Anesthesiology
81:229–237, 1994

Effects of Subanesthetic Concentrations of Isoflurane and Their Interactions with Epinephrine on Acquisition and Retention of the Rabbit Nictitating Membrane Response

H. M. El-Zahaby, M.D., M. M. Ghoneim, M.D.,† G. M. Johnson, B.A.,‡ I. Gormezano, Ph.D.§*

Background: **Evidence concerning the concentrations of volatile anesthetics that prevent learning and recall is limited. Epinephrine is believed to enable learning during anesthesia. We investigated the effects of isoflurane and its interaction with epinephrine on learning and subsequent retention of the rabbit's classically conditioned nictitating membrane response.**

Methods: **In experiment 1, a tone (conditioned stimulus, CS) preceded paraorbital shock (unconditioned stimulus, US) during 60-min daily sessions of 60 presentations of these paired stimuli for 6 days of acquisition training under 0, 0.4%, or 0.8% isoflurane (n = 8, 13, and 9, respectively). Responses were recorded as conditioned responses (CRs) if they occurred during the CS and before the onset of the US. After 1 day of rest, the animals were given 3 days of extinction consisting of 60 presentations of CS-alone and without isoflurane to assess the retention of CRs from acquisition training. In experiment 2, epinephrine in a dose of 0, 0.01, or 0.1 mg/kg was injected subcutaneously in rabbits receiving 0.4% isoflurane. Two types of epinephrine were used, a sustained release form and epinephrine hydrochloride. Acquisition and retention were tested in the same way as in experiment 1. No isoflurane or epinephrine was used during retention testing.**

Results: **Learning was significantly suppressed during 0.4% isoflurane (≅0.2 MAC) treatment and eliminated during 0.8% (≅0.4 MAC). Information learned during administration of 0.4% isoflurane was not retained ($P < 0.05$). Although the low dose of epinephrine improved learning during the last day of the acquisition phase ($P < 0.05$), there were no differences between the treatment groups on any of the remaining acquisition or extinction days.**

Conclusions: **There was no learning during treatment with 0.8% concentration. Even a 0.4% concentration, which allowed some learning, abolished CRs in extinction, perhaps because of state-dependent retrieval. Epinephrine did not alter substantially the rates of CR acquisition or resistance to extinction. (Key words: Anesthetics, volatile: isoflurane. Conditioning. Memory. Sympathetic nervous system, catecholamines: epinephrine.)**

EVIDENCE concerning the concentrations of volatile anesthetics that prevent learning and recall is limited. Defining these concentrations is necessary because there are many clinical situations in which patients can tolerate only "light" anesthesia, such as during cesarean section operations, major trauma cases, and cases complicated by severe cardiovascular and other systemic diseases. It is possible that these patients may become conscious while totally paralyzed because there is no measurement that guarantees unconsciousness in the paralyzed patient.[1]

Isoflurane is the most commonly used volatile anesthetic in clinical practice. We wanted to assess its effects on learning and retention using a classical conditioning paradigm. Classical conditioning is one basic category of associative learning whose essential feature is a set of experimental operations involving an unconditioned stimulus (US) reliably evoking a measurable unconditioned response (UR), along with a conditioned stimulus (CS) that has been shown by test not to elicit the UR. The CS and US are presented repeatedly to the organism in a specified order and temporal spacing, and a response similar to the UR develops to the CS that is called the conditioned response (CR). Later, if the CS is presented repeatedly without US, the occurrence of CRs will decline gradually. This decline is called extinction.[2] We tested retention during this extinction phase.

Classical conditioning occurs even in the simplest organisms. The rabbit's nictitating membrane response

* Research Fellow of Anesthesia, Department of Anesthesia. Current position: Department of Anesthesia, Ain Shams University, Cairo, Egypt.

† Professor of Anesthesia, Department of Anesthesia.

‡ Senior Research Assistant, Department of Anesthesia.

§ Professor of Psychology, Department of Psychology.

Received from the Departments of Anesthesia and Psychology, University of Iowa College of Medicine, Iowa City, Iowa. Accepted for publication March 29, 1994.

Address reprint requests to Dr. Ghoneim: Department of Anesthesia, University of Iowa College of Medicine, Iowa City, Iowa 52242.

(NMR) is the most widely used model system for studying associative learning in mammals. There is a wealth of data for both humans and animals on the behavioral properties of these elementary learned responses. The parameters governing the acquisition of the behavioral response of eyelid-blink responses are well defined and understood for rabbits and humans. Within certain boundaries, acquisition of the eyelid-blink response in rabbits (nictitating membrane response) and humans is governed by the same parameters and follows the same set of laws.[3–5] Although classical conditioning is considered the simplest form of associative learning, it has been argued that both the behavioral and the neurobiologic mechanisms underlying classical conditioning are applicable to more complex types of learning.[6] Therefore, assessing the effects of isoflurane upon acquisition and retention of conditioned responses would constitute an assessment of its effects upon a model response system.

