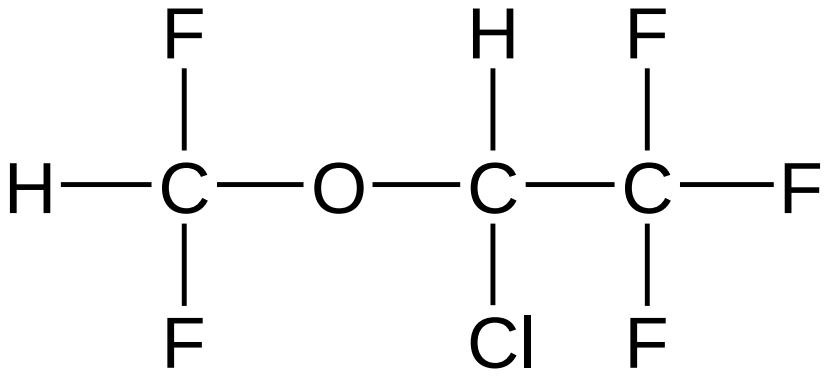
Figure 1

(A)



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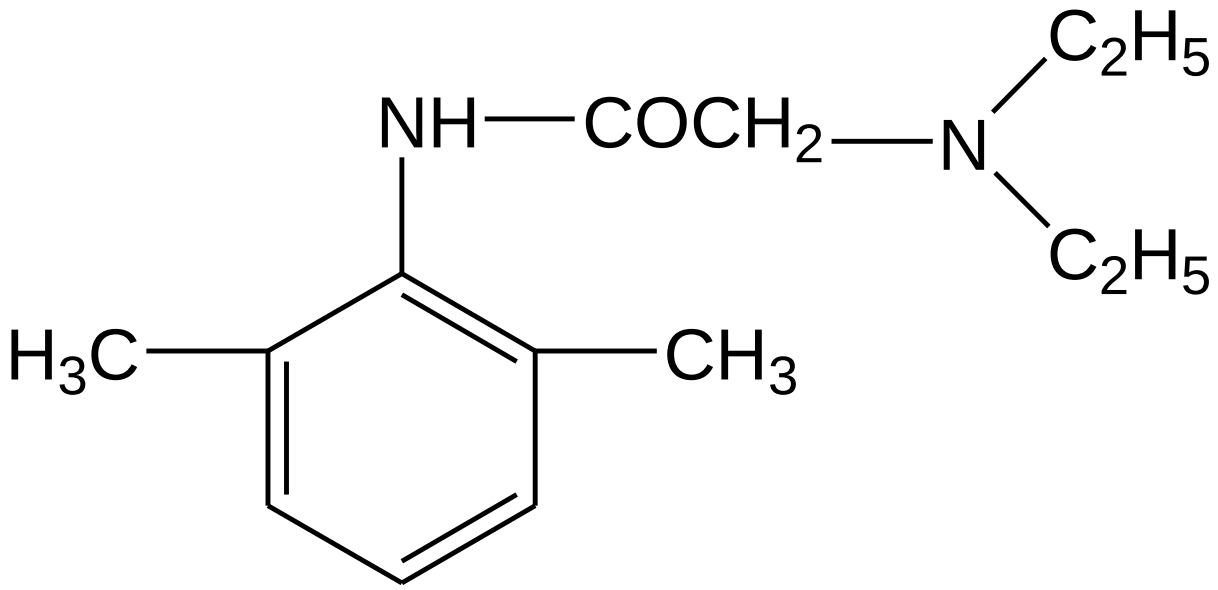

Fig. 1.

