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Isoflurane Potency in Mice from the First and Second Parity

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As an approach to investigating mechanisms of anesthetic action, studies using selective breeding of animal stocks with different anesthetic sensitivity have increased during recent years. Mice are an ideal model for such studies due to their small size, short reproductive cycle, well-established behavioral endpoints of anesthesia, and well-known genetic background. Because single litters are not large enough for simultaneous selective breeding and conservation of the stock, mice must be used from successive litters. However, the stability of isoflurane anesthetic potency across successive litters has not been reported. In the present study, 24 (12 male and 12 female) outbred ICR mice were mated. Each pair was allowed to produce 2 successive litters. Offspring were separated by sex after weaning at 21 d of age. Reproductive characteristics were documented, including litter size at birth, sex ratio at weaning, and neonatal mortality. At 65 to 75 d of age, the median effective dose (ED_{50}) of inhaled isoflurane was measured in mice from the 2 parities by using a bracketing design. Loss of righting reflex was chosen as the criterion for successful anesthesia. The 2 parities did not differ significantly with regard to reproductive parameters and isoflurane ED_{50} of offspring. These results indicate that offspring in the second litter from the same parents can be used for stock conservation and anesthesia research.

Abbreviation: ED_{50}, median effective dose.

The use of genetics to study volatile anesthetic action has gained more attention over the past 2 decades.[12] One approach in forward genetics is to exploit naturally occurring polymorphisms by using selective breeding of variants with different sensitivities to volatile anesthetics in a genetically heterogenous population.[6] Because selective breeding requires screening large numbers of individuals, simple organisms are most often used due to their high reproductive capacity, such as *Caenorhabditis elegans*[10,14] and *Drosophila melanogaster*.[1,5,8] However, behavioral analysis in these simple organisms may be difficult to relate to humans under anesthesia. Mice have unequivocally similar behaviors to anesthetic action in humans, and more than 80% of mouse gene functions are the same as those in humans.[11] The mouse may be an ideal surrogate for human beings in the study of anesthetic action. Mouse stocks with different sensitivities to volatile anesthetics can be produced by selective breeding, but doing so requires enormous resources. Until now, only limited efforts have been made to establish mouse stocks with different anesthetic sensitivities.[9]

In our preliminary study, we sought to develop stocks of mice with different sensitivities to isoflurane from an outbred population by selecting and mating mice that were sensitive or resistant to isoflurane anesthesia.[19] We found that it was difficult to have sufficient mice from a single litter to perform both selective breeding and experiments to determine isoflurane potency. One possible solution is to collect the second litter from the same parents to increase the number of available offspring. However, use of this option requires that mice in successive litters have the same sensitivity to isoflurane. In the present study, we compared the potency of inhaled isoflurane between mice from the first and second litter produced by the same parents.

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Materials and Methods

Ethical considerations. This study was approved by the Committee of Scientific Research and the Committee of Animal Care in Capital Medical University (Beijing, China). Throughout the study, the animals were cared for in accordance with the Regulations for the Administration of Affairs Concerning Experimental Animals[17] promulgated by the State Science and Technology Commission in China.

Animals. The study was carried out in the Laboratory Animals Center of Capital Medical University (Beijing, China). We used outbred Vr:CD1 (ICR) mice (Vital River, Beijing, China) in this study. Mice were kept in a specific-pathogen-free animal facility, where quarterly sentinel surveillance was conducted. Sentinel mice were negative for *Salmonella* spp., *Mycoplasma* spp., *Corynebacterium kutscheri*, Tyzzer disease virus, *Pasteurella pneumotropica*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, ectromelia virus, mouse hepatitis virus, Sendai virus, pneumonia virus of mice, reovirus type 3, minute virus of mice, Theiler mouse encephalomyelitis virus, mouse adenovirus, polyoma virus, and hantavirus. Trained animal care staff provided daily health monitoring for the mice. The breeding room was maintained at approximately 20 to 23 °C with a relative humidity of 50% ± 5% and 15 to 20 air changes per hour. The room was on a 12:12-h light:dark cycle with lights on at 0700. The animals were housed in cages (320 × 180 × 150 mm, Laboratory Animals Instrument, Suzhou, China) with chipped wood bedding. The mice had ad libitum access to commercial chow and tap water (HCl-acidified, pH < 2.5) in polycarbonate bottles.