Injection of epinephrine during training of rats under anesthesia resulted in the acquisition of conditioned fear, as shown 10 days later by conditioned suppression of water drinking.[7] Learning did not occur in control animals that did not receive epinephrine. There are also anecdotal reports in humans that sympathetic stimulation may enhance learning during anesthesia.[8,9] In particular, the aims of the current study were to measure the effects of isoflurane on acquisition and retention of NMR and the effects of epinephrine as a possible factor modulating its actions. We used two concentrations of isoflurane in oxygen, 0.4% and 0.8%, and two doses of epinephrine, 0.01 and 0.1 mg/kg. Minimum alveolar concentration for isoflurane in the New Zealand white rabbit is 2.05%.[10]

Materials and Methods

Subjects

Experimentally naive New Zealand white albino rabbits of either sex weighing approximately 2 kg upon arrival were obtained from a local supplier. Animals were housed individually with free access to tap water and given 60 g of Teklad (Harlan Teklad, Madison, WI) rabbit chow daily. Consistent with their rearing conditions, animals were kept in constant light.

Apparatus and General Procedures

The apparatus and procedures have been described in detail.[11] Briefly, on the day after receipt, the rabbits were prepared for the experiments by placement of a suture loop (6-0 Ethilon Monofilament, Ethicon, Somerville, NJ) in the posterior margin of the nictitating membrane. Fur surrounding the right eye was removed, and two wound clips (Autosuture, Norwalk, CT) were attached to the skin over the paraorbital region at a distance 10 mm apart and 15 mm posterior to the dorsal canthus.

On the next day (adaptation day), rabbits were positioned in Plexiglas restrainers and placed in individual sound-attenuated chambers breathing 100% O_2 for 90 min. A muzzle headmount containing a photosensitive Polaroid transducer[11] was positioned and secured on the animal's head. The rotary armature of the transducer was attached to the nictitating membrane with a horizontal bar (22-G needle) with one end hooked into the suture loop on the nictitating membrane and the other end fixed with a set screw to the end of the rotary armature. NMR was defined as an extension of the nictitating membrane of at least 0.5 mm. Resolution of the phototransducer was determined to be 0.06 mm movement (extension).

Animals were trained over 6 days (acquisition) during which CS-US pairings were used and treatments were given. The CS consisted of a 1-KHz tone of fixed duration (400 ms) and intensity (84 dB). An audio-oscillator with 11.4-cm-diameter speaker for delivery of the CS was positioned approximately 20 cm above and 8 cm in front of the rabbit's head. Electrodes for delivery of the US were attached to the wound clips. The US consisted of an electric shock (60 Hz) of fixed intensity (3 mA) and duration (100 ms). The time lapse between the onset of the CS and that of the US, which is defined as the interstimulus interval, was fixed at 400 ms. Analog-to-digital conversion, response analysis, and experimental control were done using an Apple II/FIRST computer system.‖

Each day of conditioning consisted of an initial 30-min administration of anesthetic to achieve equilibration between the inspired and alveolar concentrations. For 10 min, the animals received twice the assigned isoflurane concentration followed by 20 min of the assigned concentration. This "over-pressure" was used to reduce the time required to attain the target concentrations. After equilibration, 60 CS-US pairings (trials) were presented with an average intertrial interval of 60 s (randomly varied from 50–70 s). The

‖ Scandrett J, Gormezano I: Microprocessor control and A/D data acquisition in classical conditioning. Behavior Research Methods & Instrumentation 12:120–125, 1980.

trial consisted of a baseline recording period (400 ms immediately preceding tone onset), followed by presentations of tone and shock stimuli (fig. 1). Amplitudes (extension, mm) and latencies (ms) of the responses were recorded. Responses were recorded as CRs if they occurred during the CS, but before US onset, whereas those that occurred after US onset were recorded as URs. A response was defined as an nictitating membrane extension of at least 0.5 mm.

After 1 day of rest (animals in their home cages), animals went into 3 days of extinction, in which no treatments were administered except 100% O_2. The trials were the same as in acquisition sessions except that no shock (US) was delivered.

Anesthetic Delivery

Isoflurane was administered using Isotec 3 vaporizers (Ohmeda, Madison, WI) and delivered mixed with oxygen to individual animals at flow rates of 3 l/min. The composition of inspiratory gas was confirmed by a gas analyzer that was calibrated daily before use. Each animal was fitted with a specially designed anesthesia mask attached to a Jackson-Rees modified Ayre's T-piece. Expired gases were scavenged and exhausted outside the building.

End-expired and arterial blood levels of isoflurane resulting from spontaneous breathing through the anesthesia system used were measured in an initial group of rabbits (n = 9). After placement of the mask, animals were allowed to breathe isoflurane in oxygen at 4.1% for 10 min followed by 20 min at 2.0%. After this 30-min equilibration period, 3-ml samples of arterial blood were drawn at 0, 30, and 60 min for quantitation of isoflurane by gas-liquid chromatography. After the final sample was drawn, the mask was removed and the trachea intubated with a cuffed tracheal tube, which was attached to the Jackson-Rees modification of the Ayre's T-piece. The rabbit was allowed to continue breathing 2.0% isoflurane spontaneously for 15 min to compensate for intubation time, after which five sequential 1.0-ml gas aliquots were drawn through a needle that was placed in the lumen of the tracheal tube at the entrance to the mouth for measurement of end-expired concentrations. Hamilton gas-tight syringes fitted with Teflon plungers were used for sampling. Samples and standards were made soluble in n-heptane and separated on a 30-m capillary column (0.54 mm ID) with AT-624 liquid phase (Alltech, Deerfield, IL) using argon/methane (95%/5%) as the carrier gas. Chromatographic conditions included injection port at 100°C, column at 35°C, and detector at 225°C. We treated the animals with higher concentrations of isoflurane than those used in studying learning to allow us to intubate the trachea.

