

Transmission through the Dorsal Spinocerebellar and Spinoreticular Tracts

Wakefulness versus Thiopental Anesthesia

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Background: Most of what is known regarding the actions of injectable barbiturate anesthetics on the activity of lumbar sensory neurons arises from experiments performed in acute animal preparations that are exposed to invasive surgery and neural depression caused by coadministered inhalational anesthetics. Other parameters such as cortical synchronization and motor outflow are typically not monitored, and, therefore, anesthetic actions on multiple cellular systems have not been quantitatively compared.

Methods: The activities of antidromically identified dorsal spinocerebellar and spinoreticular tract neurons, neck motoneurons, and cortical neurons were monitored extracellularly before, during, and following recovery from the anesthetic state induced by thiopental in intact, chronically instrumented animal preparations.

Results: Intravenous administration of 15 mg/kg, but not 5 mg/kg, of thiopental to awake cats induced general anesthesia that was characterized by 5–10 min of cortical synchronization, reflected as large-amplitude slow-wave events and neck muscle atonia. However, even though the animal behaviorally began to reemerge from the anesthetic state after this 5–10-min period, neck muscle (neck motoneuron) activity recovered more slowly and remained significantly suppressed for up to 23 min after thiopental administration. The spontaneous activity of both dorsal spinocerebellar and spinoreticular tract neurons was maximally suppressed 5 min after administration but remained significantly attenuated for up to 17 min after injection. Peripheral nerve and glutamate-evoked responses of dorsal spinocerebellar and spinoreticular tract neurons were particularly sensitive to thiopental administration and remained suppressed for up to 20 min after injection.

Conclusions: These results demonstrate that thiopental administration is associated with a prolonged blockade of motoneuron output and sensory transmission through the dorsal spinocerebellar and spinoreticular tracts that exceeds the duration of general anesthesia. Further, the blockade of glutamate-evoked neuronal responses indicates that these effects are due,

in part, to a local action of the drug in the spinal cord. The authors suggest that this combination of lumbar sensory and motoneuron inhibition underlies the prolonged impairment of reflex coordination observed when thiopental is used clinically.

ADMINISTRATION of ultra-short-acting barbiturate anesthetics, such as thiopental, can impair reflex coordination for several hours after administration, a period that greatly exceeds their duration of clinical anesthesia.^{1,2} One possible explanation for this effect is that these anesthetics may cause a prolonged suppression of the excitability of lumbar sensory tract neurons, such as the dorsal spinocerebellar tract (DSCT) neurons, which convey ascending proprioceptive sensory information. However, the effect of barbiturate anesthetics on identified lumbar sensory tract neurons, such as DSCT neurons, has only been studied in acutely anesthetized animals.^{3–8}

In all of these acute recording experiments, animals are first subjected to invasive surgical procedures using other anesthetic agents.^{9–11} Some procedures intended to eliminate the need for continued anesthesia in these experiments, including decerebration or spinal cord transection, make it impossible to identify ascending tract neurons and can dramatically alter neuronal excitability.^{12,13} Thus, it is unclear from acute recording experiments whether certain spinal cord ascending sensory pathways are more sensitive to the effects of barbiturate anesthetics than others.

In the current study, we tested the hypothesis that thiopental administration suppresses the excitability of antidromically identified DSCT neurons and spinoreticular tract (SRT) neurons, which convey principally proprioceptive, exteroceptive, and nociceptive sensory information, respectively, in chronically instrumented, drug-free cats.^{3–8} The spontaneous discharge and peripherally evoked responses, as well as responses to juxtacellular application of glutamate to DSCT neurons, were quantified during wakefulness, thiopental-induced general anesthesia, and recovery of consciousness from thiopental-induced anesthesia. The use of chronically instrumented, intact animals also allowed us to test the hypothesis that suppression of proprioceptive information contributes to prolonged reflex impairment after thiopental administration by permitting the examination of the relation between thiopental-induced changes in DSCT and SRT neuron excitability and muscle atonia *versus* cortical electroencephalographic activity.

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Materials and Methods

Chronically Instrumented Animal Preparation

The chronic intact cat preparation used in these studies provides optimal conditions for assessing the actions of injectable anesthetics on cellular function *in vivo*. This preparation has been used to examine the neural mechanisms of atonia during naturally occurring active or rapid-eye-movement (REM) sleep as well as the dampening of ascending sensory transmission through proprioceptive and nociceptive pathways during REM sleep when compared with wakefulness.¹⁴⁻¹⁷ The chronic preparation requires the aseptic implantation of head- and lumbar-restraining devices that permit stabilization of the neuraxis essential for long-term extracellular or intracellular recordings of identified spinal neurons.^{18,19}

