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Temporal Summation Governs Part of the Minimum Alveolar Concentration of Isoflurane Anesthesia

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Background: **General anesthesia may delay the onset of movement in response to noxious stimulation. The authors hypothesized that the production of immobility could involve depression of time-related processes involved in the generation of movement.**

Methods: **The delays (latencies) between onset of tail clamp (n = 16) or 50-Hz continuous electrical stimulation (n = 8) and movement were measured in rats equilibrated at 0.1–0.2% increasing steps of isoflurane. In other rats (n = 8), the isoflurane concentrations just permitting and preventing movement (crossover concentrations) in response to trains of 0.5-ms 50-V square-wave pulses of interstimulus intervals of 10, 3, 1, 0.3, or 0.1 s during the step increases were measured. These measures were again made during administration of intravenous MK801, an *N*-methyl-D-aspartate receptor antagonist that can block temporal summation (n = 6). Temporal summation refers to the cumulative effect of repeated stimuli. Crossover concentrations to 10- and 0.1-s interstimulus interval pulses ranging in voltage from 0.25–50 V were also measured (n = 4).**

Results: **The increase in concentrations from 0.6 to nearly 1.0 minimum alveolar concentration progressively increased latency from less than 1 s to 58 s. Shortening the interstimulus interval (50 V) pulses from 10 to 0.1 s progressively increased crossover concentrations from 0.6 to 1.0 minimum alveolar concentration. In contrast, during MK801 administration shortening interstimulus intervals did not change crossover concentrations, producing a flat response to change in the interstimulus interval. Increasing the voltage of interstimulus interval pulses increased the crossover concentrations but did not change the response to change in interstimulus intervals for pulses greater than 1 V.**

Conclusions: **Increasing the duration or frequency (interstimulus interval) of stimulation increases the concentration of isoflurane required to suppress movement by a 0.4 minimum alveolar concentration MK801 blocks this effect, a finding consistent with temporal summation (which requires intact *N*-methyl-D-aspartate receptor activity) at concentrations of up to 1 minimum alveolar concentration isoflurane.**

THE mechanical or electrical stimulation used to determine MAC (the minimum alveolar concentration producing immobility in 50% of subjects receiving noxious stimulation) is applied continuously for up to 1 min.[1–5] This period allows for a delay between the onset of stimulation and the beginning of movement. The delay seems to increase as the anesthetic concentration approaches MAC, suggesting that time-related processes underlie the generation of the movement and that increasing anesthetic concentrations are required to suppress the response to increasing durations of stimulation.

Such processes may be assessed by application of temporally graded electrical stimuli of the type used to create neuronal windup.[6–10] Typically, these stimuli are trains of brief (0.5 ms) high-intensity square-wave pulses with relatively large interstimulus intervals (ISI), often up to 3 s. Such stimuli delivered to spinal cord afferents of spinal cord-transected rats can produce a cumulative depolarization of dorsal and ventral horn neurons. After several seconds, this progressive depolarization can reach a threshold, triggering a sustained burst of action potentials, giving rise to the term "windup." *N*-methyl-D-aspartate (NMDA) receptor activity underlies at least part of the cumulative depolarization and subsequent windup.[11,12]

We hypothesized that temporal summation, the cumulative effect of repeated stimulation, might govern part of the MAC for isoflurane anesthesia. Although electrophysiologic and electromyographic effects of temporal summation have been investigated during isoflurane and halothane anesthesia in humans and rodents,[13–16] the involvement of temporal summation in the generation of movement during anesthesia has not been investigated.

To document the time-related dose-dependent effect of isoflurane on the generation of movement responses, we measured the latency to movement after tail clamp and 50-Hz continuous stimulation. To test for temporal summation, we measured the effect of the ISI on the latency to movement responses, on the concentrations required to achieve immobility, and on the observed buildup of muscle tone and hindlimb electromyogram. To determine if disruption of temporal summation impaired the generation of movement, we measured the effect of the NMDA antagonist, MK801, on isoflurane concentrations required to achieve immobility during the ISI pulses. To study the dose-dependent effect of isoflurane on temporal summation, we studied the effect of ISIs of increasing voltage and the corresponding increasing concentrations required to achieve immobility.