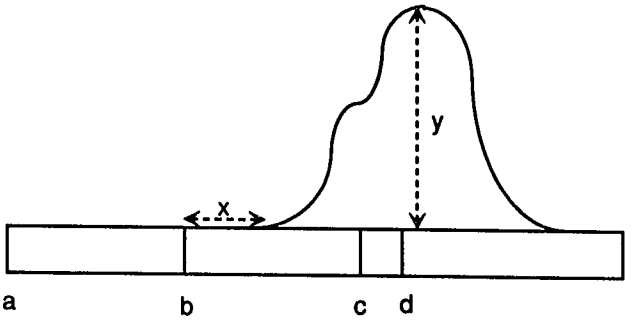

Fig. 1. Schematic diagram showing a complete conditioning trial with a conditioned response (CR). The period from a to b is the period for recording the baseline responses, from b to c is the period when tone is presented, and from c to d is when shock is presented. The distance (x) in ms is the onset latency, and (y) in mV is the amplitude of the CR.

Experiment 1. Effect of Isoflurane on NMR

Thirty rabbits were randomized to receive either 0% (control; n = 8), 0.4% (n = 13), or 0.8% (n = 9) isoflurane. Rabbits went into an adaptation day followed by 6 days of acquisition (paired CS-US) training. After 1 day of rest, retention was tested over 3 days using only CS and without isoflurane treatment.

Experiment 2. Interactions of Isoflurane and Epinephrine on NMR

On each of the 6 days of acquisition training, two groups of animals received daily injections of epinephrine. Two types of epinephrine were used, a sustained release form (Sus-Phrine, Forest Pharmaceuticals, St. Louis, MO) and epinephrine hydrochloride. Sus-Phrine has a rapid action due to the epinephrine in solution, while the sustained activity is due to the crystalline epinephrine-free base in suspension.[12] Epinephrine was administered randomly in three doses, either 0 (saline), 0.01, or 0.1 mg/kg subcutaneously using 25-G needles. The sites of injections were varied daily to avoid producing local necrosis. The drug was injected 5 min before each acquisition session. Our use of Sus-Phrine allowed us to avoid disturbing the animals during the acquisition sessions. Because of the short action of epinephrine hydrochloride, its administration was repeated every 15 min. There were 28 Sus-Phrine-treated animals (n = 9 each for the controls

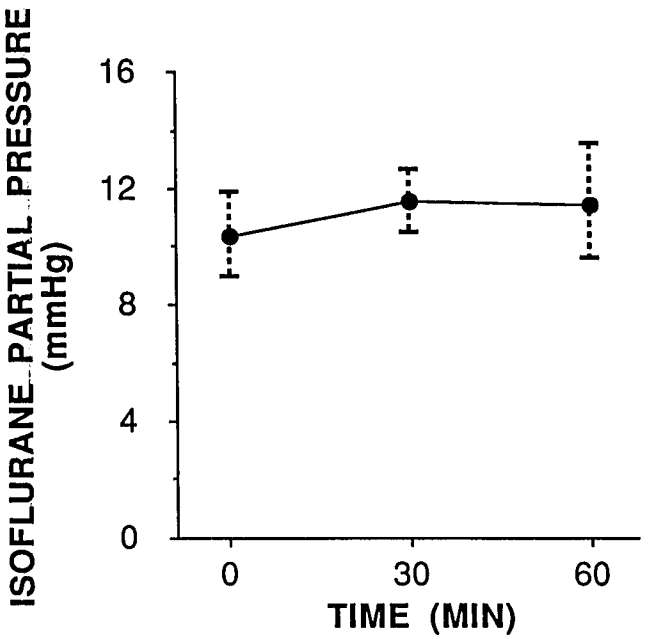

Fig. 2. Comparison of mean (±SE) arterial isoflurane partial pressures over time during spontaneous ventilation with 2% isoflurane (n = 9). Time 0 starts after 10 min of administration of 4.1% followed by 20 min of 2% isoflurane in oxygen.

and the 0.01 mg/kg group and n = 10 for the 0.1 mg/kg group). Sixty-two rabbits received epinephrine hydrochloride (n = 20 for the controls and n = 21 for each of the groups treated with epinephrine). All animals were treated with 0.4% isoflurane. The delivery of stimuli was identical to that of experiment 1 for the same 6 days of acquisition. During the 3 days of extinction, neither shock, isoflurane, nor epinephrine were used.

Statistical Analysis

A repeated measures analysis of variance was performed separately on the data of acquisition and extinction for each experiment. Each day of acquisition and extinction was composed of 60 trials, and the data of the trials were further subjected to a blocks analysis consisting of 12 blocks of 5 trials. Follow-up analyses were conducted to localize significant sources of variation and were carried out by the method of Tukey's[13] honest significant difference (*hsd*). The level of significance was set at $P < 0.05$.