Surgical Implant Procedures

Experiments were conducted in eight intact, chronically instrumented cats that were implanted during deep gaseous anesthesia (45–60% N₂O in a 1.5–2.5% halothane-oxygen mixture) with a head-restraining device and lumbar recording chamber.^{14,18} Electrodes were also implanted into the frontal sinus (electroencephalogram), lateral geniculate nucleus of the thalamus (ponto-geniculate-occipital), the orbital plate (electrooculogram), and neck muscles (electromyogram). Through the use of these electrodes, each animal's behavioral state of wakefulness and thiopental-induced anesthesia as opposed to sleep could be distinguished. An indwelling jugular catheter was chronically implanted and used for the intravenous administration of thiopental. Full details regarding these surgical implant procedures and those for implanting stimulating electrodes onto the sciatic nerve for peripheral nerve stimulation may be found elsewhere.^{14,18} All surgical and chronic restraint procedures reported complied with (inter)national^{20,21} and institutional (University of British Columbia Animal Care Committee) guidelines.

Procedures for Behavioral State Recording and Antidromic Identification of Dorsal Spinocerebellar and Spinoreticular Tract Neurons

Cats were allowed to recover from all surgical procedures for a minimum of 6 weeks before any recordings occurred. During this time period, they were gradually introduced to the recording room environment and slowly adapted to stereotaxic head and lumbar restraint.¹⁸ During recording sessions, cats were loosely wrapped in a soft canvas bag and were able to make limited postural adjustments as necessary. Behavioral state was constantly monitored by recording electroencephalogram, electrooculogram, ponto-geniculate-occipital, and nuchal electromyogram activities. Behavioral states of drowsy wakefulness and quiet sleep were scored using previously established criteria.^{14,18} Briefly,

quiet wakefulness was characterized by desynchronized, low-voltage cortical electroencephalogram with occasional low-amplitude oscillations of 4–6 Hz. Quiet sleep was characterized by high-voltage electroencephalogram activity (7–15 Hz) with cortical spindles and intermittent slow waves, and tonic electromyogram activity that is less than that during drowsy wakefulness. During quiet sleep, eye movements are few and much slower than those observed during wakefulness, and pontogeniculo-occipital wave activity is minimal or absent. The electroencephalogram parameters of quiet sleep resemble those observed following recovery from thiopental anesthesia (see Results). Cats normally cycled between quiet drowsy wakefulness and quiet sleep, as well as episodes of active sleep. We have previously reported that the spontaneous spike activity of most DSCT and SRT neurons was suppressed during active sleep, while their firing rate during wakefulness and quiet sleep did not differ.¹⁴⁻¹⁶ Accordingly, the behavioral state of quiet wakefulness served as a control state for testing the effects of thiopental.

The extracellular spike activity of Clarke's column DSCT and SRT neurons was recorded with glass micropipettes containing a carbon fiber (2 M Ω) and amplified with an AC-coupled amplifier (bandpass 0.3–10 KHz, 1,000 \times ; Model 1800, A-M Systems Inc., Sequim, WA). Prior to recording spinal cord neuronal activity, stimulating electrodes (A-M Systems, Cat. # 5755, 5–8 M Ω , AC) were stereotaxically lowered bilaterally into the brain, in two tracks located 3.5 mm lateral to the midline to permit antidromic identification of spinal cord neurons. One electrode was lowered 1 mm anterior to the primary fissure and positioned at a final depth of 10–11 mm below the cerebellar surface ipsilateral to the site of neuronal recording in the spinal cord. The cerebellar electrodes trajectories were directed at lobules III and IV using an angle of 36° posterior to the vertical plane (fig. 1). The other electrode was lowered to the ventrolateral reticular formation contralateral to the site of recording at an angle of 20° posterior to vertical. The tip of this second electrode (HC coordinates, P8–10, L3.5, H-8 to -8.5) was targeted toward ascending fiber tracts ventral to the VII motor nucleus, which contains axons of passage from lumbar spinoreticular, spinomesencephalic, and spinothalamic tract neurons (fig. 1).²²⁻²⁹

Criteria for antidromic activation included constant latency spikes evoked by consecutive low-intensity monopolar stimuli (0.2 ms, 0.5 Hz, 70–540 μ A), the ability to faithfully follow a short train (2–4 pulses) of high-frequency stimuli (500 Hz), and collision with spontaneous or peripheral nerve-evoked action potentials (fig. 1; see also fig. 1 in Soja *et al.*^{14,17}). Neurons were considered to comprise the DSCT if they could be antidromically identified from the electrode positioned within the ipsilateral cerebellar lobule.^{14,17} Those cells antidromically activated by contralateral reticular formation stim-