Materials and Methods

Subjects

The Committee on Animal Research of the University of California, San Francisco approved our study of 34

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male specific-pathogen–free Sprague-Dawley rats weighing 275–325 g and obtained from Charles River Laboratories (Hollister, CA). Animals were housed in our animal care facility under 12-h cycles of light and dark, three per cage, and had continuous access to standard rat chow and tap water for more than 1 week before study. The effect of isoflurane concentration on latency after tail clamp was studied in 16 animals (latency in eight of these animals also was determined with 50-Hz electrical stimulation; in the other eight we also determined whether varying time intervals between tail clamps influenced latency), after temporally graded stimuli in 12 (the effect of 50-V pulses in eight and the effect of varying the voltage of pulses in four), and after MK801 administration at various ISIs in six rats.

Apparatus

Each rat was placed in a gas-tight clear plastic cylinder sealed with rubber stoppers. A rectal temperature probe was inserted, and for most studies the temperature probe and the tail of the rat were separately drawn through holes in the rubber stopper. Ports through the rubber stoppers allowed delivery of gases at the head end of the cylinder and exit at the tail. Each cylinder received an average flow of 1 l/min oxygen and isoflurane. For studies that included electromyographic monitoring, cylinders were modified to provide access to the hindlimbs by applying a thin flexible diaphragm around the end of the cylinders. The hindlimbs were then drawn though a hole in the diaphragms, which fit snugly around the animal's lower abdomen creating a gas seal.

Procedures

For studies of the anesthetic dose-response to tail clamp and 50-Hz electrical stimulation, latency was determined in eight rats concurrently. The groups were studied at increasing steps of isoflurane, beginning at 0.0% for one group receiving tail clamp, at 1.1% for a second group receiving tail clamp, and at 0.7% for the group receiving 50-Hz electrical stimulation. Animals were equilibrated with a given isoflurane concentration for 30 min. Then, the tail clamp or 50-Hz stimulation was applied and the isoflurane concentration was measured by gas chromatography (the concentration determined by gas chromatography was used as the defining concentration in all studies). The isoflurane concentration was then increased by 0.1% to 0.2%. After equilibration for 30 min, the stimulation was applied again and the isoflurane concentration measured. This procedure was repeated until we achieved a concentration at which the animals did not move.

For all studies, isoflurane concentration in a representative cylinder was continuously monitored with an infrared analyzer (Capnomac II, Datex, Helsinki, Finland). Isoflurane concentrations after stimulation were measured with a Gow-MAC 750 flame ionization detector gas chromatograph (Gow-MAC Instrument Corp, Bridgewater, NJ) as previously described.[5] The rectal temperature was maintained near 37°C by applying a heat lamp as needed. The tail clamps were applied with an alligator clip oscillated at approximately ± 45° and 1 Hz for 60 s or until the animal moved.[2,3,5]

For the 50-Hz electrical stimulation studies, a 15-V biphasic 6.5-ms pulse was delivered for 60 s or until the animal moved through a pair of subcutaneous platinum needle electrodes (type E2, Grass Instruments, Quincy, MA) inserted into the distal third of the tail.[2]

For studies of the effect of varying time intervals between tail clamp applications, the MAC of each animal was first determined. The concentration of isoflurane then was decreased to 1.2% for 30 min. Then, while maintaining this concentration, each animal received tail clamps at intervals of 1, 2, 4, 8, and 16 min in a randomized order after the proceeding tail clamp.

For the temporally graded stimulation studies using 50-V pulses, animals were briefly anesthetized with 2% isoflurane for placement of stimulation and recording needles and then equilibrated at 0.75%. Equilibration and step increases in isoflurane concentration were the same as those described previously. The temporally graded stimuli were 0.5-ms square-wave pulses with ISIs of 10, 3, 1, 0.3, or 0.1 s (BIOPAC Systems Inc., Santa Barbara, CA). A train of stimuli of the selected ISI was delivered through a pair of platinum needles for 120 s or until the animal moved. After delivering a train of one ISI, a period of 2 to 4 min was allowed before delivering the next train of stimuli. The trains were delivered in order of descending ISIs, after which the tail clamp was applied. When a train produced a latency of less than 10 s, trains with shorter ISIs were omitted.

