Published in final edited form as:

Neuroreport. 2011 September 14; 22(13): 655–659. doi:10.1097/WNR.0b013e32834a20f2.

Anesthetic effects on fictive locomotion in the rat isolated spinal cord

Steven L. Jinks¹, Jason Andrada¹, and Omar Satter¹

¹Department of Anesthesiology and Pain Medicine, University of California, Davis CA 95616

Abstract

General anesthetic mechanisms are poorly understood. Anesthetic immobilizing effects occur in the spinal ventral horn. However, a detailed analysis of anesthetic effects on ventral motor networks is lacking. We delivered isoflurane, desflurane, or propofol during NMDA/5-HT-induced, or noxious tail stimulus-evoked, fictive locomotion in neonatal rat isolated spinal cords. Anesthetics changed the frequency, amplitude, and regularity of fictive locomotion with little effect on phase-lag. Isoflurane abolished pharmacologically-induced vs noxious stimulus-induced motor output at similar concentrations. Propofol abolished pharmacologically-induced fictive locomotion via a GABA_A-receptor mechanism. Anesthetic effects on pharmacologically-elicited fictive locomotion appear clinically-relevant, and support a ventral horn immobilizing effect on locomotor rhythm generation.

Keywords

Anesthetics; immobility; locomotion; isoflurane; desflurane; propofol; GABA; Central pattern generator; minimum alveolar concentration; neonate

INTRODUCTION

General anesthetics have a narrow safety margin. However, the mechanisms and neural sites of anesthetic action remain unclear, and volatile agents alter multiple membrane channels [1]. Immobility, a desired anesthetic endpoint and gauge of potency, is determined by the minimum alveolar concentration (MAC) that abolishes movement evoked by supramaximal noxious stimuli [2] (e.g., tail clamp or surgical incision). Such movements are typically locomotor in nature [3]. The spinal cord is the primary site of anesthetic immobilizing action [4], which occurs predominantly in the ventral horn [5,6]. The ventral horn contains motoneurons, as well as excitatory and inhibitory interneurons that form locomotor networks termed the central pattern generator (CPG). The CPG can establish and coordinate rhythmic locomotor activity without descending or sensory input [7].

Some evidence suggests anesthetics affect the CPG [6,8] and motoneurons [9], but the extent that each effect contributes to immobility is unclear. Accessible yet clinically-relevant models are necessary to study anesthetic effects on components of sensorimotor processing. The neonatal rodent isolated spinal cord is used to study mammalian locomotion [10]. Neuropharmacological agonists applied to the spinal cord activate the CPG and elicit prolonged stable “fictive” locomotion [7]. Locomotor activity consists of coordinated rhythmic bursting among ventral root motor axons, and some classes of putative CPG

neurons have been studied [11]. Moreover pharmacologically-induced locomotion preferentially activates ventral horn neurons [7]. Therefore the isolated spinal cord may represent a tractable model in which to conduct anesthetic studies restricted to most relevant neural sites.

We studied anesthetic effects on pharmacological and sensory-evoked locomotion in neonatal rat isolated spinal cords. We hypothesized that clinical concentrations of anesthetics block locomotion in both cases, that propofol immobilizes fictive locomotion via a GABA_A receptor mechanism (as occurs in vivo [4]), and that anesthetic effects on locomotion are consistent with CPG disruption.

METHODS

This study was approved by the UC Davis IACUC.