Results

In the preliminary group used for determining equilibration between the inspired, end-expired, and arterial concentrations of isoflurane, analyses of inspiratory gas samples gave a mean result of 2.05 ± 0.15% (SE) (14.1 mmHg) with corresponding end-tidal isoflurane concentration of 1.87 ± 0.08% (13.26 mmHg). The mean arterial partial pressures of isoflurane were 10.44 ± 0.54 mmHg at time 0, 11.54 ± 0.39 mmHg at 30 min, and 11.40 ± 0.7 mmHg at time 60 min. Time 0 started after 10 min of administration of 4.1% isoflurane followed by 20 min of 2% (fig. 2). End-expiratory to inspiratory gas partial pressure ratio was 0.94, and arterial to end-expiratory partial pressures ratio was 0.84.

Experiment 1

Figure 3 presents the mean percentage of CRs to tone-CS across the 6 days of acquisition training as a function of isoflurane dosage (0, 0.4%, and 0.8%). The number of trials to the first CR was a direct function of drug dosage (table 1); whereas, subsequently, the overall frequency and terminal level of CRs was greatest for the control group, the 0.4% dose revealed a lower overall frequency and a lower terminal level of CR, and the 0.8% dose revealed little or no evidence of conditioning. Specifically, on the 1st day of training,

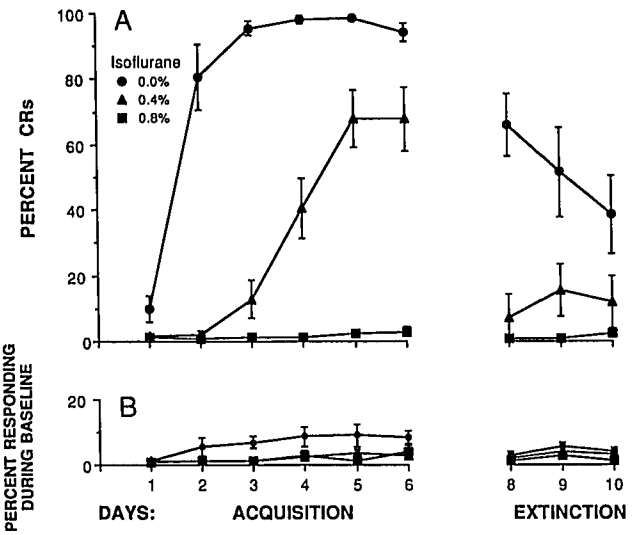

Fig. 3. Effects of isoflurane (0.0%, 0.4% and 0.8%) on acquisition (*left*) and extinction (*right*) of CRs to a tone CS (experiment 1). (*A*) The mean percentage of CRs on each of the 6 acquisition days. The bars indicate standard errors. (*B*) The mean percentage of baseline responses during the 400 ms immediately before the tone and are a measure of random eye-blinking independent of delivered stimuli. Treatment with 100% O_2 (control group) is presented as circles, 0.4% isoflurane as triangles, and 0.8% isoflurane as squares. Each point is the mean of 8, 13, and 9 animals per treatment, respectively.

Table 1. Number of Trials to 1, 5, and 10 Consecutive Conditioned Responses

	Conditioned Response Criterion		
Isoflurane Dosage (%)	1	5	10
0.0	33	74	83
0.4	133*	227*	255*
0.8	157*	360†	360†

The total number of trials was 360 (60 trials/day × 6 days). Animal groups that did not reach a criterion were assigned a value of 360.

* The number is greater than that for the controls ($P < 0.01$).

† The number is greater than those for both the control and the low dose groups ($P < 0.01$).

the level of CRs for the control group (0.0% isoflurane) was 9.9 ± 4.4% and was less than 2% in both groups receiving isoflurane. Over subsequent acquisition days, the rate and level of CRs of the control group increased substantially, reaching an asymptote of 99.1 ± 0.5% CRs on day 4 of acquisition. Conversely, the 0.4% (≅0.2 MAC) isoflurane group reached terminal CR level of 68 ± 8.7%, whereas the 0.8% (≅0.4 MAC) isoflurane group showed no evidence of CR acquisition across the 6 days of training. A four-factor analysis of variance (ANOVA; trial blocks, days, subjects, and doses) revealed a significant effect of dose ($F(2,25) = 83.752$, $P < 0.001$) and dose × days interaction ($F(10,25) = 19.693$, $P < 0.001$). Tukey's *hsd* test indicated that the control group had significantly higher level of CR acquisition than the other two groups ($P < 0.01$). The 0.4% group had a higher level of CR acquisition than the 0.8% group ($P < 0.01$). ANOVA for the rate of NMRs during the baseline period (the 400 ms just before the CS onset) showed a main effect of dose ($F(2,25) = 10.073$, $P < 0.001$). Tukey's *hsd* follow-up test indicated that the control group had significantly higher levels of responding than both the 0.4% and the 0.8% group ($P < 0.01$). Furthermore, ANOVA revealed no significant difference between the levels of CR acquisition for the 0.8% group and their baseline response rates. ANOVA for the amplitudes of URs before the occurrence of the first CR indicated a main effect of dose ($F(2,25) = 6.117$, $P < 0.01$), which the Tukey's *hsd* test localized to the control group's significantly higher levels than the other two groups ($P < 0.01$).