Twenty-four virgin mice (age, 65 to 70 d; weight, 28 to 40 g; 12 male and 12 female) were selected and numbered. We paired animals randomly to compose 12 pairs of parents. Each pair was

housed in a single cage. The mating was confirmed by observing vaginal plug formation. After mating, female and male mice were separated and housed individually. Cages were labeled to identify the original mates. Signs of pregnancy were observed in female mice. After 18 to 22 d of gestation, first litters were born. Reproductive parameters were documented, including litter size at birth, sex ratio at weaning, and neonatal mortality. Neonatal mortality was calculated by subtracting the number of pups surviving to weaning age from the number born, and expressed as percentages per litter. Pups were separated by gender after weaning at 21 d of age. Littermates of the same gender were housed in a single cage. One week after weaning the first litter, the original pair of parental mice were placed together again to produce the second litter. Mice were weighed (JA2003 electronic balance; maximal weighing capacity, 200 g; readability, 0.001 g; Hangzhou Yingda Energy Technology, Hangzhou, China) at birth, weaning, and the time of anesthetic potency determination.

System for the delivery of isoflurane. A chamber system was used to deliver the gas mixture of isoflurane. During anesthesia, mice were placed individually in transparent acrylic chambers (internal diameter 6 cm, length 30 cm). Both ends of the chamber were fitted with rubber stoppers with stainless steel tubes (internal diameter, 0.9 cm) through them for circuit connection and a 16-gauge metal needle with a nylon stopcock for connection to an infrared gas analyzer (90518 Multigas Analyzer, Spacelabs Medical, Redmond, WA). The stopcocks were closed when not in use. We connected 4 chambers in parallel with 2 tiers of T-tubes, thus there was a single inflow and outflow tube. The inflow tube (length, 20 cm) was connected to the common gas outlet of an anesthesia machine (model 7300, Aeon Medical Instrument, Beijing, China). The outflow tube (length, 150 cm) was directed to a T-piece, with 1 end connected to a central vacuum and the other open to room air. An anesthetic gas absorber (TA Medical Equipment, Nanjing, China) was added between the T-piece and vacuum and was replaced every hour during anesthesia. The schematic representation of this system is shown in Figure 1. Isoflurane (stock number 11613Z8, Abbott Laboratories, Queensborough, Kent) was vaporized (MVP300 isoflurane vaporizer, Aeon Medical Instrument) in oxygen (flow rate, 2 L/min), and gas mixture passed continuously through the chambers during anesthesia. The concentrations of isoflurane and carbon dioxide were monitored continually by the infrared gas analyzer. The analyzer was calibrated once a month by using the manufacturer's standards.

Determination of isoflurane potency. The potency of inhaled isoflurane was measured in offspring from the first and second parities at age 65 to 75 d. Before measurement, all animals were habituated to the anesthetic chamber for 1 h each day on 4 successive days. All mice were fasted for 4 h before experiments but were given water to drink. Determination of potency was performed in a room adjacent to the breeding room.

Male and female mice were tested separately. Mice were placed individually in the chambers, and isoflurane was administered at an initial concentration of 0.9%. After a period of 30 min at this concentration, the chamber was rotated gently to place the mouse in a supine position. Loss of the righting reflex was confirmed if the mouse failed to right itself onto all 4 feet within 15 s after placement on its back. All mice experienced loss of righting reflex at the 0.9% concentration. The concentration of isoflurane was decreased by 0.1% for another 30-min period, and righting reflex was tested again. Concentration reduction continued until all mice regained the righting reflex. The median effective dose (ED_{50}) for loss of righting reflex was calculated by averaging the 2 concentrations at which the mouse either regained or lost the righting reflex.

Rectal temperature was measured with a probe connected to a digital vital sense monitor (Model 90303B, Spacelabs Medical, Redmond, WA) before and after each determination of righting reflex, and body temperature was maintained between 36 °C and 38 °C by warming with heat lamp over the chambers. During the entire experiment, the concentration of carbon dioxide was lower than 0.2%.

Statistics. Data are presented as mean ± 1 SD. The Student *t* test was used to compare litter size, body weight, and isoflurane ED_{50} in mice from the first and second parities. A χ^2 test was used to compare the sex ratio of pups within parity and between the 2 parities. All statistical analyses was carried out by using the SPSS 10.0 software package (SPSS, Chicago, IL). Statistical significance was inferred at a P value of less than 0.05.

Results

No parental infertility or prenatal death occurred during the study. Numbers of newborns were 109 in the first and 115 in the second parity; 5 pups died before weaning in the first parity and 6 in the second. No more than 1 neonatal death occurred with the same breeder pair. No mice died after weaning. We used these 104 first-parity and 109 second-parity offspring for determination of isoflurane ED_{50}. Reproductive characteristics were not significantly different between the 2 parities (Table 1). Body weights did not differ significantly across parities for either male or female mice, but male mice were heavier ($P < 0.05$) than female mice at weaning and ED_{50} determination (Table 2).

No mice died during isoflurane ED_{50} measurement. Significant differences were not detected in isoflurane ED_{50} across parities or between sexes within parity (Table 3).