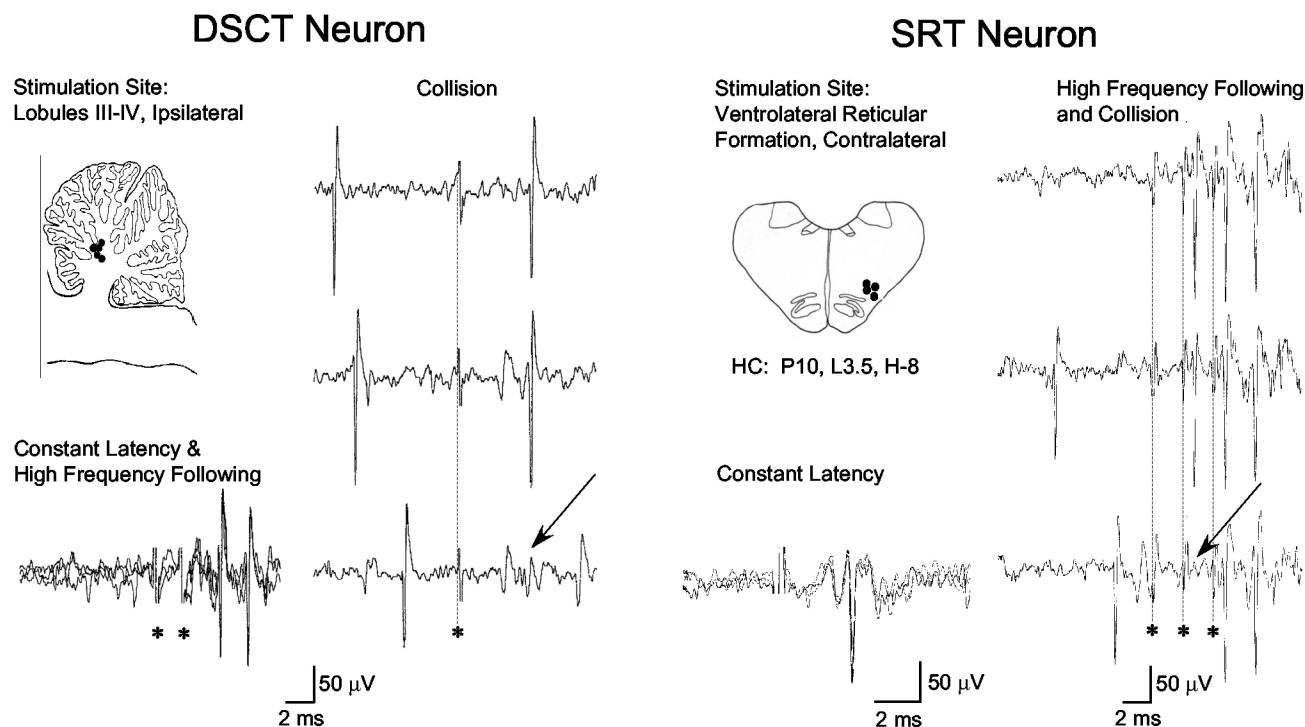


Fig. 1. Electrophysiologic criteria for identifying L3 dorsal spinocerebellar tract (DSCT) and spinoreticular tract (SRT) neurons in the chronic unanesthetized cat preparation. Low-intensity stimulation of the ipsilateral anterior cerebellar lobule or ventrolateral reticular formation, contralateral to the recording site, evokes an action potential at a constant latency using either a double pulse train (DSCT cell) or a single shock stimulus (SRT neuron). The SRT cell responded faithfully to a short-duration, high-frequency stimulus train (500 Hz). Collision of antidromically propagated action potentials with spontaneously occurring action potentials is evident in both the DSCT and SRT neuron. Collision occurs when a spontaneous action potential fires within the critical period for each cell. Arrows denote the point in time where antidromically propagated action potentials should have occurred.

ulation were identified as SRT neurons.^{6,7,15,29} For each neuron, identification procedures were performed first, and then the spontaneous and evoked spike activity was monitored before, during, and after thiopental anesthesia.

Stimulation Procedures

Synaptic activation of DSCT neurons was achieved by applying low-intensity stimuli (0.04 ms, 50–300 μ A) to the ipsilateral sciatic nerve *via* an indwelling cuff electrode.¹⁸ Low-intensity stimulation of the sciatic nerve produced a twitch that was not accompanied by any aversive behavior, *e.g.*, vocalization or electroencephalogram desynchronization. In one chronic animal preparation, the quadriceps muscle ipsilateral to the site of recording was implanted with a bipolar wire electrode for providing muscular afferent input to DSCT and SRT neurons.