To determine isoflurane's effects on the initiation of CR acquisition, calculations were made on the mean number of trials required to achieve the criteria of 1, 5, and 10 consecutive CRs. Table 1 presents the mean number of trials to each of these CRs at different isoflurane doses. As dosage increased, the number of trials required to attain the successive criteria increased. ANOVA on the number of trials revealed significant effects of dose [$F(2,25) = 61.798$, $P < 0.001$], criterion ($F(9,225) = 36.826$, $P < 0.001$) and dose × criterion ($F(18,225) = 5.668$, $P < 0.001$). Tukey's *hsd* test indicated that the mean number of trials to each criterion was significantly greater for 0.4% isoflurane than for the control group ($P < 0.01$) and greater for 0.8% than for 0.4% groups ($P < 0.01$). In both control and 0.4% groups, 100% of the animals achieved each criterion. In contrast, for the 0.8% group, no animal achieved more than two successive CRs.

During extinction, there was a main effect of trial blocks ($F(11,275) = 6.12$, $P < 0.001$). Tukey's *hsd* test indicated that the first four trial blocks were higher than the last four with regard to percentage of CR. There was also a main effect of dosage between groups with regard to percentage of CRs across the 3 days ($F(2,25) = 12.27$, $P < 0.001$). Tukey's *hsd* test indicated that the percentage of CRs for the control group were higher than both isoflurane groups ($P < 0.01$ for both). There was no significant difference between the two isoflurane groups nor between each and its baseline response rates. Interaction of trial blocks × dose was significant ($F(4,50) = 6.431$, $P < 0.001$). Figure 4 shows percentage of CRs as a function of 12 trial blocks for the control group and the low isoflurane dose group during the last day of acquisition and all days of extinction. Although the low dose of isoflurane group had a percentage of CR value of 67.7 ± 9.6% on the last day of acquisition, it started the 1st day of extinction at a value of 0 for the first trial block. This unexpected finding led us to use a higher number of animals in this group as a precaution. We also examined the percentage of CR values for the last trial block on the last day of acquisition and the first trial block on the 1st day of extinction before the CRs naturally declined because of the absence of US. The mean values on the acquisition and extinction days for the control group were 87.5 and 96.9, for the low dose of isoflurane group 55.0 and 0.0, and for the high dose of isoflurane group 0 and 0, respectively.

Experiment 2

Both groups that were treated with epinephrine hydrochloride and Sus-Phrine displayed similar percentage of CR acquisition and extinction functions. ANOVA comparing CR percentages for both groups were done for individual days of acquisition and extinction in 12 five-trial blocks. There were no sig-

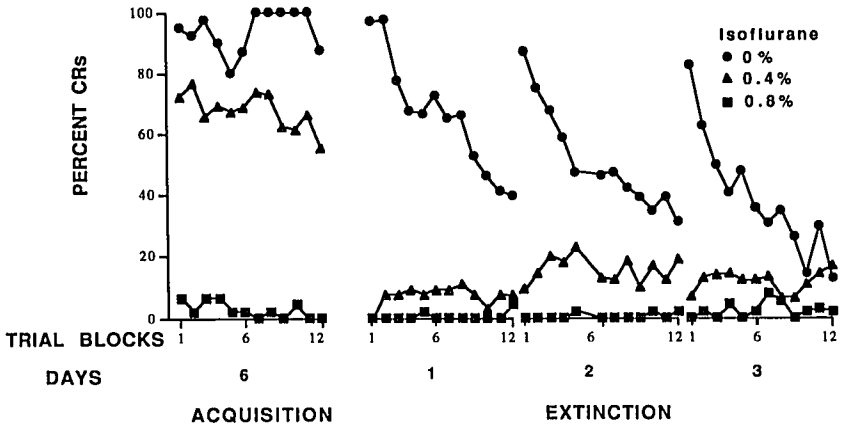

Fig. 4. Percentage of CRs for the control and the two isoflurane groups during the last day of acquisition and the 3 days of extinction (experiment 1). Animals received 60 trials per day, which were divided into 12 blocks of 5 trials. Each data point is the mean of five trials. Number of animals are 8, 13, and 9 for the control, low, and high isoflurane groups, respectively. The symbols for treatments are the same as in figure 3.

nificant differences (P range 0.65–0.96). Figure 5 shows percentages of CRs for the epinephrine chloride groups. During acquisition days, the control, low-dose epinephrine, and high-dose epinephrine groups reached terminal levels of responding on day 6 of 56.6 ± 8.8, 78.3 ± 5.7, and 63.3 ± 8.3%, respectively. The main effect of dose and dose × days interaction was not significant. ANOVAs conducted on the percentage of CRs in acquisition and extinction in 12 five-trial blocks revealed only a significant effect of days in acquisition that was localized to a significantly greater frequency of CRs for the low-dose epinephrine hydrochloride group than the control group on day 6 ($P < 0.05$). There were no differences in percentage of CRs between groups on any of the remaining acquisition days or extinction days. In addition, ANOVAs revealed there were no significant differences between the three groups in CR amplitudes, latencies, or baseline response during acquisition and extinction.

Power analyses were conducted to examine whether the inability to detect substantial differences between the epinephrine-treated and saline groups was due to an inadequate sample size. At a power of 0.8 and a significance level of 0.05, our sample size could have detected differences in percentages of CRs of 29 to 32 and 24 between the groups during acquisition and extinction, respectively.

