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Validation and Insights of Anesthetic Action in an Early Vertebrate Network: The Isolated Lamprey Spinal Cord

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Abstract

Background—The lamprey spinal cord is a well-characterized vertebrate network that could facilitate our understanding of anesthetic action. We tested several hypotheses concerning the lamprey's clinical application to anesthesia, and the sites/mechanisms of anesthetic action.

Methods—In isolated lamprey spinal cords, minimum immobilizing concentrations (MIC) were determined for halothane, isoflurane, sevoflurane, desflurane, propofol, or the nonimmobilizer F6 (1,2-dichlorohexafluorocyclobutane)- applied during D-glutamate-induced fictive swimming or noxious tail stimulation. Isoflurane and propofol effects on fictive swimming were tested in the presence and absence of strychnine and/or picrotoxin.

Results—Volatile anesthetic MICs were clinically comparable. Isoflurane MIC for fictive swimming and noxious stimulus-evoked movement were the same. F6 did not produce immobility, but decreased the amplitude and phase lag of fictive swimming. Isoflurane decreased fictive swimming cycle frequency, amplitude, autocorrelation, rostrocaudal phase lag, and coherence. Strychnine and picrotoxin elicited only disorganized motor activity under isoflurane and caused small increases in MIC. Propofol's effects differed from isoflurane for all locomotor rhythm variables except amplitude. The propofol MIC was much larger in lampreys compared to mammals. However, picrotoxin reversed propofol-induced immobility by reinitiating coordinated locomotor activity and increasing MIC >8-fold.

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DISCLOSURES

Name: Steven L. Jinks, Ph.D.

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Attestation: I have reviewed and approved the final manuscript, data and its analysis, and serve as archival author.

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Conclusions—The lamprey spinal cord is a relevant and tractable vertebrate network model for anesthetic action. Isoflurane disrupts interneuronal locomotor networks. Gamma-aminobutyric acid A and glycine receptors play marginal roles in isoflurane-induced immobility in lampreys. Propofol's selective gamma-aminobutyric acid A_A-receptor-mediated immobilizing mechanism is conserved in lampreys. The differential immobilizing mechanisms of isoflurane versus propofol reflect those in mammals, and further suggest different network modes of immobilizing action.

Introduction

The sites and mechanisms by which anesthetics disrupt neuronal networks to abolish memory, consciousness, and movement remain poorly understood. The well-characterized vertebrate locomotor network of the lamprey could promote greater understanding of anesthetic mechanisms. Our aims were to validate the lamprey as a model for anesthetic immobilizing action, and investigate some of the sights and mechanisms of anesthetic-induced immobility in the lamprey spinal cord.

Anesthetic-induced immobility is gauged by the minimum alveolar concentration (MAC) necessary to block intersegmentally mediated movement in response to a supramaximal noxious stimulus (1). Although the degree of supraspinal effects varies across anesthetic classes (2-4), the spinal cord is considered the primary locus for anesthetic-induced immobility (5,6). Spinal immobilizing effects appear to be mediated primarily within the ventral horn (7-9), where there reside motor neurons and locomotion-generating interneuronal networks, termed the central pattern generator (CPG). In isolation, spinal locomotor networks of both lamprey and mammals produce coordinated rhythmic motor output (10,11), which is typically assessed in MAC determination (12).

The lamprey has the best understood vertebrate locomotor system and shares basic anatomical, functional, and neuropharmacological properties with other vertebrates including mammals (13). The spinal cord is less than 300 μ m thick and avascular (14), allowing rapid drug equilibration. Lamprey swimming is produced by a laterally displaced wave that undulates rostrocaudally along the body, with a constant intersegmental phase lag regardless of swimming speed (cycle frequency). Generating this behavior is rhythmic activity of excitatory and inhibitory CPG interneurons. The CPG in turn establishes rhythmic membrane potential oscillations in motoneurons and appropriate segmental timing of these oscillations to achieve left-right and rostrocaudal coordination (15). Glutamate receptor agonists applied to isolated spinal cords elicit a pattern of activity among ventral roots called "fictive locomotion" that closely matches the in vivo swimming pattern (16), analogous to isolated mammalian spinal cords (13). Fictive swimming remains stable for hours, permitting detailed examination of anesthetic effects on organized movement. The spinal cord contains relatively few neurons, and several classes of locomotor interneurons have been identified and characterized (17). Therefore the lamprey is a tractable vertebrate network for anesthetic studies. Versus 1 is a simplified lamprey locomotor network depicting essential CPG interneurons and their projections to motoneurons (see (18) for a recent review).