Microiontophoretic Procedures

Four-barrel micropipettes were used in several experiments to microiontophoretically apply the excitatory amino acid glutamate to individual DSCT and SRT neurons before and after thiopental administration.¹⁶ One or two barrels contained glutamate (0.5 M, pH 8.0). The remaining barrels contained NaCl (160 mM) for automatic current balancing. Anodal retention currents

(10 nA) were applied to minimize leakage.^{15,18,30} Glutamate was applied in short 10–15-s epochs at 20–30-s intervals. Retention and ejection currents were applied using a programmable current generator (Dagan, 6400; Dagan Corp., Minneapolis, MN). Ejection currents were adjusted so that responses were approximately twice the baseline firing rate. Microiontophoretic ejection of glutamate excited DSCT and SRT neurons without causing any change in behavioral state.¹⁶

Intravenous Drug Administration

Prior to the administration of thiopental, ongoing and evoked spike activity was obtained during the state of quiet wakefulness. Normal saline (0.5 ml) was then slowly injected intravenously over 1 min, and a second control response was collected. Finally, an anesthetic dose of thiopental (15 mg mg/kg diluted to a 1% solution in normal saline; Pentothal®; Rhone Merieux, Quebec, Canada) was slowly injected over 1.5–2 min and neuronal activity monitored for up to 1 h after drug administration. Several cells were “lost” prior to the 1-h time limit. Summarized data presented in this report were based on the longest time point following thiopental for all cells. In five experiments, dose-response studies were performed with thiopental on the spontaneous firing rate of DSCT neurons. Briefly, thiopental (5 mg/kg, intrave-

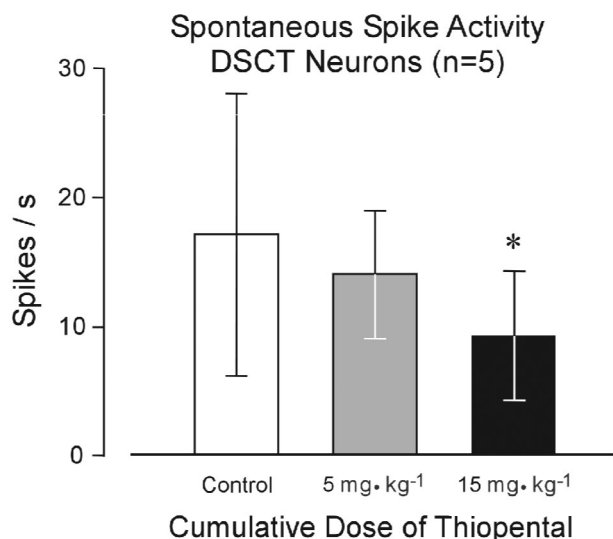


Fig. 2. The relation between systemic dose of thiopental and spontaneous discharge of five dorsal spinocerebellar tract (DSCT) neurons is shown. Note that only a cumulative dose of 15 mg/kg was effective in suppressing DSCT neuronal activity and inducing anesthesia. Each bar represents the mean \pm SD. * $P < 0.05$, repeated-measures analysis of variance and Dunnett test.

nous) was injected over a 30-s time period and the behavioral state and cell activity monitored for 5 min. Then an additional bolus (10 mg/kg, intravenous) was injected and the behavioral state and cell activity monitored for up to 1 h. Based on the results of the dose-response relation of DSCT neuron spontaneous spike activity to thiopental, all other experiments were undertaken with a dose of 15 mg/kg. A maximum of two experiments were performed per week. The minimum time period between successive drug administrations during each week was 72 h. The patency of the catheter was maintained with daily flushing with 10% heparinized saline.

Data Analysis

Spike Activity of Dorsal Spinocerebellar and Spino-reticular Tract Neurons. All behavioral state and neuron spike activity was archived on tape and routed to a computer data acquisition system (1401-plus; Cambridge Electronic Design, Inc., Cambridge, United Kingdom) equipped with spike analysis software (Spike 2[®], version 3.19; Cambridge Electronic Design, Inc.).

Spontaneous spike activity of DSCT and SRT neurons was sampled over a period of 1 min at regular intervals (2.5 min) before, during, and following the administra-