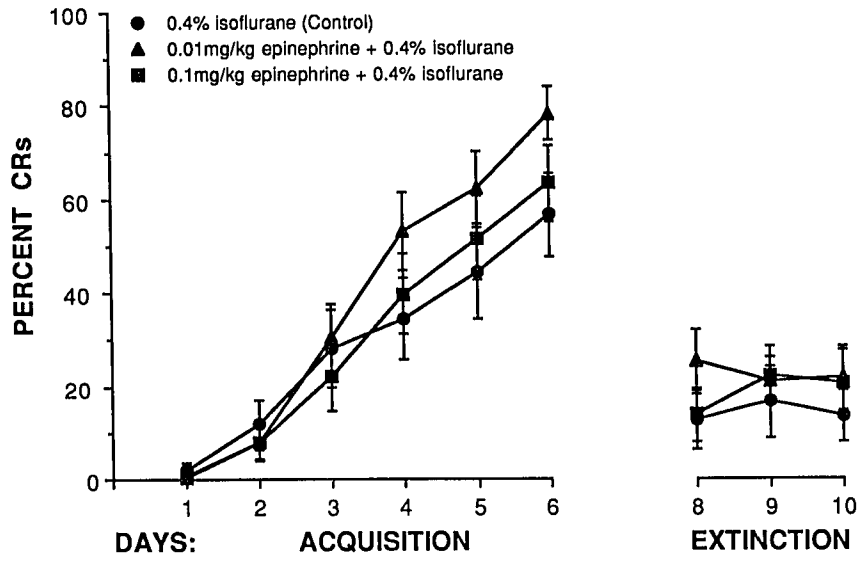

Fig. 5 Effects of epinephrine hydrochloride on acquisition (*left*) and extinction (*right*) of CRs to a tone CS (experiment 2). Data represent the means + SE. All rabbits received 0.4% during isoflurane during acquisition but not during extinction. Treatment with saline (control group) is presented as circles (n = 20), 0.01 mg/kg epinephrine as triangles (n = 21), and 0.1 mg/kg as squares (n = 21).

Discussion

To provide consistent conditions for all animals, we used face masks, because animals receiving 100% O_2 and low isoflurane concentrations would not tolerate laryngeal masks or tracheal tubes. In the preliminary measurements of inspired, end-expired, and arterial partial pressures of isoflurane, the end-expired to inspired partial pressures ratio and the mean arterial to end-expired ratio were greater than those obtained by Landon *et al.* and Frei *et al.* in adult patients.[14,15] These two studies had end-expired to inspired partial isoflurane pressure ratios less than 0.8 and arterial to end-expired ratios of 0.66 and 0.78, respectively. The rate of isoflurane uptake was enhanced in our study probably because of the use of small animals like rabbits, with smaller functional residual capacity per unit of body weight and a greater tissue blood flow, especially to vessel-rich group, compared to human adults.[16] The close correlation between inspired, alveolar, and arterial anesthetic concentrations allowed us to proceed using inspired measurements only.

Isoflurane showed a dose-dependent effect on acquisition of NMRs. The results are similar to those obtained by Chortkoff *et al.* in human volunteers.[17] They found complete suppression of learning at 0.4 MAC and an ED_{50} of 0.2 MAC isoflurane. Our low dose of isoflurane suppressed the rate of CR acquisition and the final asymptotic level of performance. Retention of the information learned was suppressed, as described later. The high dose eliminated learning as evidenced by calculating percentage of CRs and by numbers of trials to achieve certain criterion. The dose effect on the motor component of conditioning was apparent in decreasing the amplitude of URs before occurrence of any CR. However, localization of the exact neural site(s) of action of isoflurane upon URs would require electric brain stimulation of different sites of the NMR circuit or implantation of electrodes in specific brain sites and recording their neuronal activities.[18]

We conducted a more detailed analysis of isoflurane's impairment of CR acquisition with a determination of the anesthetic's effect on the initiation of conditioning. The analysis revealed a dose-dependent increment in the number of trials to the occurrence of the 1st, 5th, and 10th consecutive CRs. The dose-dependent effects of isoflurane on trials to criterion and the overall level of CRs indicate that the pattern of isoflurane's effects were essentially the same before and after CR occurrence. This similarity suggests that a principal effect of the drug was to impair the entry of conditioning components (CS, US, and/or UR) into the process governing acquisition.