The lamprey spinal cord has been subjected to few anesthetic studies (19,20). Furthermore, the lamprey's clinical relevance is not established, and it has not been used to its full potential regarding anesthetic mechanisms. We hypothesized that anesthetic immobilizing action in the lamprey spinal cord resembles that in mammals. If so, anesthetics should have similar immobilizing potencies to those in mammals, and nonimmobilizing agents (21) should behave as such in lampreys. MAC should not substantially differ for fictive locomotion (where ventral locomotor circuits are selectively activated) compared to noxious stimulus evoked movement. The gamma-aminobutyric acid A (GABA_A) antagonist

microtoxin should produce a greater reversal of propofol's immobilizing action compared to isoflurane (22). We further explored potential molecular and network modes of anesthetic action in lamprey locomotor circuitry, by comparing isoflurane versus propofol effects on several variables of the locomotor rhythm, and by testing the ability of inhibitory receptor antagonists to restore such variables after anesthetic-induced immobility.

Methods

The University of California, Davis institutional animal care and use committee approved this study. Adult silver lampreys (*ichthyomyzon unicuspis*) were housed in continuously filtered and aerated fresh-water aquaria, and kept cooled 1-4 deg C to produce torpor.

Drugs

D-glutamate, microtoxin, and strychnine were purchased from Sigma (St. Louis, MO). Anesthetics were purchased from the following manufacturers: desflurane (Baxter, Deerfield, IL), halothane (Halocarbon Laboratories, River Edge, NJ), isoflurane (Minrad, Bethlehem, PA), sevoflurane (Abbot Labs, Chicago, IL), and propofol (Hospira, Lake Forest, IL). The nonimmobilizer F6 (1,2-dichlorohexafluorocyclobutane) was purchased from Synquest Labs (Alachua, FL).

Isolated spinal cord preparation and ventral root recording

Lamprey spinal cords and attached notochords were excised and pinned through the notochord to a sylgard-bottom bath containing Ringer's solution (in mM: 91.0 NaCl, 2.1 KCl, 2.6 CaCl₂, 1.8 MgCl₂, and 20 NaHCO₃). The Ringer's solution was bubbled with 95% O₂/ 5% CO₂ (mixed with or without anesthetics/drugs) in a cooled water-jacketed chamber. F6 was delivered using an enflurane vaporizer calibrated to F6 using gas chromatography (Gow-MAC Instruments, Bridgewater, NJ) according to previously published methods (21). Solutions were delivered to a cooled water-jacketed bath containing the spinal cord and suctioned off to create laminar flow across the full length of the spinal cord. Bath temperature was kept 7-9° C throughout the experiment. Some preparations included a small piece of attached tail end (~2 cm) to permit noxious stimulation. Silver-wire / glass pipette suction electrodes were placed on ventral roots to record motor activity. Ventral root activity was amplified and band-pass filtered 10 Hz-5 kHz (model 1700 differential amplifier, A-M Systems, Carlsborg, WA) and acquired on a PC at 3 kHz sampling rate (power 1401 with Spike2 software, Cambridge Electronic Design, Cambridge, UK).

Experimental Design

Fictive swimming was induced with 450- 750 μ M D-glutamate, which equilibrated for at least 30 min before data collection began. Noxious mechanical stimulation was applied with an arterial clamp placed on varying locations of the tail end. Anesthetics or F6 were delivered to isolated spinal cords to test their effects on fictive swimming or motor responses to noxious tail stimulation. Equilibration times were at least 20 minutes for desflurane, isoflurane, and sevoflurane, at least 30 min for halothane and F6, and at least 45 minutes for propofol. In pilot experiments we did not find a significant difference in minimum immobilizing concentrations (MIC) when equilibration time was increased to twice that of the above minimum values.

We use the term "minimum immobilizing concentration" (MIC) in place of "MAC" to define anesthetic immobilizing potency, as both volatile and IV anesthetics were delivered in vitro to cooled Ringer's solution in the present study. Anesthetic concentrations were incrementally increased 15-30% until ventral root activity was abolished. In some

experiments we bath-applied picrotoxin or strychnine under 1.1-1.2 MIC. If activity returned we again increased the anesthetic concentration until immobility occurred.