For temporally graded stimulation studies using ISI pulses of varying voltage, the stimulating needles were placed while the animals were awake. After determining the threshold voltage that produced movement, isoflurane was increased and equilibrated in steps of 0.1–0.2% isoflurane as previously described. At each step, 10- and 0.1-s ISI pulses of 0.25, 0.5, 1, 2.5, 5, 10, 20, 30, and 50 V were applied as needed to just produce and suppress movement.

The electromyogram was measured from platinum needles placed 1 cm apart in the biceps femoris and in the gastrocnemius-soleus muscles. A differential amplifier was used to record the electromyogram with 80-Hz to 260-kHz bandpass filtering and 1-k/s sampling (BIOPAC Systems Inc.).[17]

For the MK801 studies, 2.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$ MK801 (Sigma-Aldrich, Inc., St. Louis, MO) was administered through an intravenous catheter placed the previous day during isoflurane anesthesia.[18] Previous studies in our laboratory demonstrated that this dose produced a 30–40% decrease in MAC to tail clamp.[18] Two animals were studied concurrently. After placing the stimulation nee-

dles during a brief period of 2% isoflurane administration, we allowed 60 min of equilibration with MK801 and 0.75% isoflurane. Then, we applied 0.5-ms square-wave 50-V pulses of 10, 3, 1, 0.3, or 0.1 s ISI and the tail clamp stimulations at step increases in isoflurane as described previously. MAC for these animals (without MK801) was determined separately the next day.

Statistical Analysis

The MAC value was taken as the MAC to tail clamp for the tail clamp and temporally graded stimulation studies, and as the MAC to 50-Hz stimulation in the 50-Hz stimulation study. The MAC fraction at a crossover for a response was calculated as the midpoint of the concentrations just permitting and suppressing the response for that animal divided by the MAC. Group MAC fraction means ± SD were calculated.

Motor responses to stimulation were classified as muscle tone or purposeful movement. Tone was defined as the progressive buildup of muscle contraction that produced sustained lifting of the head or pelvis, usually in a ratcheting motion, appearing after several stimuli. Consistent with previous MAC studies, movement was defined as a complex rhythmic response such as pawing, head shaking, or generalized body shudder, or as a rapid limb withdrawal or extension, neck flexion, or spine arching. A positive electromyographic response to stimulation was defined by sustained electromyographic activity (*i.e.*, that which continued during the interstimulus interval) at more than 1.0 μV root-mean-square.[17]

Latency was measured from the onset of stimulation to the onset of movement. Two investigators measured latencies; one investigator applied the stimulation and the other measured the latency with a stopwatch, and both judged the onset of movement.

We used one- and two-factor ANOVA (StatView, Abacus Concepts, Inc., Berkeley, CA) to determine the significance of effects of time intervals, ISI, MK801 administration, stimulus voltage, and response endpoints. A P value of < 0.05 was regarded as significant for all comparisons.

Results

Latencies from onset of tail clamp and 50-Hz stimulations to onset of movement were less than 1 s at concentrations of up to 0.6 MAC (fig. 1). Further increases in concentration increased the latencies with abolition of movement at concentrations greater than 1.0 MAC. MAC to tail clamp (n = 16) was 1.54 ± 0.13% isoflurane (mean ± SD), and to 50-Hz stimulation (n = 8) was 1.56 ± 0.14%. No animal moved when we extended the period of tail clamp stimulation to 120 s, at the concentration step that just suppressed movement in eight animals (*i.e.*, for an additional 60 s), indicating that the stimulation period for tail clamp need not exceed 60 s.