Discussion

Mice and rats are the most widely used laboratory animals in biomedical research.[18] Mice have several advantages for selective breeding studies related to anesthesia research, including small size, short reproductive cycle, well-established behavioral endpoints of anesthesia, and well-known genetic background. In this study, we compared the anesthetic potency of isoflurane in mice from first and second parities and found that the ED_{50} for loss of righting reflex did not differ significantly between these 2 groups of offspring.

An earlier study reported that monogamous breeding resulted in the highest production per female mouse, the highest mean litter size, and the lowest neonatal mortality.[3] We found no significant differences in litter size, sex ratio, and body weight between the 2 parities of the same parents. Reproductive parameters of ICR mice in this study were comparable to those of outbred NIH/S mice.[3]

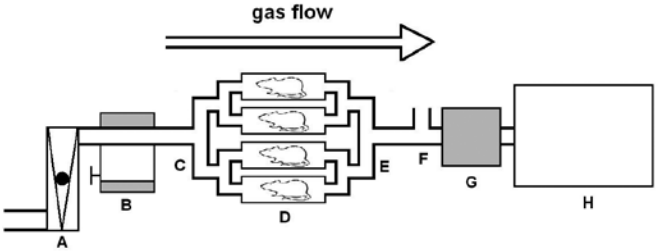

Figure 1. Schematic representation of delivery system for gas mixture of isoflurane. (A) Oxygen flowmeter. (B) Isoflurane vaporizer. (C) Inflow tube. (D) Chambers. (E) Outflow tube. (F) T-piece. (G) Anesthetic gas absorber. (H) Central vacuum.

Table 1. Reproductive parameters of the first and second parity

	First parity	Second parity	*P*
No. of pups born (mean ± 1 SD)	9.1 ± 1.2	9.6 ± 0.8	0.13
No. of pups surviving to weaning (mean ± 1 SD)	8.7 ± 0.9	9.1 ± 0.7	0.11
No. of pups that died before weaning (mean ± 1 SD)	4.1 ± 6.5	4.9 ± 6.5	0.78
Sex ratio at weaning (male:female)	54:50	57:52	0.96

Table 2. Body weight (no.) of mice at birth, weaning, and ED_{50} determination

		Body weight in grams (no. of mice)		
Parity	Sex	Birth	Weaning	ED_{50} determination
First	Male	1.5 ± 0.2 (109)	10.4 ± 1.5 (54)	32.2 ± 4.9 (54)
	Female		9.8 ± 1.4 (50)[a]	27.2 ± 4.6 (50)[c]
Second	Male	1.5 ± 0.2 (115)	10.5 ± 1.6 (57)	33.5 ± 5.1 (57)
	Female		9.9 ± 1.4 (52)[b]	28.1 ± 4.9 (52)[d]

Data are shown as mean ± 1 SD. Mice were 65 to 75 d old at ED_{50} determination.
[a]$P = 0.038$ between values for male and female mice in the first parity at weaning.
[b]$P = 0.031$ between values for male and female mice in the second parity at weaning.
[c]$P < 0.0001$ between values for male and female mice in the first parity at ED_{50} determination.
[d]$P < 0.0001$ between values for male and female mice in the second parity at ED_{50} determination.

Table 3. Isoflurane ED_{50} by sex and parity

	Male mice	Female mice	*P* across sex
First parity	0.574% ± 0.061% (54)	0.578% ± 0.067% (50)	0.756
Second parity	0.573% ± 0.060% (57)	0.579% ± 0.070% (52)	0.627
P across parity	0.912	0.950	

Data are shown as mean ± 1 SD (no. of mice).

Isoflurane was chosen as the experimental agent in this study because of its wide use in clinical anesthesia and laboratory animal research. The righting reflex was chosen as the endpoint of successful induction of anesthesia because it is a behavioral measurement commonly used to assess unconsciousness in rodents. We used a bracketing design to determine the anesthetic potency by averaging the 2 concentrations at which the mouse either regained or lost the righting reflex. Bracketing is a well-established method for determining anesthetic potency and fewer animals are required than for best-fit probit analysis.[15] Several studies have been performed to measure isoflurane ED_{50} in mice with different mutations and in wild-type controls. In those studies, the ED_{50} ranged from 0.50% to 0.63%.[2,4,7,13] Our results (0.573% to 0.579%) are comparable to these values.

Because the main purpose of this study related to the selective breeding of a genetically heterogenous population, we used mice of the outbred ICR stock as parents. Several studies have demonstrated that variabilities in reproductive characteristics and volatile anesthetic potency exist in different inbred mouse strains and between inbred and outbred mouse stocks.[3,16] Further studies are needed in inbred mice.

Isolflurane anesthetic potency was identical in mice from 2 succeeding parities. The results of this study indicated that offspring in the second litter from the same parents could be used for selective breeding and stock conservation in anesthesia research.

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