Figure 2

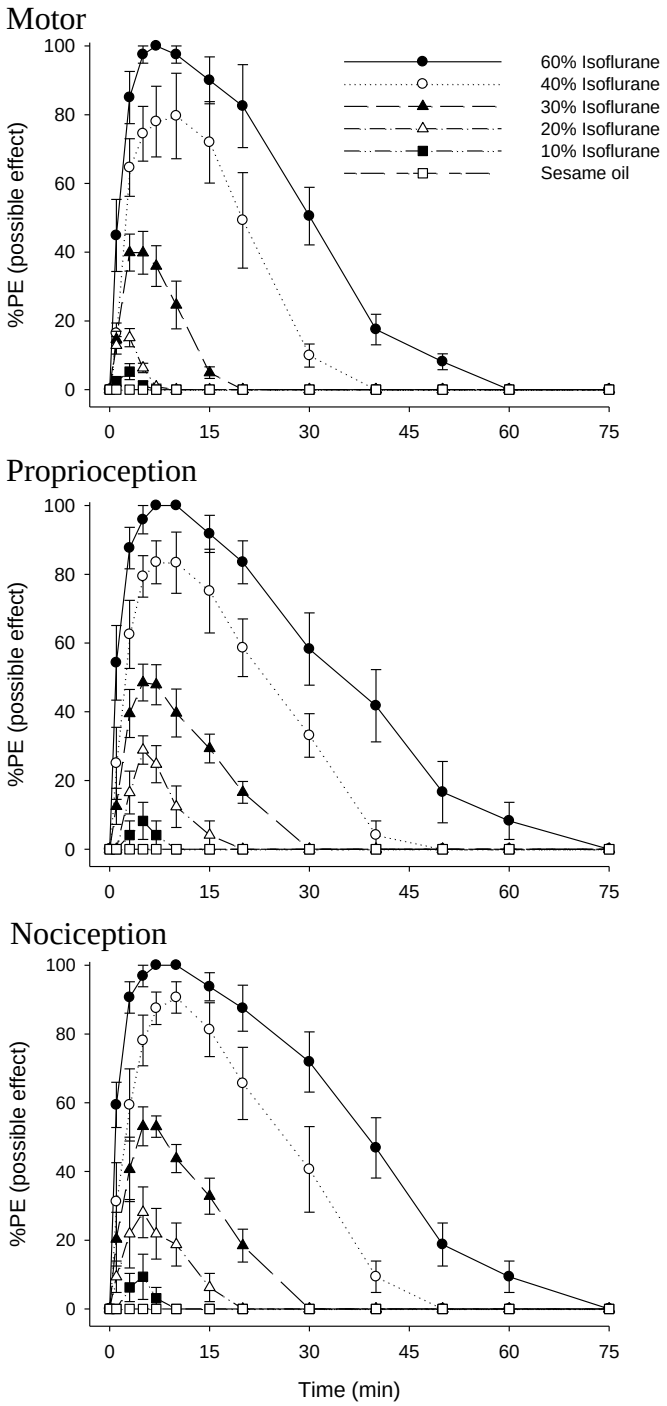

Fig. 2.