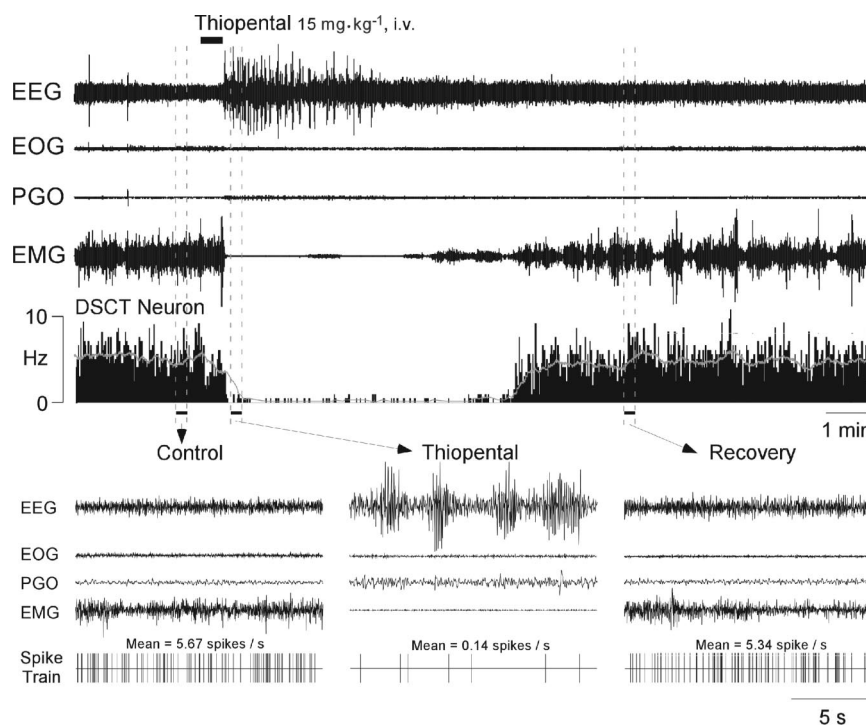


Fig. 3. Effect of thiopental on spike activity of a dorsal spinocerebellar tract (DSCT) neuron. The first four traces represent 19-min epochs of electroencephalogram, electrooculogram, ponto-geniculo-occipital, and electromyogram activity characteristic of quiet wakefulness, thiopental-induced anesthesia, and recovery. Calibration bars: 100 μ V. Below these traces is a ratemeter histogram plot of spontaneous spike activity of this DSCT neuron in spikes per second (Hertz). Thiopental (15 mg/kg, intravenous) was administered during the period indicated by the short solid bar. Note the emergence of large-amplitude, slow-wave oscillations in the electroencephalogram wave activity and abolition of electromyogram activity immediately following the injection of thiopental, which is accompanied by a profound reduction in the spike activity of this cell from 5.7 to 0.14 spikes/s. The spike rate of this cell remained suppressed for approximately 7 min, after which it returned to predrug control levels. The three panels below this 19-min record are time expansions illustrating behavioral state and the recorded DSCT cell's spike train pattern before, during, and following recovery of consciousness from thiopental anesthesia. EEG = electroencephalogram; EMG = electromyogram; EOG = electrooculogram; PGO = ponto-geniculo-occipital spike.

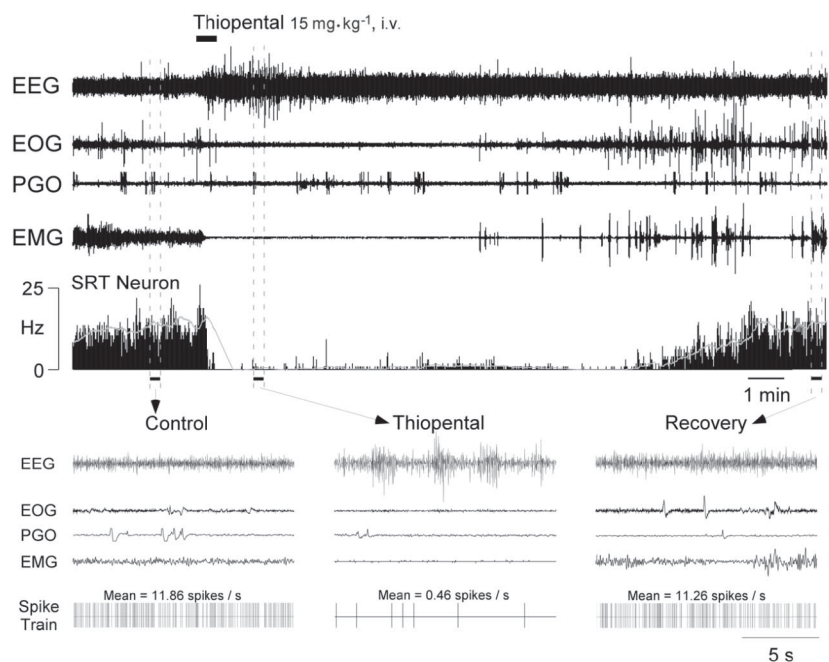


Fig. 4. Effect of thiopental on spike activity of a spinoreticular tract (SRT) neuron. The first four traces represent 38-min epochs of electroencephalogram, electrooculogram, ponto-geniculo-occipital, and electromyogram activity as described in fig. 3. Calibration bars: 100 μ V. Thiopental (15 mg/kg, intravenous) induced atonia, the appearance of large-amplitude slow waves in the electroencephalogram, and a cessation of eye movement activity. The cell's spike rate decreased from 11.86 to 0.46 spikes/s during thiopental-induced anesthesia and recovered after 28 min. The cell's spike train patterns before, during, and after thiopental were similar to those observed for dorsal spinocerebellar tract cells (fig. 3). EEG = electroencephalogram; EMG = electromyogram; EOG = electrooculogram; PGO = ponto-geniculo-occipital spike.

tion of thiopental. The group mean firing rate at each time point for all cells was determined. The group mean rate before drug ejection was equated to 100%, and subsequent values were calculated based on this value.