Dissection and Recording

Neonatal Wistar rats (0–4 days old) were deeply anesthetized by isoflurane or hypothermia. Anesthetized rats were decapitated, eviscerated and placed in cold low Ca⁺⁺, high Mg⁺⁺ oxygenated Ringer's [12]. The animal was pinned ventral side up, and the spinal cord isolated from mid-thoracic to sacral levels. The spinal cord recovered for at room temperature for 1 hr in normal Ringer's (in mM: 118 NaCl, 4.0 KCl, 25 NaHCO₃, 1.2 KH₂PO₄, 1.25 MgSO₄, 2.5 CaCl₂, and 20 glucose). Glass pipette suction electrodes were attached to lumbar ventral roots to record motor activity. Activity was amplified and acquired on a PC at 3kHz using a Power 1401 and Spike2 software (CED, Cambridge, UK). Locomotor activity was induced with bath-applied NMDA (4.5–8 μM) and 5-HT (7–21 μM), purchased from Sigma (St. Louis, MO). Concentrations were adjusted and permitted to equilibrate for at least 20 min until we achieved a steady baseline of coordinated ventral root bursting. Temperature was 22± 1 C throughout the experiment. Isoflurane (Minrad, Bethlehem, PA) or Desflurane (Baxter, Deerfield, IL) was bubbled in 95% O₂/ 5% CO₂ in a temperature-controlled reservoir. Propofol (Hospira, Lake Forest, IL) was diluted directly in Ringer's. Anesthetic solutions were delivered from the reservoir to the spinal cord bath.

Experimental Design and Data Analysis

Locomotor activity was recorded under baseline conditions and anesthetic. Anesthetics were increased in 20–33% increments until fictive locomotion was abolished ("immobility"), defined by a >90% reduction in burst amplitude. The minimum immobilizing concentration (MIC) was the average of the two concentrations bracketing immobility. At each concentration, isoflurane and desflurane equilibrated in the spinal cord for 20 min, and propofol left on for 45 min, before data measurement. The GABA_A antagonist picrotoxin (10–20 μM; Sigma, St. Louis, MO) was applied during propofol-induced immobility (1.2 MIC). In some animals, we determined isoflurane MIC in with the limbs/tail attached [13]. In this case, no NMDA/5HT was added, and we applied noxious mechanical stimulation to the tail with forceps. Isoflurane was delivered as described above. The response was negative if 1 min of tail stimulation failed to evoke motor activity [2].

Data measurements were conducted in Spike2 using 2–3 min of rectified, integrated activity for each condition: baseline, 50% of MIC ("0.5 MIC") and 80% of MIC ("0.8 MIC"). We measured burst amplitude, cycle period, and autocorrelation for each channel. We measured phase-lag and spectral coherence between channels of activity (L2 and L5 on the same side, or L2 on opposite sides). Figure 1 depicts how we obtained data measurements. Statistical analysis was conducted using a general linear model (SPSS, Inc, Chicago, IL). Overall significance warranted posthoc Tukey multicomparisons. Single comparisons were analyzed

by a student's t-test where indicated. A p-value <0.05 was considered statistically significant.

RESULTS

Anesthetic-induced immobility in the neonatal rat isolated spinal cord

For pharmacologically-induced locomotion, isoflurane MIC was $1.1 \pm 0.2\%$ SD (n= 15), desflurane MIC was $6.7 \pm 1.8\%$ SD (n= 12), and propofol MIC was $7.9 \pm 2.5 \mu\text{M}$ SD (n= 7). Examples of anesthetic effects on fictive locomotion are shown in figure 2. Picrotoxin restored coordinated locomotion under immobilizing doses of propofol (figure 2C). Isoflurane MIC for noxious tail stimulus evoked locomotor activity was $1.0 \pm 0.1\%$ SD (n= 6), which was not different from MIC for pharmacologically-induced locomotion (p= 0.42; unpaired t-test). Figure 3 is an example of isoflurane effects on noxious tail stimulus-evoked ventral root activity.

Effects of anesthetics on locomotor rhythm parameters

Table 1 shows mean anesthetic effects on locomotor rhythm parameters, and mean effects of picrotoxin on propofol-induced immobility. Isoflurane, desflurane, and propofol significantly increased cycle period, decreased amplitude, and decreased autocorrelation at both 0.5 MIC and 0.8 MIC (p< 0.02 in all cases). No anesthetic decreased rostrocaudal (L2–L5) coherence at 0.5 MIC, but at 0.8 MIC all anesthetics modestly reduced coherence by 14–22% (p< 0.03 in all cases). Isoflurane and desflurane did not change rostrocaudal phase lag at 0.5 and 0.8 MIC. Propofol did not change rostrocaudal phaselag at 0.5 MIC, but caused a modest phase delay at 0.8MIC (p< 0.02). Picrotoxin restored all parameters of fictive locomotion to baseline levels, except for cycle period (p= 0.018). We also measured left-right coordination in L2 under isoflurane, which was unchanged up to 0.8 MAC.