In tail-attached experiments, isoflurane MIC for noxious stimulus-evoked motor responses and MIC for fictive swimming were determined in the same animal. When testing MIC for fictive locomotion, we placed a Vaseline barrier 5 segments rostral to the intact tail end, which was bathed in D-glutamate-free Ringer's solution to prevent tail movement (and thus sensory feedback). The Vaseline barrier was removed for isoflurane MIC determination for noxious stimulation (in D-glutamate-free Ringer's solution) commenced. After testing MIC under one condition, we washed out isoflurane for at least one hour before testing MIC under the other condition. The order of MIC testing under the two conditions was switched from experiment to experiment.

Data Analysis

MIC was calculated as the average of the two concentrations that bracketed immobility. For isoflurane and propofol experiments, at least two ventral roots were recorded on the same side. Rhythmic ventral root bursting was rectified and integrated. Two-minute segments of data were analyzed for each treatment using script language in Spike2 (Cambridge Electronic Design, Cambridge, UK). We measured isoflurane's and propofol's effect on several variables of the lamprey locomotor rhythm (cycle frequency, cycle amplitude, autocorrelation, rostrocaudal phase lag, and rostrocaudal coherence). Versus 2 depicts how we obtained these measurements from ventral root activity during fictive swimming. We measured cycle frequency and amplitude using peak detection in rectified and integrated waveforms of ventral root activity (Fig. 2A). Cycle frequency (Hz) is the number of cycles ("bursts") per second, or $1/T$, where T is a period of the waveform. Amplitude is the trough-to-peak height of each burst averaged across all cycles in the time segment analyzed. We also measured the autocorrelation peak for each channel. Autocorrelation is a point-by-point correlation of a given channel with itself and is essentially a measure of rhythm regularity. That is, given the occurrence of a ventral root burst, how predictable the following burst is across all cycles in the analyzed time segment. Rostrocaudal phase lag was calculated as the proportion lag/T , where lag is the within-cycle time lag between the two channels of bursting activity (Fig. 2A). Lag was calculated by obtaining the time when the first peak occurred in the cross-correlation between the two channels (Fig 2B). Swimming in intact lampreys is executed with one wavelength placed across the entire 100 body segments at all times. Thus, rostrocaudal phase lag per segment is approximately 0.01, or 1% of the cycle period. Finally, we measured rostrocaudal spectral coherence between two channels. Coherence measures the similarity between the frequency spectra of two channels and is a measure of coordination (phase-locking). Coherence values range from 0 to 1, where 0 indicates the activity between two channels is completely independent, and 1 indicates that the phase lag and amplitude ratios between the two channels are constant at a given frequency (Fig. 2B). Equation 1 shows the basic formula for coherence, which is essentially the ratio of the cross-spectral density to the within channel spectral densities. Coherence analysis has been previously applied to lamprey locomotor activity (19,23) as well as other biological rhythms.

Equation 1. Coherence at frequency f is given by:

$$\text{coh}(f) = \frac{|\sum \text{csd}_{ab}(f)|^2}{\sum \text{psd}_a(f) \sum \text{psd}_b(f)}$$

Where, csd is the cross power spectral density between the two channels (a,b), and psd is the within-channel power spectral density.

Statistical Analysis

For data involving multiple treatments, we used a general linear model with treatment set as a fixed factor and animal designated a random effects factor. Overall significance warranted post hoc Tukey multicomparison tests. Single-pair comparisons were analyzed by a student's t-test. An F-test was used to compare variances. A p-value <0.05 was considered statistically significant. Graphical data are presented as mean $\pm$ SEM.

Results

Anesthetic immobilizing requirements in lampreys

Relative immobilizing potencies among four volatile anesthetics paralleled those determined in mammals. Lamprey MIC values for four volatile anesthetics and propofol are shown in Table 1. Note that MIC partial pressures are lower compared to mammals because anesthetics were delivered to 7-9° C Ringer's solution. Interpolation of a gas chromatograph analysis curve from our prior study (19) yielded a mean aqueous isoflurane MIC value of 0.38 mM for the present study. In contrast to volatile anesthetics, propofol MIC in lampreys was well above that in mammals (Table 1). However, picrotoxin produced a robust reversal of propofol-induced immobility (see below, "Effects of inhibitory blockade on isoflurane and propofol-induced immobility").