Poststimulus time histograms of sciatic nerve stimulation were constructed from a minimum of 50 consecutive responses, and the mean activity in spikes/stimulus was calculated. Evoked activity was sampled every 5 min over the first 20 min after drug infusion and then every 10 min thereafter, for a total of 50 min or until the cell was lost. The magnitude of the control response at time zero was equated to 100%, and subsequent values were calculated based on this value.

Consecutive responses ($n = 5$) to glutamate (bin width 0.25 s) were computer-averaged and plotted as perievent histograms around drug applications using specialized scripts integrated into the Spike 2 program. Baseline spike activity was subtracted from values obtained during the glutamate ejection to indicate the magnitude of "glutamate-evoked activity."¹⁶

Cortical Electroencephalogram and Nuchal Electromyogram Activity. The Spike 2 program was also used to quantify cortical neuron and spinal motoneuron activity concurrently with sensory tract neuron activity. Briefly, the electroencephalogram waveform activity was filtered (bandpass 7–15 Hz), full-wave rectified, and the resultant signal passed through a window discriminator set to detect voltage crossings of thiopental-induced large-amplitude slow waves. The voltage crossings were converted to logic pulses that were then quantified around thiopental injections in events per second. Similarly, nuchal electromyogram activity was converted into window discriminated logic pulses corresponding motoneuron spike discharges. Both electro-

encephalogram and electromyogram data were sampled at the same time points as sensory tract neurons.

In each experiment, thiopental-related changes in neuronal excitability were assessed for each sensory tract neuron in conjunction with electroencephalogram and electromyogram data by comparing the mean activity during quiet wakefulness with that obtained during and on recovery from thiopental-induced anesthesia. A minimum postinfusion recording time of 30 min was used to accept data from individual sensory neurons. A repeated-measures analysis of variance and Dunnett test were applied to determine whether thiopental exerted significant changes in the magnitude of evoked responses over time. A paired Student *t* test was used to investigate differences in the spike activity of DSCT neurons tested with microiontophoretic juxtacellular applications of glutamate during wakefulness *versus* thiopental anesthesia. For all tests, α was set at 0.05. Values are reported as means \pm SD.

Results

Dose-response experiments were conducted to determine the effect of different doses of thiopental on the spontaneous activity of five DSCT neurons. Injection of a low dose (5 mg/kg, intravenous) of thiopental did not result in unconsciousness and muscle atonia or in a significant change in the spontaneous spike activity of DSCT neurons compared with quiet wakefulness. However, injection of an additional bolus (10 mg/kg, intravenous) of thiopental 5 min later (for a total cumulative dose of 15 mg/kg) did induce anesthesia and significantly suppressed the spontaneous spike activity of DSCT neu-

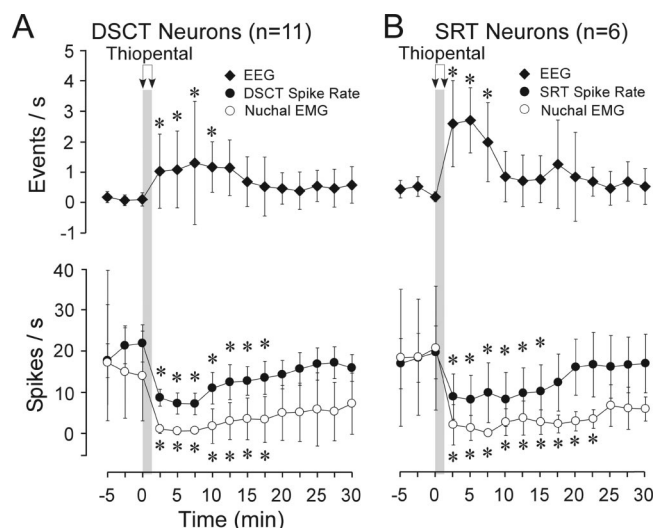


Fig. 5. Summary of thiopental's action on the spontaneous firing rate of (A) dorsal spinocerebellar tract (DSCT) neurons ($n = 11$) and (B) spinoreticular tract (SRT) neurons ($n = 6$) concurrently with the induction of slow, spindle-like wave events in the electroencephalogram waveform and firing rate of neck motoneurons. Time relative to zero is indicated in minutes. Each point on each graph represents the group mean (\pm SD) activity (spikes per second for DSCT, SRT, and nuchal electromyogram [motoneuron] activity and events per second for slow waves in the electroencephalogram waveform induced by thiopental). Thiopental exerted depressant actions on DSCT and SRT neurons. Motoneuron activity was also depressed to a greater extent than DSCT or SRT neuron activity. The duration of slow, spindle-like events in the electroencephalogram waveform was transient when compared with the drug's depressant action on sensory tract and motoneuron activity. *Statistically significant differences from control ($P < 0.05$, repeated-measures analysis of variance and Dunnett test). EEG = electroencephalogram; EMG = electromyogram.

rons compared with quiet wakefulness (fig. 2). As a result, only the 15-mg/kg (accepted clinical anesthetic) dose of thiopental was examined throughout this study.