Extinction refers to the experimental procedure in which, subsequent to acquisition training, the CS is presented repeatedly without US. Accordingly, in the absence of the US, a decline in responding occurs. The overall frequency of CRs during extinction and/or rate of decline are taken as measures of the strength of acquisition without the possible confounding of performance factors operating in acquisition. Moreover, the level of responding also can be used as a measure of retention of CRs learned during acquisition. In particular, an excellent measure of retention is obtained in the early blocks of trials in extinction before there is a substantial decrement in CRs. The control group showed gradual decline in percentage of CRs during extinction with higher CR levels at early trial blocks than at later ones. Both drug groups started at very low levels, although the low-dose isoflurane group reached terminal acquisition levels of 68%. Both drug groups started each day of extinction at the lowest CR levels for these days. Percentage of CRs for both groups during extinction did not differ from their percentage responding during the baseline periods. This behavior of the isoflurane-treated groups suggests two explanations. The first is that the drug may block the consolidation of new memories. The second is that poor memory retention may be due to the difference in pharmacologic state between acquisition and extinction. In the former, it is assumed that proper memory traces are not created, whereas in the latter, it may be the case that memory traces were established but cannot be accessed because of the change in pharmacologic state. A look at figure 4 suggests that lack of consolidation is not an adequate explanation for the effects of isoflurane. For example, animals that received the low dose of isoflurane had a percentage of CRs in the first trial block of the last day of acquisition of 74.5, and the mean percentage of CRs for that day was 67.7. If isoflurane had blocked consolidation, the percentage of CRs in the first trial block for that day should have started at a much lower percentage and increased in the following trials. A more plausible explanation for the dismal extinction levels with isoflurane is that the drug altered the properties of the tone CS so that, when there was no isoflurane, the animals did not recognize the tone as before. This is known as state-dependent memory, where memories formed in one drug state will be better recalled in the same drug state than in a different one. Mismatching

of drug states during acquisition and extinction may decrease the accessibility of information.[19] Adam *et al.*[20] showed that state-dependent memory occurred in subjects who received isoflurane. We also have evidence that nitrous oxide produces state-dependent retrieval in the same animal preparation that we used in the current study.#

Whatever is the explanation for the fragile nature of memories acquired during anesthesia, this fragility may account for the often reported failures to elicit recall for intraoperative events despite the fact that patients were known to be awake during the surgery, and the claims that reported cases of recall in the anesthesia literature may be the tip of the iceberg.[21,22]

Catecholamines modulate learning and memory functions.[23,24] There is evidence that epinephrine influences memory storage. Thus post-training injections of epinephrine produce dose-dependent and time-dependent enhancement of retention.[23,24] The key role of the adrenal gland in animal studies correlates with the profound effect of emotional states on the ability of humans to remember experiences.[25] Epinephrine probably acts through the release of central norepinephrine.[24] Weinberger *et al.*[7] reported that injection of epinephrine during training of rats under anesthesia resulted in the acquisition of conditioned fear, which was retained until at least 10 days later, as revealed by the ability of the CS to produce conditioned suppression of drinking water. There also are anecdotal reports that arousal or sympathetic stimulation during anesthesia in humans may explain the sporadic incidence of awareness and learning during surgery, when learning cannot be demonstrated in many studies.[9] It is possible that some anesthetic or surgical manipulations resulted in the release of epinephrine and that, in such cases, patients can learn and remember events taking place during anesthesia.

We used the same doses of epinephrine as Weinberger *et al.*,[7] except that we used two types of epinephrine. One is epinephrine aqueous suspension (Sus-Phrine), which produces both rapid and sustained epinephrine activity. The rapid action is due to the epinephrine in solution, while the sustained activity is due to the crystalline epinephrine-free base in suspension.[12] The longer duration of action provided by this preparation compared to the aqueous solution was of importance because Weinberger *et al.* used only 10 paired trials of CS and US presented over a 10-min interval, whereas we used 60 trials over a 60-min session. In another set of rabbits, we also used aqueous epinephrine to simulate closely the drug treatment provided by Weinberger *et al.*, however, because of our longer trial sessions, we repeated the injections every 15 min. (It should be noted that we did not study the whole spectrum of actions of epinephrine, which would have included treating a group of rabbits receiving 100% O_2 with epinephrine. This would have added to an already long and expensive study. It also would have been difficult to see an improvement over animals receiving 100% O_2 with no epinephrine because of the rapid rate of acquisition in the latter group. Our only aim was to replicate the work of Weinberger *et al.*) We found no improvement of learning or retention caused by epinephrine. A small significant enhancement was observed only during the last day of acquisition in the group which was treated with 0.01 mg/kg epinephrine. This is probably meaningless in the context of enabling learning in anesthetized or semi-anesthetized subjects, because it was not reflected in the retention performance. Power analysis of the data revealed that our sample size was adequate to detect practically significant differences between the groups, *e.g.*, one standard deviation difference. We used a different species than did Weinberger *et al.* Another difference in our methods is the type of anesthetic used and its administration. Weinberger *et al.* used a mixture of pentobarbital and chloral hydrate injected in a bolus mode achieving unknown concentrations in the brain; we used isoflurane with some control over its delivery to the arterial blood and its sites of action in the CNS.

Our NMR preparation is well established and widely used for studying associative learning and its interaction with drugs. The CS-CR functions are obtained in circumstances in which the CS and US are completely under the experimenter's control. Both the acquisition and retention of CRs can be observed from the start of training. Conversely, the conditioned suppression (or fear) paradigm used by Weinberger *et al.* involves a transfer of training design in which the stimulus pairings of classical conditioning are followed by the presentation of the CS during some instrumental conditioning task. The fear CR is not identical to the UR to shock. Therefore, the effect of CS-US pairings must be measured indirectly. Also, no URs are recorded. Therefore, it is unlikely that the conditioned suppression paradigm would be more sensitive to experimental variables than our Pavlovian paradigm. It also should

Manuscript in preparation.

be noted that, critical to the findings of Weinberger *et al.*, is their observation of the retention of conditioned fear CRs over a 10-day interval. Yet, that basic finding of 10-day retention does not appear to the authors' knowledge to have been replicated in the conditioned suppression literature. Moreover, no published studies have appeared replicating the findings of Weinberger *et al.* with the same experimental paradigm and experimental procedures.