Isoflurane MIC for noxious tail stimulation and fictive swimming

Isoflurane MIC (n=9) was not significantly different for fictive swimming (0.64% $\pm$ 0.07 SD) compared to supramaximal noxious mechanical tail stimulation (0.63% $\pm$ 0.09% SD). Six of nine animals had identical MIC values for both conditions. Furthermore, under the minimum isoflurane concentration that abolished D-glutamate-induced fictive swimming, the additional application of noxious tail stimulation did not result in a significantly higher MIC value (0.67% $\pm$ 0.09 SD). In this situation the addition of tail stimulation failed to produce any motor response in seven of nine animals. An individual example of isoflurane effects on fictive swimming and noxious tail stimulation is shown in Versus 3.

Nonimmobilizer effects on fictive swimming

The nonimmobilizer F6 did not produce immobility (n=6) up to 6.4% alone, or up to 4.3% in combination with 0.5 MIC isoflurane (n=3). However, F6 significantly decreased burst amplitude and rostrocaudal phase lag of fictive swimming. F6 had no effect on cycle frequency, autocorrelation, or coherence. An individual example of F6 effects on fictive swimming are shown in Figure 4A, and mean effects of F6 on locomotor rhythm variables are shown in Figure 4B.

Isoflurane versus propofol effects on fictive swimming

In whole-cord experiments, we measured isoflurane (n=14) and propofol (n=13) effects on several variables of the locomotor rhythm-defining fictive swimming. Isoflurane significantly affected all measured variables, including burst amplitude, cycle frequency, autocorrelation, rostrocaudal phase lag, and spectral coherence. At 0.5 MIC, isoflurane significantly decreased cycle frequency (p= 0.027), burst amplitude (p= 0.008) and autocorrelation (p= 0.043) while increasing phase lag (p= 0.010). At 0.8 MIC, isoflurane reduced cycle frequency (p= 0.048), burst amplitude (p< 0.001), autocorrelation (p< 0.001), and coherence (p< 0.001). At 0.8 MIC isoflurane, apparent phase-locking failures led to a significant increase in phase lag variance (p< 0.003). This was supported by the significant, substantial decrease in coherence at 0.8 MIC isoflurane (but not under 0.5 MIC). An example of isoflurane effects on fictive swimming are shown in Figure 5, and mean effects are shown in Figure 6 (solid bars).

Propofol produced a different profile of effects on locomotor rhythm variables compared to isoflurane. The exception was propofol's reduction in burst amplitude at 0.5 MIC ($p=0.006$) and 0.8 MIC ($p<0.001$), which was similar to isoflurane at equipotent concentrations. Propofol did not produce a significant change in cycle frequency or rostrocaudal phase lag up to 0.8 MIC. Propofol at 0.8 MIC caused small but significant reductions in autocorrelation ($p=0.010$) and coherence ($p=0.006$). However autocorrelation and coherence were reduced significantly less by propofol than by isoflurane at equipotent concentrations ($p<0.001$ in both cases; unpaired t-tests). The mean effects of propofol on fictive swimming are shown in Figure 6 (open bars).

Effects of inhibitory blockade on isoflurane and propofol-induced immobility

To test the potential roles of GABA_A and glycine receptors in mediating isoflurane immobilizing effects in lampreys, we bath-applied picrotoxin ($n=6$) or strychnine ($n=6$) once immobility was achieved. Both picrotoxin and strychnine caused small but significant (16-20%) increases in isoflurane MIC ($p=0.012$ and $p=0.007$, respectively; paired t-test). However with either antagonist, any activity that returned consisted of highly irregular bursting or tonic activity. An individual example of strychnine and picrotoxin's effect on isoflurane-induced immobility is shown in Figures 7A and 7B, respectively. The mean antagonist effects on isoflurane MIC are shown in Figure 7D.