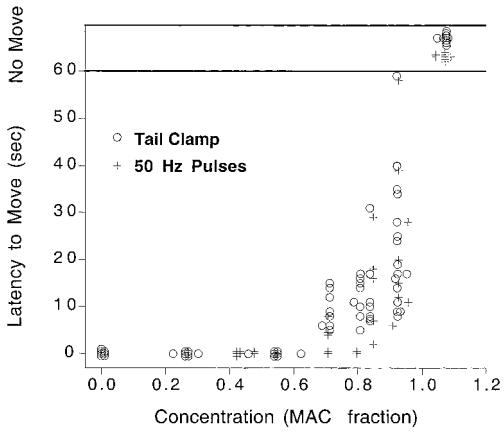

Fig. 1. Latency from the onset of noxious stimulation to the onset of purposeful movement progressively increases as the isoflurane concentration increases from 0.6 to nearly 1.0 MAC (the minimum alveolar concentration producing immobility in 50% of subjects receiving noxious stimulation); *i.e.*, longer periods of stimulation are required to compensate for the increasing suppressing effect of isoflurane concentrations approaching MAC. Each animal contributed data points at several concentrations.

For six of the eight animals studied (one animal did not move to tail clamping and there were technical problems with another) at various intervals between stimulation, we found that intervals of 2–16 min between successive tail clamps provided a latency that did not differ from that found with the initial tail clamp (fig. 2). ANOVA yielded an insignificant effect of time ($F_{5,30} = 1.4$, $P = 0.24$).

For each ISI, latency increased with increasing isoflurane concentration (fig. 3). The upswing in latency appeared to have the same steepness at a given ISI, but occurred at greater anesthetic concentration with decreasing ISI. Data for 3- and 0.3-s ISI pulses were intermediate between the 10- and 1-s and the 1- and 0.1-s pulses, respectively, and have been omitted for clarity. In a separate test in four rats, 10-s ISI pulses were continued for 4 min at the concentration step that had

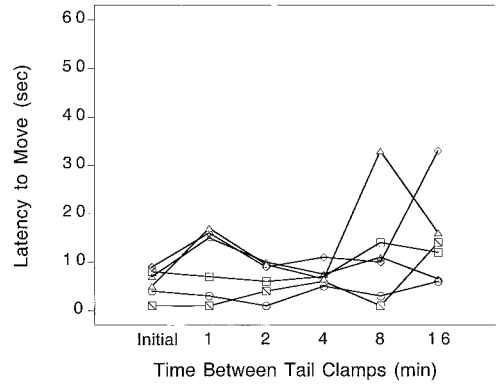

Fig. 2. Latency from onset of tail clamp to onset of movement does not differ as a function of the interval between successive tail clamp stimulations. *Lines* connect the data points of individual animals.

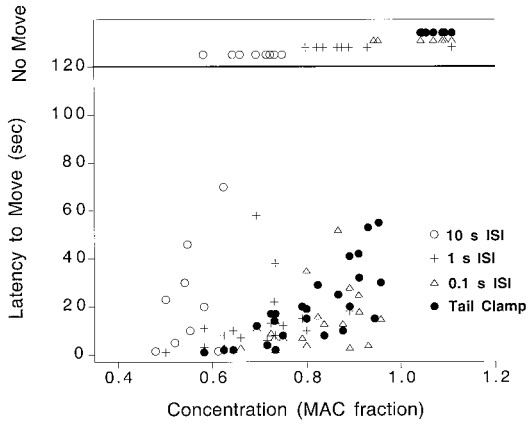

Fig. 3. Latency from onset of stimulation to onset of movement increases as a function of increasing isoflurane concentration and increasing interstimulus interval (*ISI*). For a given concentration, shorter ISIs produce shorter latencies, showing that more frequent stimulation restores the generation of movement. Each animal provided data for each ISI and tail clamp determination.

just suppressed movement; no movement occurred, indicating that (as with tail clamp) the stimulation period for electrical stimulation need not exceed 60 s. In summary, for a given isoflurane concentration within the concentration range of 0.6 to nearly 1.0 MAC, pulses with shorter ISIs (*i.e.*, more frequent stimulation) produced shorter latencies. Decreasing the ISI compensated for the effects of increasing concentration.