In summary, we found that isoflurane suppresses learning in a dose-dependent manner and impairs retention, perhaps due to state-dependent retrieval. We failed to detect evidence that epinephrine improves learning or retention impaired by isoflurane. Two reports in the literature have influenced the recent surge of interest in learning during anesthesia and have been cited often. One of them is Weinberger *et al.*'s work in animals, and the other is Levinson's[8] study in humans in which he exposed patients to a faked crisis during anesthesia and the majority of the patients recalled the crisis while hypnotized in the postoperative period. It is therefore disturbing that we could not replicate the essential aspects of one study and another group could not replicate the other.**

The authors thank Anaquest Company for supplying part of the isoflurane used in this study and Merry Howell for preparation of the manuscript.

References

1. Ghoneim MM, Block RI: Learning and consciousness during general anesthesia. ANESTHESIOLOGY 76:279–305, 1992

2. Gormezano I: Classical conditioning, Experimental Methods and Instrumentation in Psychology. Edited by Sidowski JB. New York, McGraw-Hill, 1966, pp 385–420

3. Woodruff-Pak DS: Aging and classical conditioning: Parallel studies in rabbits and humans. Neurobiol Aging 9:511–522, 1988

4. Solomon PR, Beal MF, Pendleburg WW: Age-related disruption of classical conditioning: A model systems approach to memory disorders. Neurobiol Aging 9:535–546, 1988

5. Thompson RF: Classical conditioning: The Rosetta stone for brain substrates of age-related deficits in learning and memory? Neurobiol Aging 9:547–548, 1988

6. Hawkins RD, Kandel ER: Is there a cell biological alphabet for simple forms of learning. Psychol Rev 91:375–391, 1984

7. Weinberger NM, Gold PE, Sternberg DB: Epinephrine enables Pavlovian fear conditioning under anesthesia. Science 223:605–607, 1984

8. Levinson BW: States of awareness during general anesthesia. Br J Anaesth 37:544–546, 1965

9. Trustman R, Dubovs S, Titley R: Auditory perception during general anesthesia: Myth or fact? Int J Clin Exp Hypn 25:88–105, 1977

10. Drummond JC: MAC for halothane, enflurane, and isoflurane in the New Zealand white rabbit and a test for the validity of MAC determinations. ANESTHESIOLOGY 62:336–338, 1985

11. Gormezano I, Kehoe EJ, Marshall-Goodell B: Twenty years of classical conditioning research with the rabbit, Progress in Psychobiology and Physiological Psychology. Volume 10. Edited by Sprague JM, Epstein AN. New York, Academic, 1983, pp 197–275

12. Physicians' Desk Reference. Montvale, Medical Economics Data, 1994, p 957

13. Winer BJ: Statistical Principles in Experimental Design. New York, McGraw-Hill, 1971, p 198

14. Landon MJ, Maston AM, Royston BD, Hewlett AM, White DC, Nunn JF: Components of the inspiratory-arterial isoflurane partial pressure difference. Br J Anaesth 70:605–611, 1993

15. Frei FJ, Zbinden AM, Thompson DA, Reider HU: Is the end-tidal partial pressure of isoflurane a good predictor of its arterial partial pressure? Br J Anaesth 66:331–339, 1991

16. Dhasmana KM, Prakash O, Saxena PR: Effects of fentanyl and the antagonism by naloxone on regional blood flow and biochemical variables in conscious rabbits. Arch Int Pharmacodyn Ther 260:115–129, 1982

17. Chortkoff BS, Bennet HL, Eger EL: Subanesthetic concentrations of isoflurane suppress learning as defined by the category-example task. ANESTHESIOLOGY 79:16–22, 1993

18. Thompson RF, Steinmetz JE: The Essential Memory Trace Circuit for a Basic Form of Associative Learning, Learning and Memory: The Behavioral and Biological Substrates. Edited by Gormezano I, Wasserman EA. Hillsdale, Lawrence Erlbaum, 1992, pp 369–386

19. Overton DA: Historical context of state dependent learning and discriminative drug effects. Behav Pharmacol 2:253–264, 1991

20. Adam N, Castro AD, Clark DL: State-dependent learning with a general anesthetic (isoflurane) in man. TIT Life Sci 4:125–134, 1974

21. Russel IF: Midazolam-alfentanil: An anesthetic? An investigation using the isolated forearm technique, Memory and Awareness in Anesthesia. Edited by Sebel PS, Bonke B, Winograd E. Englewood Cliffs, Prentice Hall, 1993, pp 317–321

22. King H-K, Ashley S, Brathwaite D, Decayette J, Wooten DJ: Adequacy of general anesthesia for cesarean section. Anesth Analg 77:84–88, 1993

23. Gold PE: Memory modulation: Roles of peripheral catecholamines, Neuropsychology of Memory. Edited by Squire LR, Butters N. New York, Guilford, 1984, pp 566–578

24. McGaugh JL: Dissociating learning and performance: Drug and hormone enhancement of memory storage. Brain Res Bull 23:339–345, 1989

25. Thompson RF: The neurobiology of learning and memory. Science 233:941–947, 1986

** Chortkoff BS: Personal communication. 1994.