Effect of Thiopental on Electrophysiological Indices of Behavioral State

Behavioral state indices were monitored throughout each experiment. Control neuronal activity was collected during the state of quiet wakefulness, which was characterized by desynchronized, low-voltage cortical electroencephalogram, tonic electromyogram activity, and a paucity of eye movement and ponto-geniculo-occipital wave activity.^{14,18,31,32} In contrast, the anesthetic state induced by thiopental was clearly differentiated from quiet wakefulness, primarily by the presence of rhythmic high-voltage, slow-wave oscillations in electroencephalogram activity and by an atonic electromyogram. Representative traces of electroencephalogram, electrooculogram, ponto-geniculo-occipital, and electromyogram activity during wakefulness, thiopental anesthesia, and recovery in two separate animals are shown in figures 3 and 4.

Effect of Thiopental on Sensory Tract Neurons Spontaneous Spike Activity.

Dorsal Spinocerebellar Tract Neurons. All of the DSCT neurons examined in this study displayed spontaneous spike activity during the control states of wakefulness and quiet sleep (mean: 21.8 ± 4.5 spikes/s). An example of the effects of thiopental on behavioral state and spontaneous firing rate of an individual DSCT neuron is illustrated in figure 3. In this particular cell, thiopental rapidly induced a state of general anesthesia characterized by electroencephalogram synchronization and muscle atonia. Electromyogram activity reappeared approximately 5 min following drug infusion. Spontaneous firing rate decreased 98% from a mean of 5.7 to 0.14 spikes/s. The cell's spike rate recovered to 5.34 spikes/s within 10 min.

The effect of thiopental anesthesia was examined on a total of 11 DSCT neurons in five animals (animal no. 1: $n = 3$, animal no. 2: $n = 4$, animal no. 3: $n = 3$, animal no. 5: $n = 1$; fig. 5A). The baseline firing rate for these DSCT neurons was significantly reduced by 59% to 7.2 ± 2.6 spikes/sec 5 min following injection of thiopental. The suppression of DSCT neuron spike activity was statistically different up to 17.5 min following the injection when compared with control values at time zero (fig. 5A; $P < 0.05$). As depicted in figure 5A, the duration of DSCT neuronal suppression was identical to motoneuron suppression. Motor outflow was abolished for 7.5 min and gradually recovered. The depressant action of thiopental on motoneuronal and DSCT neuronal discharge outlasted the drug's action to increase synchronous large-amplitude slow electroencephalogram wave activity. These data suggest that the return of consciousness precedes the recovery of proprioceptive and motoneuron activity and are consistent with the idea that the recovery from thiopental anesthesia is associated with sensorimotor deficits.

Spinoreticular Neurons. Spinoreticular neurons in the chronic cat preparation exhibited moderate spontaneous spike activity comparable to those described for DSCT neurons.¹⁴ In the current study, the mean spontaneous spike rate (19.8 ± 6.5 spikes/s) for SRT neurons monitored did not differ during the control states of wakefulness and quiet sleep.

Spontaneous spike activity of SRT neurons was also reduced during thiopental anesthesia. The time course and peak effect of suppression was comparable to that observed for DSCT neurons. An example of the effect of thiopental on SRT neuron spike rate is presented in figure 4. In this particular example, the cell's spike rate decreased by approximately 96% from 11.9 to 0.5 spikes/s.

Figure 5B summarizes the effect of thiopental on a total of six SRT neurons recorded in two animals (animal no. 5: $n = 5$; animal no. 7: $n = 1$). Overall, thiopental-induced motoneuron suppression lasted approximately