Figure 3

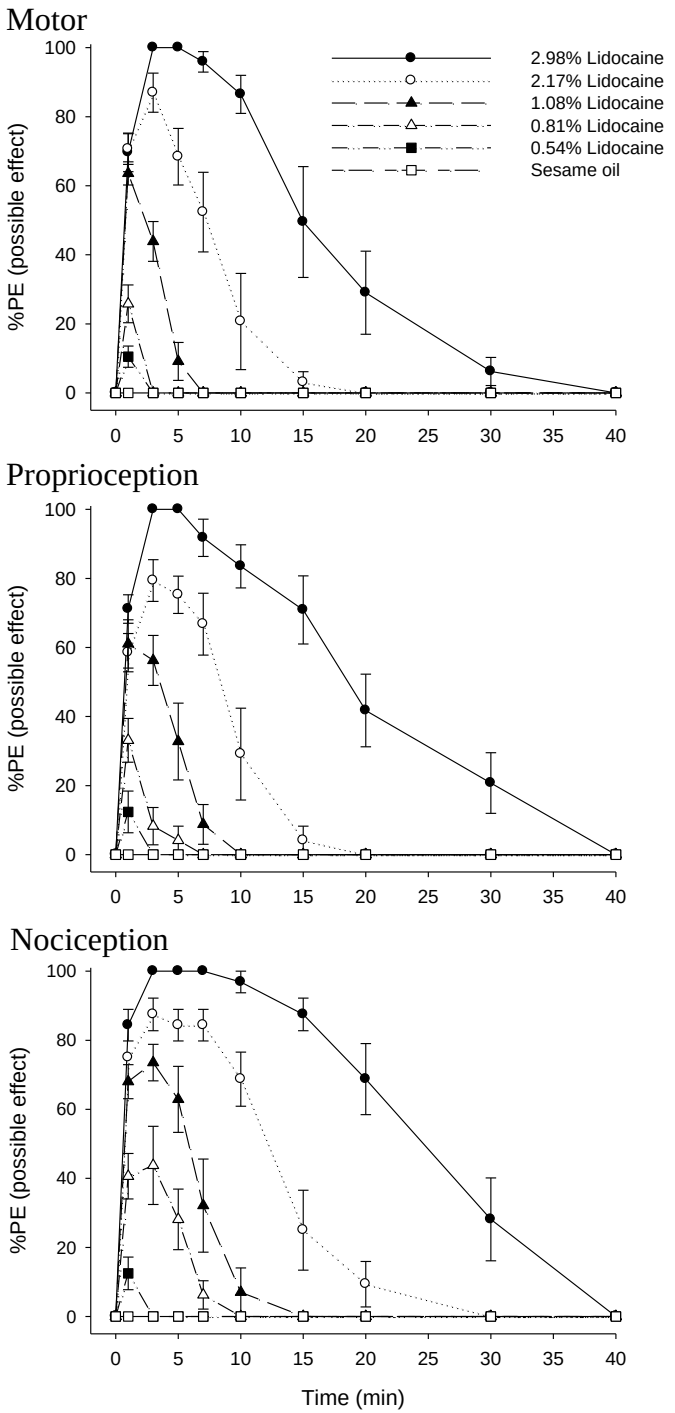

Fig. 3.

Figure 4

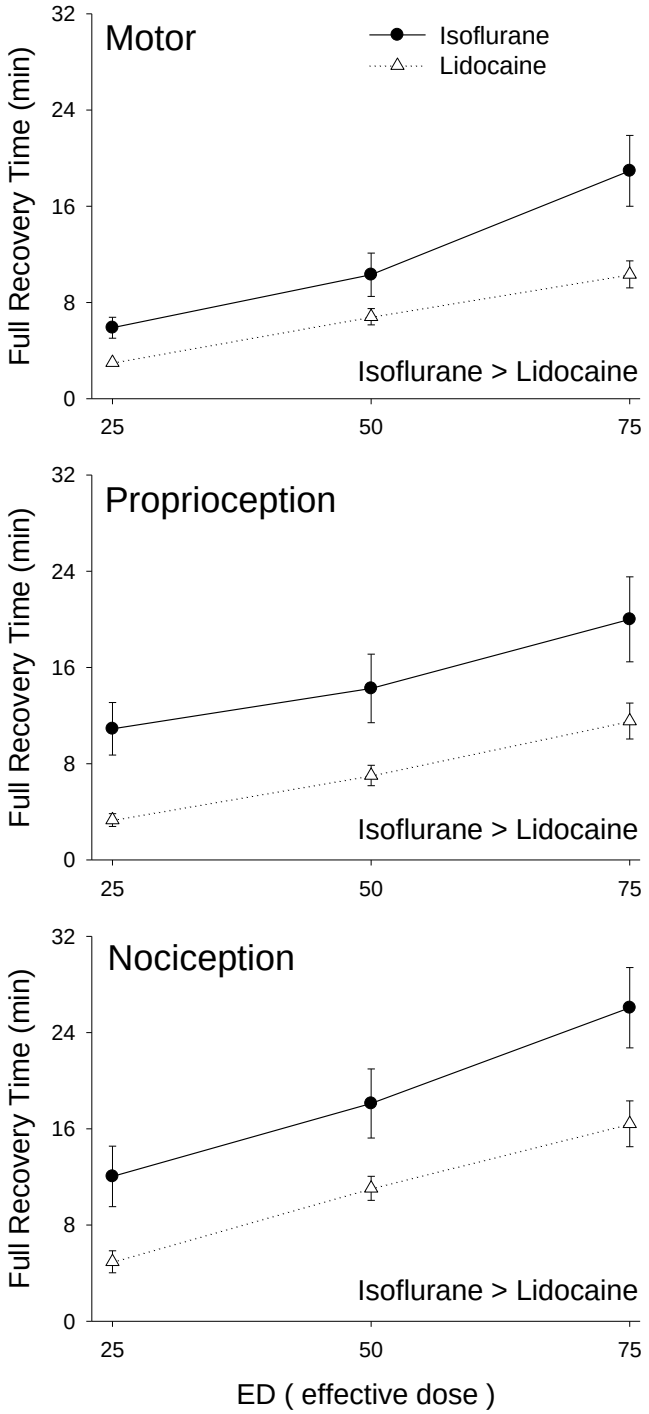

Fig. 4.