The crossover concentrations (expressed as fractions of the MAC to tail clamp) required to achieve immobility were a function of the ISI (fig. 4). For the train of pulses with a 10-s ISI, the crossover concentration was 0.62 ± 0.04 of the MAC to tail clamp. Single-factor ANOVA yielded a significant effect of ISI ($F_{4,35} = 33.5$, $P < 0.001$).

MK801 flattened the response slope to decreasing ISIs (fig. 4). The response slope refers to the change in

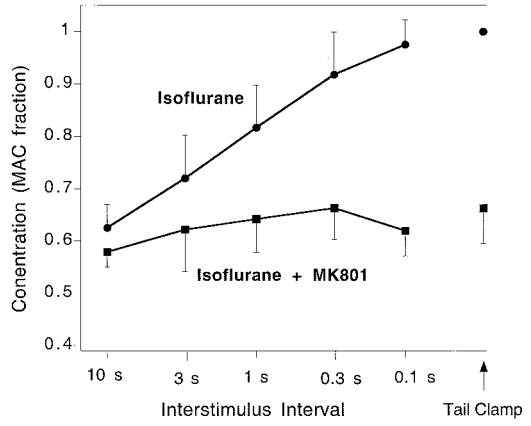

Fig. 4. Isoflurane concentrations (mean ± SD) required to produce immobility in response to noxious stimulation increase with decreasing interstimulus interval. *MK801* (an *N*-methyl-D-aspartate antagonist that impairs temporal summation) administration added to isoflurane administration prevents shorter interstimulus intervals from provoking movement at greater isoflurane concentrations.

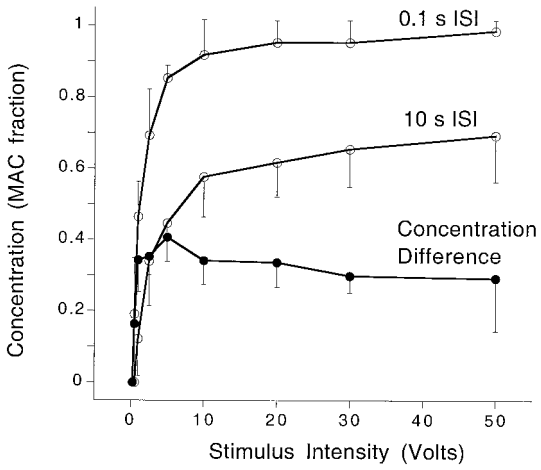

Fig. 5. Isoflurane concentrations (mean ± SD) required to produce immobility increase with increasing stimulation voltage. However, for a given voltage greater than 1 V, the concentration differences between 0.1- and 10-s interstimulus interval (*ISI*) pulses remained unchanged. The finding of an unchanging concentration difference across an isoflurane range from 0.0 to nearly 1.0 *MAC* (the minimum alveolar concentration producing immobility in 50% of subjects receiving noxious stimulation) suggests that isoflurane may not be altering the process of temporal summation itself. The 50-V ISI pulses provided supramaximally intense stimulation.

crossover concentration as a function of change in ISI. Single-factor ANOVA yielded an insignificant effect of ISI during MK801 infusion ($F_{4,25} = 1.7$, $P = 0.18$). Two-factor ANOVA comparing the administration of MK801 plus isoflurane to isoflurane alone yielded significant main effects of MK801 administration ($F_{1,60} = 139.4$, $P < 0.001$) and ISI ($F_{4,60} = 22.1$, $P < 0.001$), and significant interaction between the two ($F_{4,60} = 12.2$, $P < 0.001$).

During the varying voltage studies, the isoflurane concentrations required to achieve immobility to 10- and 0.1-s ISI pulses increased with increasing voltage (fig. 5). But for voltages greater than 1 V, the difference between crossover concentrations for the 10- and 0.1-s pulses remained unchanged, an increment of about 0.35 MAC. Therefore, the response slopes to the ISI pulses did not change with increasing voltage or concentration of isoflurane ($P > 0.05$). The 50-V stimulus was supramaximal for the 10- and 0.1-s ISI pulses, providing no greater crossover concentrations than the 30-V stimulus.