DISCUSSION

The results confirm studies suggesting anesthetics immobilize through effects in the spinal ventral horn, and further suggest this mode of action is conserved across postnatal development. Results are discussed in relation to the sites/mechanisms of anesthetic-induced immobility and spinal locomotor circuits, with reference to clinical relevance.

Correcting for temperature [14], isoflurane and desflurane MIC values for fictive locomotion are in excellent accord with in vivo MAC determined by noxious stimulation in neonates, which have approximately 60% greater MAC compared to adults [15,16]. Furthermore isoflurane MIC for NMDA/5-HT-induced locomotion was nearly identical to MIC for noxious stimulus-evoked locomotion (a scenario similar to MAC determination). Propofol abolished fictive locomotion at low micromolar concentration (6.7 μM), but somewhat higher than expected, likely due to propofol's hours long equilibration [17], which we could not afford. However, picrotoxin restored coordinated fictive locomotion under immobilizing concentrations of propofol (figure 2). Propofol acts via a GABA_A mechanism on ventral horn activity in cultured spinal slices [18], and in vivo using MAC determination [4]. Because pharmacologically-induced locomotion selectively activates ventral, but not dorsal horn neurons [7,19], the results suggest anesthetics act on ventral horn neurons to abolish fictive locomotion.

Anesthetics modulated the locomotor rhythm, becoming slower, reduced in amplitude, and less regular (Table 1). While amplitude decreases alone could imply motoneuron effects, decreased burst rate and rhythm regularity suggest CPG disruption. Future studies could delineate CPG vs. motoneuron contributions to anesthetic-induced immobility by exploiting the greater rhythm-generating capacity in the rostral lumbar segments [12,20].

Interestingly, phase relationships were largely unaffected, although modest decreases in coherence at higher anesthetic concentrations suggests less stable phase-locking. In mammals, the CPG is proposed to consist of at least two tiers- a rhythm generator, and a pattern formation circuit [10]. Our present results suggest anesthetics act within the CPG to impede rhythm generating interneurons [21], but flexor-extensor and left-right pattern formation networks are resistant. Glycinergic neurotransmission is a major contributor to synaptic inhibition impinging on motoneurons during locomotion [22], and believed to play a critical role in left-right and flexor extensor coordination [23]. Therefore it is possible that volatile anesthetics suppress excitation while leaving glycinergic inhibition unchanged. In support, pre- and postsynaptic volatile anesthetic effects on rat motoneurons led to decreased glutamatergic neurotransmission with less effect on glycinergic inhibition [9,24]. Thus such effects could explain the lack of phase-lag changes presently observed, as well as the limited role of glycine receptors in volatile anesthetic-induced immobility [25].

In conclusion, the present results suggest that studying anesthetic effects on fictive locomotion in isolated spinal cords is clinically relevant. A predominant ventral horn anesthetic immobilizing action appears conserved throughout development, as well as propofol's GABAergic mechanism. However, anesthetic mechanisms in neonates could differ in other respects from adults, as MAC exhibits developmental changes. Suppression of rhythm generation might contribute to general anesthetic immobilizing capacity. Future anesthetic studies in identified classes of spinal neurons are needed to elucidate mechanisms underlying immobility.

Acknowledgments

Grant Support: This study was supported by a National Institutes of Health Grant R01 GM 078167 (SLJ)