The effects of picrotoxin under propofol were in stark contrast to the effects of picrotoxin under isoflurane. Picrotoxin increased propofol MIC values to 836% of control ($p<0.001$; paired t-test). In addition, 2-5 μ M picrotoxin elicited stable, coordinated fictive swimming under 1.2 MIC propofol. Cycle frequency, phase lag, autocorrelation and coherence values returned to baseline levels. Picrotoxin increased burst amplitude to 52% of baseline, which was significantly less than baseline ($p<0.001$), but significantly more than burst amplitude under 0.8 MIC propofol alone ($p<0.001$). An individual example of picrotoxin's effect on propofol-induced immobility is shown in Figure 7C. The mean effects on MIC are shown in Figure 7D, and mean variables of fictive swimming under 1.2 MIC propofol after picrotoxin reversal are shown in Figure 7E.

Discussion

A major finding was that key sites and processes of anesthetic immobilizing action are conserved throughout vertebrate evolution. The data support the lamprey isolated spinal cord as a relevant model to investigate anesthetic-induced immobility, and extend findings concerning the mechanisms and network modes of isoflurane and propofol-induced immobility. The data also suggest that fictive swimming is relevant to the study of anesthetic effects in locomotor networks, where many anesthetics appear to exert major immobilizing effects (see Introduction).

Anesthetic immobilizing requirements in lampreys

Relative immobilizing potencies among four volatile anesthetics closely matched those in mammals. Aqueous-phase isoflurane immobilizing concentrations were interpolated from our prior study (19) using gas chromatography analysis of aqueous-phase isoflurane concentrations obtained from lamprey Ringer's solution at the same temperature. This calculation yielded a value approximately 20% higher than typical mammalian MIC values (24), but lower than some mammals including juvenile rats (25) and adult rabbits (26). Unlike volatile anesthetics, propofol MIC in lampreys was several-fold higher than in mammals (3,27). However as in mammals (22), the GABA_A receptor antagonist picrotoxin reversed immobility, producing large increases in propofol MIC (see below, "Effects of inhibitory blockade on anesthetic-induced immobility"). The reported long equilibration

time of propofol could in part account for this difference (28), but changing the equilibration time for propofol from 45 min to 90 min did not change MIC values in lampreys. The relative specificity of propofol might contribute to large MIC differences among vertebrate orders, compared to volatile anesthetics with multiple molecular targets (29). A prior study determined that the ketamine MIC was larger in lampreys than in mammals, yet ketamine retained its selective N-Methyl-d-Aspartate-receptor-mediated immobilizing mechanism (20). Another possibility is that propofol has greater supraspinal effects compared to volatile anesthetics. Studies in rat spinal cord slices show that propofol suppresses ventral horn neuronal activity, but with limited efficacy compared to sevoflurane (30,31).

We found that isoflurane requirements to block fictive swimming were the same as those required to block organized motor responses elicited through activation of nociceptive sensory pathways (Figure 3). During fictive locomotion in both lampreys and mammals, ventral locomotor neurons are selectively activated with sensory neurons remaining inactive (11,32,33). This result implicates “ventral horn” locomotor circuits as the primary spinal site for anesthetic-induced immobility in lampreys. In rats, ventral horn neurons are substantially more sensitive to volatile anesthetics and propofol compared to dorsal horn neurons (7-9). Therefore both vertebrate orders exhibit similar differences in anesthetic sensitivity between sensory and motor components of nociceptive sensorimotor circuits. Additionally, more detailed information may be gleaned from testing anesthetic effects on fictive locomotion, which is highly stable, ongoing and involves selective activation of the spinal circuits most relevant to anesthetic-induced immobility.

Effects of a nonimmobilizer on lamprey fictive swimming

F6 is classified as a “nonimmobilizer,” despite its prediction to behave as an anesthetic based on its oil/gas partition coefficient according to the Meyer-Overton hypothesis (21). As in mammals, F6 failed to halt motor output in lamprey spinal cords above its predicted MAC value of 4.2% (34). Again, delivery of F6 to cold Ringer’s solution likely resulted in much higher aqueous concentrations than achieved in mammals. These data also support the relevance of testing anesthetic effects on fictive swimming networks in the lamprey isolated spinal cord. However, F6 caused a modest reduction in amplitude and phase lag (Figure 4). In general, such modulation of network timing could underlie F6’s known convulsant (21) and/or amnestic (34) effects.