The latency to activation of the electromyogram decreased as the ISI decreased. For example, at 0.79 MAC, after a 33-s stimulation with pulses separated by 3 s, the electromyographic activity began to build up after each pulse, and then faded away (fig. 6). These pulses did not produce movement. In contrast, the shorter ISI pulses produced a buildup of electromyographic activity eventually culminating in a burst of sustained electromyographic activity and movement.

The crossover concentrations that abolished buildup of muscle tone and electromyographic activation slightly exceeded those that abolished purposeful movement,

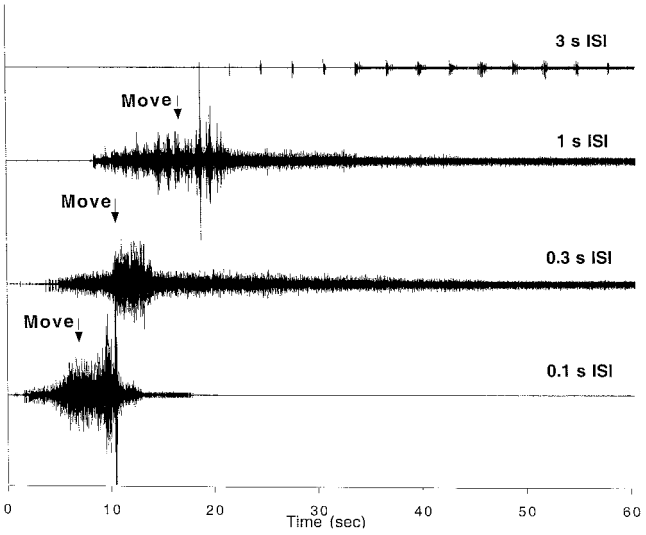

Fig. 6. Interstimulus interval (*ISI*) duration during 0.79 MAC (the minimum alveolar concentration producing immobility in 50% of subjects receiving noxious stimulation) isoflurane influences electromyographic responses (results for one animal). By 33 s of stimulation with 3-s ISI pulses, electromyographic activity develops after each pulse but then fades and movement does not occur. During stimulation with shorter ISI pulses, the latency to electromyographic activation decreases and movement occurs. Stimulation was discontinued after movement occurred.

ranging from 0.00 ± 0.00 to 0.18 ± 0.06 MAC, for the two responses and the five different ISIs. Pooling the concentrations differences for the ISIs for each response yielded overall concentration differences to tone and electromyographic activation *versus* movement of 0.05 ± 0.09 and 0.05 ± 0.07 MAC, respectively. Two-factor ANOVA showed that the main effects of crossover concentrations for tone and electromyographic activation *versus* movement were significant ($P = 0.03$ and $P < 0.001$, respectively). Taken together, these results show that although movement is slightly more vulnerable to the suppressive effects of isoflurane, all three responses are similarly affected.

Discussion

The physiologic processes underlying the production of movement in response to noxious stimulation remain unclear, as do the mechanisms underlying the anesthetic-induced disruptions of these processes. The noxious stimulation used to evoke movement for MAC determinations is supramaximal in intensity and continuous in duration.[1–5] By applying electrical pulses of graded ISI, we separated the temporal and intensity components of the stimulation and found that generation of movement involves temporal summation.

We have shown the effect of isoflurane on temporal summation in three ways. First, latency increases with increasing concentration, a finding consistent with an increasing need to accumulate the effects of stimulation in order to generate movement. For supramaximally intense stimuli, this latency increase begins at concentrations exceeding 0.6 MAC. Second, shortening the ISI increases the isoflurane concentration required to suppress movement. Shortening the ISI increases the rate at which the stimulus effects are accumulated, and additional isoflurane is required to counterbalance this accumulation. Put differently, for a given concentration, decreasing the ISI restores the capacity to generate movement. However, even the continuous simulation provided by a tail clamp could not produce movement at a sufficient concentration of isoflurane (*i.e.*, at MAC). Third, blocking temporal summation decreases the isoflurane concentration required to suppress movement. Administration of MK801, a NMDA antagonist that impairs temporal summation,[11,12] decreases the MAC to tail clamp and blocks the capacity of shorter ISI pulses to restore generation of movement to the pulses. The results to tail clamp are to be expected since NMDA antagonists reduce MAC.[18–20] However, the MK801 effect progressively decreases as the ISI increases, having no effect on the generation of movement to 10-s ISI pulses. These findings are consistent with MK801 blockade of temporal summation.