References

1. Krasowski MD, Harrison NL. General anaesthetic actions on ligand-gated ion channels. *Cell Mol Life Sci.* 1999; 55:1278–1303. [PubMed: 10487207]
2. Quasha AL, Eger EI 2nd, Tinker JH. Determination and applications of MAC. *Anesthesiology.* 1980; 53:315–334. [PubMed: 6107067]
3. Antognini JF, Wang XW, Carstens E. Quantitative and qualitative effects of isoflurane on movement occurring after noxious stimulation. *Anesthesiology.* 1999; 91:1064–1071. [PubMed: 10519510]
4. Eger EI 2nd, Raines DE, Shafer SL, Hemmings HC Jr, Sonner JM. Is a new paradigm needed to explain how inhaled anesthetics produce immobility? *Anesth Analg.* 2008; 107:832–848. [PubMed: 18713892]
5. Kim J, Yao A, Atherley R, Carstens E, Jinks SL, Antognini JF. Neurons in the ventral spinal cord are more depressed by isoflurane, halothane, and propofol than are neurons in the dorsal spinal cord. *Anesth Analg.* 2007; 105:1020–1026. table of contents. [PubMed: 17898382]
6. Jinks SL, Bravo M, Hayes SG. Volatile anesthetic effects on midbrain-elicited locomotion suggest that the locomotor network in the ventral spinal cord is the primary site for immobility. *Anesthesiology.* 2008; 108:1016–1024. [PubMed: 18497602]
7. Kiehn O, Kjaerulff O. Distribution of central pattern generators for rhythmic motor outputs in the spinal cord of limbed vertebrates. *Ann N Y Acad Sci.* 1998; 860:110–129. [PubMed: 9928306]
8. Jinks SL, Atherley RJ, Dominguez CL, Sigvardt KA, Antognini JF. Isoflurane disrupts central pattern generator activity and coordination in the lamprey isolated spinal cord. *Anesthesiology.* 2005; 103:567–575. [PubMed: 16129982]
9. Cheng G, Kendig JJ. Enflurane decreases glutamate neurotransmission to spinal cord motor neurons by both pre-and postsynaptic actions. *Anesth Analg.* 2003; 96:1354–1359. table of contents. [PubMed: 12707133]

10. McCrea DA, Rybak IA. Organization of mammalian locomotor rhythm and pattern generation. *Brain Res Rev.* 2008; 57:134–146. [PubMed: 17936363]
11. Kiehn O. Locomotor circuits in the mammalian spinal cord. *Annu Rev Neurosci.* 2006; 29:279–306. [PubMed: 16776587]
12. Kjaerulff O, Kiehn O. Distribution of networks generating and coordinating locomotor activity in the neonatal rat spinal cord in vitro: a lesion study. *J Neurosci.* 1996; 16:5777–5794. [PubMed: 8795632]
13. Lev-Tov A, Delvolve I, Kremer E. Sacrocaudal afferents induce rhythmic efferent bursting in isolated spinal cords of neonatal rats. *J Neurophysiol.* 2000; 83:888–894. [PubMed: 10669502]
14. Franks NP, Lieb WR. Temperature dependence of the potency of volatile general anesthetics: implications for in vitro experiments. *Anesthesiology.* 1996; 84:716–720. [PubMed: 8659800]
15. Orliaguet G, Vivien B, Langeron O, Bouhemad B, Coriat P, Riou B. Minimum alveolar concentration of volatile anesthetics in rats during postnatal maturation. *Anesthesiology.* 2001; 95:734–739. [PubMed: 11575548]
16. Taylor RH, Lerman J. Minimum alveolar concentration of desflurane and hemodynamic responses in neonates, infants, and children. *Anesthesiology.* 1991; 75:975–979. [PubMed: 1741518]
17. Gredell JA, Turnquist PA, Maciver MB, Pearce RA. Determination of diffusion and partition coefficients of propofol in rat brain tissue: implications for studies of drug action in vitro. *Br J Anaesth.* 2004; 93:810–817. [PubMed: 15377586]
18. Grasshoff C, Antkowiak B. Propofol and sevoflurane depress spinal neurons in vitro via different molecular targets. *Anesthesiology.* 2004; 101:1167–1176. [PubMed: 15505453]
19. Buchanan JT, Cohen AH. Activities of identified interneurons, motoneurons, and muscle fibers during fictive swimming in the lamprey and effects of reticulospinal and dorsal cell stimulation. *J Neurophysiol.* 1982; 47:948–960. [PubMed: 7086476]
20. Cazalets JR, Borde M, Clarac F. Localization and organization of the central pattern generator for hindlimb locomotion in newborn rat. *J Neurosci.* 1995; 15:4943–4951. [PubMed: 7623124]
21. Hinckley CA, Wiesner EP, Mentis GZ, Titus DJ, Ziskind-Conhaim L. Sensory modulation of locomotor-like membrane oscillations in Hb9-expressing interneurons. *J Neurophysiol.* 2010; 103:3407–3423. [PubMed: 20393069]
22. Cazalets JR, Borde M, Clarac F. The synaptic drive from the spinal locomotor network to motoneurons in the newborn rat. *J Neurosci.* 1996; 16:298–306. [PubMed: 8613795]
23. Kiehn O, Butt SJ. Physiological, anatomical and genetic identification of CPG neurons in the developing mammalian spinal cord. *Prog Neurobiol.* 2003; 70:347–361. [PubMed: 12963092]
24. Cheng G, Kendig JJ. Pre- and postsynaptic volatile anaesthetic actions on glycinergic transmission to spinal cord motor neurons. *Br J Pharmacol.* 2002; 136:673–684.
25. Zhang Y, Wu S, Eger EI 2nd, Sonner JM. Neither GABA(A) nor strychnine-sensitive glycine receptors are the sole mediators of MAC for isoflurane. *Anesth Analg.* 2001; 92:123–127. [PubMed: 11133613]