Effects of isoflurane versus propofol on fictive swimming variables

Isoflurane and propofol disrupted fictive swimming in different manners. Isoflurane had significantly greater effects on cycle frequency, phase-lag, autocorrelation, and coherence compared to propofol (Figure 6). These effects support the hypothesis that isoflurane acts intrinsic to CPG interneuronal networks, which generate and coordinate rhythmic activity in motoneurons. Isoflurane potentially delayed normal phase lag, which is mediated by short-range intersegmental coupling (23), possibly excitatory CPG interneurons (35), or “E” cells (Figure 1). Higher isoflurane concentrations just below MIC disrupted phase-locking altogether as assessed by coherence. This is consistent with our prior study, showing that selective isoflurane application to the mid-region of the spinal cord blocks rostrocaudal coordination between the outlying anesthetic-free regions (19). In our prior study, selective isoflurane delivery led to an approximate two-fold higher MIC compared to the present whole-cord MIC values, thus motoneurons were still active well above normal MIC. These differences further suggest that effects on intersegmental networks (including CPG interneurons) dictate isoflurane’s immobilizing capacity. However the current and prior study cannot resolve the precise degree that any motoneuron effects contribute to immobility.

Propofol produced immobility mainly by suppression of burst amplitude, with minimal or no effect on other variables (Figure 6). Therefore propofol's network mode of immobilizing action may be an extrinsic suppression of the CPG or motoneurons. Consistent with data in cultured spinal cord slices, propofol also reduced intraburst firing without changing burst frequency in ventral horn neurons, whereas sevoflurane reduced both measures (30). However, GABA_A receptor antagonists, including picrotoxin, increase fictive swimming speed (36), yet we found that propofol failed to affect cycle frequency. This discrepancy could be attributed to propofol's preference for GABA_A receptor subtypes that generate tonic currents, whereas picrotoxin blocks both phasic and tonic currents, and gabazine selectively blocks phasic currents (37). As gabazine also increases fictive swimming speed in the lamprey, phasic GABA_A receptor currents might be primarily responsible for modulating cycle frequency. Several types of GABAergic neurons have been identified in the lamprey spinal cord (38,39), but few studies have addressed the functional role of these neurons. The present data show that isoflurane and propofol both decreased fictive swimming amplitude similarly at equipotent concentrations. Thus, different classes of anesthetics equally suppressed the magnitude of movement, but apparently through different modes of locomotor network modulation.

Effects of inhibitory blockade on isoflurane and propofol-induced immobility Because glycine receptors are enhanced by isoflurane (40), and play an important role in fictive swimming (35,41,42), we tested the effects of the glycine receptor antagonist strychnine on isoflurane-induced immobility. Although strychnine blocks left-right alternation, mediated by glycinergic C neurons (Figure 1), rhythmic bursting persists with appropriate rostrocaudal coordination and phase-lags (35). However under isoflurane, strychnine failed to restore regular, coordinated activity and only produced small increases in MIC (Figure 7). Therefore glycine receptors appear to play a limited role in isoflurane-induced immobility, as found in rats (43,44). In rat spinal cord slices, volatile anesthetics impart opposing pre- and postsynaptic effects that result in unchanged or decreased glycinergic neurotransmission to motoneurons (45), which may explain the limited role of glycine receptors in isoflurane-induced immobility.

We additionally tested the role of GABA_A receptors in isoflurane-induced immobility using picrotoxin. GABA_A receptors are also enhanced by volatile anesthetics (46), and modulate cycle frequency of fictive swimming (36). However under isoflurane picrotoxin also failed to restore coordinated bursting, or appreciably increase MIC (Figure 7). Because both antagonists elicited only highly disorganized motor output under 1.2 MIC isoflurane and produced only small MIC increases, these data further implicate excitatory CPG interneurons (E neurons) as isoflurane's primary immobilizing target.

In contrast to isoflurane-induced immobility, picrotoxin reversed propofol-induced immobility by restoring robust, regular and coordinated fictive swimming, and by increasing MIC more than eight-fold. Thus propofol's selective GABA_A-mediated immobilizing mechanism in mammals (22,47) is conserved in lampreys. However, picrotoxin did not fully reverse burst amplitude (Figure 7E). Perhaps this was simply because amplitude was most sensitive to propofol. It is also possible that propofol affected other channels (48-51), which also played a role in attenuating fictive swimming. The differential effects of picrotoxin on isoflurane versus propofol-induced immobility are consistent with findings in mammals (22), and again bolster the relevance of studying anesthetic immobilizing effects using fictive locomotion in the lamprey spinal cord.