These results might appear to suggest that isoflurane produces immobility in part by disrupting mechanisms underlying temporal summation. This notion would be consistent with the sensitivity of the slow ventral root potential, a sustained depolarization evoked in isolated fetal spinal cord motoneurons that appears to be mediated by mechanisms involved in windup, to isoflurane.[21,22] This might also be consistent with suppression of the response of NMDA receptors expressed in oocytes to the application of glutamate by about 20% at 0.5 MAC and 40% at 1.0 MAC isoflurane.[23,24] However, there is also support for an alternative hypothesis, that the mechanisms underlying temporal summation remain relatively intact during isoflurane concentrations up to 1.0 MAC. We found that the response slopes of crossover concentrations to 10- and 0.1-s ISI pulses of varying voltage remain unchanged over this concentration range (fig. 5). Petersen-Felix *et al.* found similar stability of response slopes for electromyographic evidence of temporal summation during isoflurane in humans.[13] The stable response slopes suggest that isoflurane suppresses the effect of increasing intensity of stimulation, yet the processes underlying temporal summation itself remain relatively unaltered.

Instead of blocking the mechanisms mediating temporal summation, isoflurane may suppress the stimulus effect available for summation, thereby indirectly affecting temporal summation. Alternatively, isoflurane may suppress the downstream results of temporal summation. Either hypothesis would be consistent with our finding that similar concentrations were required to sup-

press electromyographic activation, muscle tone, and purposeful movement responses, a similarity suggesting that isoflurane suppressed mechanisms common to all these responses even though they likely have different underlying mechanisms. Craniofacial electromyogram and movement responses are also suppressed by similar concentrations of isoflurane in humans.[17]

The direct impairment of processes underlying temporal summation may be of greater importance for anesthetic agents that more markedly suppress NMDA receptors, such as nitrous oxide, xenon, and ketamine.[25–27] Consistent with this concept, ketamine (whose primary effect appears to be to block NMDA receptors) suppresses temporal summation measured by electromyographic responses to electrical stimulation.[27]

Temporal summation is defined as an algebraic summation of voltage changes that occur when second and subsequent depolarizations temporally overlap previous depolarizations before they decay completely. While the specific mechanisms underlying the temporal summation involved in generating movement are unclear, some of the processes involved in temporal summation are suggested by flexor reflex studies.[7–9] For example, trains of 1-s ISI C-fiber strength pulses applied to spinal cord afferents produce excitatory postsynaptic potentials in dorsal and ventral horn neurons that, in some cells, cause a cumulative depolarization (*i.e.*, a progressive decrease in the transmembrane potential), which upon reaching a threshold results in a burst of action potentials. With cessation of stimulation, the firing gradually fades as the transmembrane potential decays back toward resting levels, with a decay half-life of several seconds.[7–9] Such cumulative depolarization produces an expansion of receptor fields and decreases the threshold of dorsal horn neurons. This depolarization can also facilitate flexor withdrawal reflexes, an indication of central sensitization.[28,29] The processes underlying the cumulative depolarization and slow ventral root potential involve mechanisms that include NMDA, neurokinin, and metabotropic receptor activity and release of substance P.[11,12,23,24,30] For ISI pulses shorter than 1 s, temporal summation may also involve short-term plasticity processes such as facilitation.[31,32]

In summary, we find that temporal summation mediates part of the movement produced in response to noxious stimulation in animals inhaling isoflurane. Immobility may result from suppression of mechanisms underlying the summation process itself or from suppression of the stimulus effect available for accumulation or the downstream results of summation.

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