Figure 1.

Analysis of fictive locomotion. A) Example of L2 and L5 ventral root activity (bottom traces) during NMDA/5-HT-elicited fictive locomotion. Rectified and integrated activity (top two traces) was used to measure cycle period (T), amplitude, autocorrelation, phase-lag and coherence across 2–3 min of activity. B) Autocorrelation of L2 activity from A. Amplitude was used as a measure of rhythm regularity. C) Cross-correlation between L2 and L5 activity from A. Time lag (Lag) was used to calculate phase-lag as a proportion of cycle period T (phaselag= Lag/T). D) Frequency plot of spectral coherence between L2 and L5 activity. Peak coherence (coh) occurs around locomotor burst frequency (1/T). Coherence is the similarity between the cross-spectral and within-channel spectral content of two channels and measures coordination (phase-locking). Details can be found in our prior study [8] using coherence to study anesthetic effects.

Figure 2.

General anesthetic effects on fictive locomotion. Examples from three individual animals showing effects of isoflurane (A) desflurane (B) and propofol (C). Thirty-seconds of ventral root activity is shown (bottom two traces) with rectified, integrated activity of corresponding ventral roots (top two traces). Isoflurane and desflurane abolished fictive locomotion at clinical concentrations, which recovered after washout. Propofol abolished fictive locomotion at low micromolar concentrations, which was reversed by picrotoxin, although cycle period remained greater than baseline.

Figure 3.

Isoflurane effects on noxious tail stimulus-evoked ventral root activity using a hindquarter-attached preparation (diagram on left). Ventral root activity (bottom two traces) is rectified and integrated (top two traces). Noxious mechanical tail stimulation elicited rhythmic L2–L5 ventral root bursting, which was abolished by isoflurane concentrations similar to those that abolished NMDA/5-HT-elicited locomotion. The response recovered after isoflurane washout.

Anesthetic effects on locomotor rhythm parameters
Effects of three general anesthetics on locomotor rhythm parameters, and effects of picrotoxin (ptx) on propofol-induced immobility (1.2 MIC propofol+ ptx).

Table 1

	Isoflurane			Desflurane			Propofol		
	0.5 MAC	0.8 MAC	Washout	0.5 MAC	0.8 MAC	Washout	0.5 MAC	0.8 MAC	1.2MAC+Ptx
Amp	74± 7 *	62± 12 *	114± 9	66± 8 *	43± 8 *	104± 11	61± 8 *	47± 11 *	118± 12
Period	129± 6 *	145± 7 *	119± 11	147± 20 *	163± 21 *	116± 13	134± 14 *	152± 16 *	126± 9 *
Autocorr	87± 5 *	66± 7 *	104± 4	82± 9 *	66± 11 *	97± 7	54± 6 *	41± 11 *	93± 8
Coherence	94± 2	86± 2 *	96± 4	92± 4	78± 4 *	96± 3	93± 3	79± 5 *	93± 5
Phaselag	103± 5	98± 9	103± 6	98± 4	100± 8	104± 4	102± 4	117± 4 *	100± 3

Values are mean± SEM (% of baseline).

* significantly different from baseline (see text in Results).