Conclusions

We demonstrated that fictive swimming in the lamprey spinal cord is a relevant model on which to base anesthetic studies. At least some sites and mechanisms of volatile and IV anesthetic immobilizing actions appear conserved across vertebrate evolution. Different classes of anesthetics produce immobility in lampreys by modulating spinal locomotor networks in different manners. Although the precise mechanism(s) of volatile anesthetics remain unknown, excitatory CPG interneurons appear to play a major role in mediating the capacity of isoflurane to produce immobility. This study paves the way to take full advantage of the lamprey isolated spinal cord, permitting detailed neurophysiological and computational investigations of anesthetic effects in a well-characterized, tractable vertebrate network.

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FIG. 1.

Schematic representation of the lamprey spinal locomotor network. Two essential classes of central pattern generator (CPG) interneurons are shown (enclosed in dotted lines) on the left and right sides of one spinal segment (dashed line indicates midline). Triangles represent excitatory synapses (primarily glutamatergic), and solid circles represent inhibitory synapses (primarily glycinergic). The CPG-motoneuron projections are absent from one hemisegment for clarity. Excitatory interneurons (E) have short rostral and caudal intersegmental axonal projections (~6 segments) to motoneurons (M) and other E neurons. Commissural interneurons (C) have crossed inhibitory projections to contralateral motoneurons and other C neurons, and some have long descending intersegmental projections (~20 segments). C neurons are excited by ipsilateral E neurons. Application of excitatory amino acids such as D-glutamate activates the CPG, which produces motoneuron membrane potential oscillations shaped by alternating rhythmic excitation and inhibition from the CPG. Motoneuron spiking atop these oscillations underlies fictive swimming, monitored by recording rhythmic ventral root (VR) bursting (top 10-second trace).

FIG. 2.

Measurement of locomotor rhythm variables. **A)** Activity was recorded from two ventral roots along the rostral (R)- caudal (C) axis of the spinal cord on the same side. Shown are actual raw traces of ventral root activity during fictive swimming in the lamprey spinal cord, taken from ventral roots #4 and #23 caudal to the last gill arch. Rectified and integrated traces are shown above raw traces. Examples of individual cycle measurements are shown: rostrocaudal time lag (Lag), cycle period (T), and cycle amplitude (A). Cycle frequency ($1/T$) and amplitudes were averaged across the total number of cycles in a two-minute time segment. **B)** Autocorrelation of ventral root #4 (Acr1#4), cross-correlation (Ccrl), and spectral coherence (Coher) graphs are plotted from two-minutes of data encompassing the traces shown above in A. Autocorrelation values were taken from the peak delineated by the arrows. Under baseline conditions the autocorrelation peak occurs at the cycle period (T; vertical dashed line). Cross-correlation was used to measure time-lag between the two channels (time-to-peak shown by dashed line). Coherence values were taken from the peak occurring around the swim cycle frequency. See “Data analysis” in Methods for a detailed explanation of locomotor rhythm variables.

FIG. 3.

Isoflurane (ISO) minimum immobilizing concentrations (MIC) for noxious stimulus-induced movement versus fictive locomotion. **A)** Top diagram shows the preparation used for testing isoflurane's effects on noxious stimulus-evoked motor responses. A small piece of the end of the tail was left attached to the isolated spinal cord. Noxious mechanical stimulation was applied to the tail and motor responses were recorded from ventral roots. Traces are raw ventral root activity under baseline, ISO and recovery conditions. **B)** Top diagram shows the preparation used for testing ISO effects on D-glutamate induced fictive swimming in the same animal. A Vaseline partition was placed several segments rostral to the cut end of the tail so that D-glutamate did not elicit tail movement or cause nociceptor activation. MIC values were the same for each condition in this animal. See text for mean values.

FIG 4.

Effects of the nonimmobilizer 1,2-dichlorohexafluorocyclobutane (F6) on fictive swimming.

A) An example of F6 on fictive swimming. Top left panel: Raw traces of ventral root activity are shown for two ventral roots (bottom traces) 7 segments apart on the right side (R#4 and R#11). The top two traces represent rectified and integrated ventral root activity of corresponding channels. Top right panel: Cross correlation (Ccr1) between the two channels used to calculate rostrocaudal phase lag between the two channels of ventral root activity. Bottom left panel: Fictive swimming under 6.4% F6. Bottom right panel: Ccr1 of fictive swimming under 6.4% F6. **B)** Mean data showing effects of 6.4% F6 (n= 6) on fictive swimming for cycle frequency (Freq), amplitude (Amp), autocorrelation (Acrl), rostrocaudal phase lag (RC Lag), and rostrocaudal spectral coherence (Cohere). *Significantly different from baseline.

FIG 5.

An example of isoflurane effects (ISO) on D-glutamate-induced fictive swimming in the lamprey spinal cord. **A)** Left panel: A 20-second trace of baseline fictive swimming monitored by ventral root recordings from the fourth (L#4) and sixteenth (L#16) segments. The bottom two traces are raw ventral root activity and the top two traces are corresponding rectified and integrated traces. Middle panel: Autocorrelation (Acr1) of baseline ventral root activity of ventral root #16. Right panel: Cross correlation (Ccr1) between the two channels.. The baseline peak spectral coherence value (Cohere) is shown to the right of the graph. **B)** Fictive swimming under 0.5 minimum immobilizing concentrations (MIC) ISO. **C)** Fictive swimming under 0.8 MIC ISO. **D)** Recovery of fictive swimming after washout of ISO.

FIG. 6.

Mean data showing the effects of isoflurane (ISO; solid bars; n= 14) and propofol (PROP; open bars; n= 13) on D-glutamate-induced fictive swimming under baseline (BL), 0.5 minimum immobilizing concentration (MIC) and 0.8 MIC **A)** Percent change in cycle frequency, which was significantly decreased by 0.8 MIC ISO ($p=0.048$), but remained unchanged by PROP. **B)** Percent change in burst amplitude, which both anesthetics decreased similarly at equipotent concentrations. **C)** Percent change in autocorrelation. Propofol's effect on autocorrelation was significantly less than ISO's effect at 0.8 MIC. **D)** Percent change in rostrocaudal phase lag, which was increased by ISO but unaffected by PROP. **E)** Percent change in spectral coherence, which was reduced by PROP significantly less than by ISO at 0.8 MIC. *Significantly different from BL; + significantly different from ISO at equipotent concentrations; +significantly increased variance compared to BL and 0.5 MIC ISO. See text for p-values and further description.

FIG. 7.

Effects of antagonism of fast synaptic inhibition on isoflurane (ISO) and propofol (PROP)-induced immobility. **A)** An example showing strychnine (Strych) effects on ISO-induced immobility. Strych caused erratic ventral root activity, which was again blocked by a small (0.2 MIC) increase in ISO concentration. **B)** An example showing picrotoxin (Picro) effects on ISO-induced immobility. Picro also caused disorganized motor activity, which was blocked by a small (0.2 minimum immobilizing concentrations (MIC)) increase in ISO concentration. **C)** An example showing Picro effects on PROP-induced immobility. Picro restored stable, coordinated fictive swimming that persisted up to 6.0 MIC PROP. **D)** Mean change in ISO MIC (solid bars) by Strych ($n=6$) and Picro ($n=6$), and mean change in PROP MIC (open bars) by Picro ($n=7$). Isoflurane MIC was marginally increased by Picro and Strych. However, PROP MIC was increased more than eight-fold by Picro. **E)** Mean locomotor rhythm variables under 1.2 MIC PROP after the application of 5 μ M Picro. Data are shown for cycle frequency (Freq), burst amplitude (Amp), autocorrelation (Acrl), rostrocaudal phase lag (RC lag), and rostrocaudal spectral coherence (Cohere).

*Significantly different from baseline; + significantly different from change in ISO MIC by Picro. See text for p-values and further description.

Table 1

Anesthetics with corresponding minimum immobilizing concentrations (MIC) values (mean $\pm$ SD) and sample size (n).

Anesthetic	MIC (mean $\pm$ SD)	n
Halothane	0.4 $\pm$ 0.03 %atm	5
Isoflurane	0.62 $\pm$ 0.10 %atm	27
Sevoflurane	1.35 $\pm$ 0.10 %atm	5
Desflurane	3.90 $\pm$ 0.64 %atm	9
Propofol	13.75 $\pm$ 2.17 μ M	13