A. DSCT Neurons: Sciatic Nerve - Evoked Responses

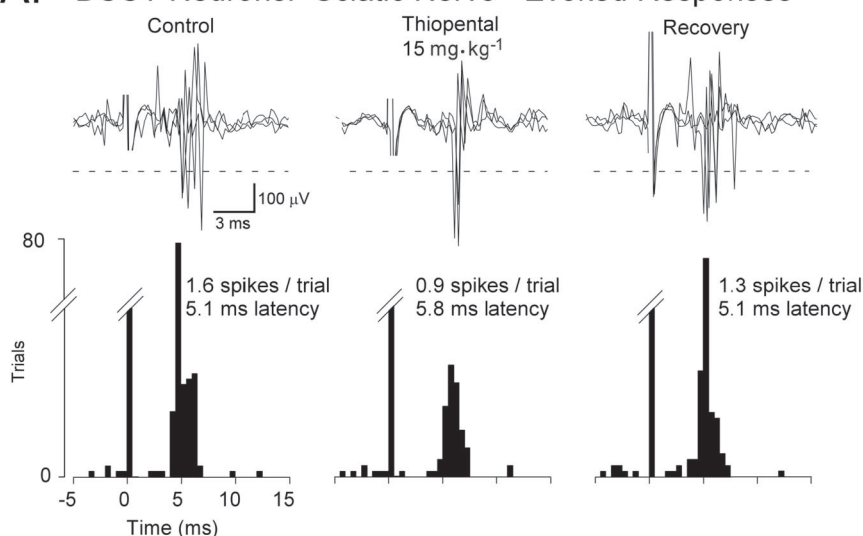
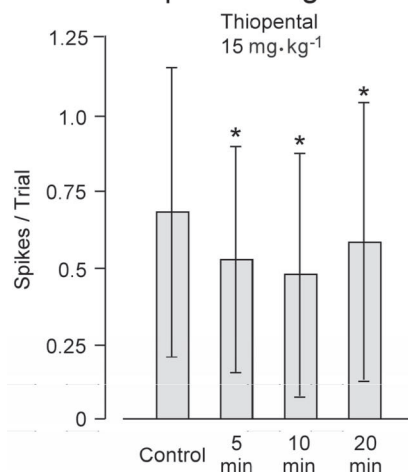
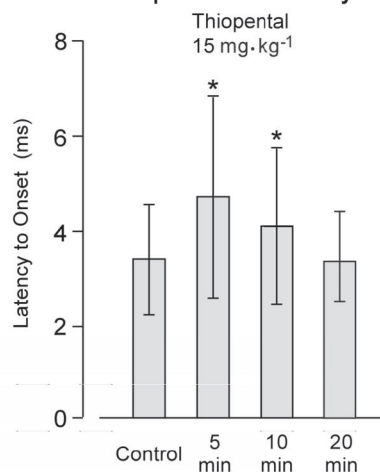


Fig. 6. (A) Effect of thiopental on sciatic nerve-evoked responses in a dorsal spinocerebellar tract (DSCT) neuron. The oscilloscope traces represent three consecutive short-latency, sciatic nerve-evoked spike responses during quiet wakefulness, thiopental anesthesia, and recovery of consciousness 20 min after drug infusion. The dashed line illustrates a voltage window discriminator that was used for counting the evoked spikes for compilation into a peristimulus time histogram that was constructed from 80 consecutive trials. The average response magnitude and latency-to-response is indicated in each peristimulus time histogram. **(B)** Summary of thiopental effects on response magnitude and latency-to-onset for DSCT neurons responding to sciatic nerve stimulation. Each bar represents the mean \pm SD response for eight DSCT neurons before and during thiopental anesthesia. Thiopental decreased the response magnitude and prolonged the latency to spike onset. *Statistically significant differences between wakefulness and thiopental-induced anesthesia ($P < 0.05$, repeated-measures analysis of variance and Dunnett test).

B. Response Magnitude



Response Latency



7.5–10 min longer and was relatively greater than SRT neuronal suppression. Here again, the drug's action on the induction of slow, spindle-like (7–15 Hz) events in the electroencephalogram waveform was brief (7.5 min) when compared with depression of sensory (15 min) and motor neuron activities (22.5 min). Finally, as indicated in figure 5, both DSCT and SRT neurons were suppressed to the same relative degree (DSCT 50% *vs.* SRT 58%) 10 min following injection of thiopental.

Peripheral Nerve Stimulation.

Dorsal Spinocerebellar Tract Neurons. Most DSCT neurons responded to low-intensity stimulation of the sciatic nerve prior to the induction of thiopental anesthesia (fig. 6A). At just suprathreshold intensity (mean stimulus intensity: $308.7 \pm 28.8 \mu\text{A}$), the neuronal response to sciatic nerve stimulation was compound in nature and characterized by a short latency response (1.5–10 ms) and a variable, longer latency response (15–34 ms). The magnitude of the short latency response was always larger than the longer latency response. There was often a period of

quiescence between the short and longer latency response components. This could either be due to oligosynaptic inhibitory pathways recruited during the stimulus presentation or to slower conducting afferent fibers. It is not possible at the moment to discern between these possibilities. For these reasons, the action of thiopental on the late component was not quantified.

An example of the action of thiopental on sciatic nerve-evoked stimulation is presented in figure 6A. In this particular example, poststimulus time histograms were constructed in response to 80 consecutive stimuli. During the state of quiet wakefulness, the magnitude of the cell's response and the average latency-to-onset measured 1.6 spikes/trial and 5.1 ms, respectively. Following the induction of thiopental anesthesia, the response magnitude was reduced by 44% to 0.9 spikes/trial and the latency-to-onset was prolonged by 29% to 5.8 ms. On partial recovery, which occurred 20 min thereafter, the response magnitude measured 1.3 spikes/trial and the latency-to-onset decreased to 5.1 ms.

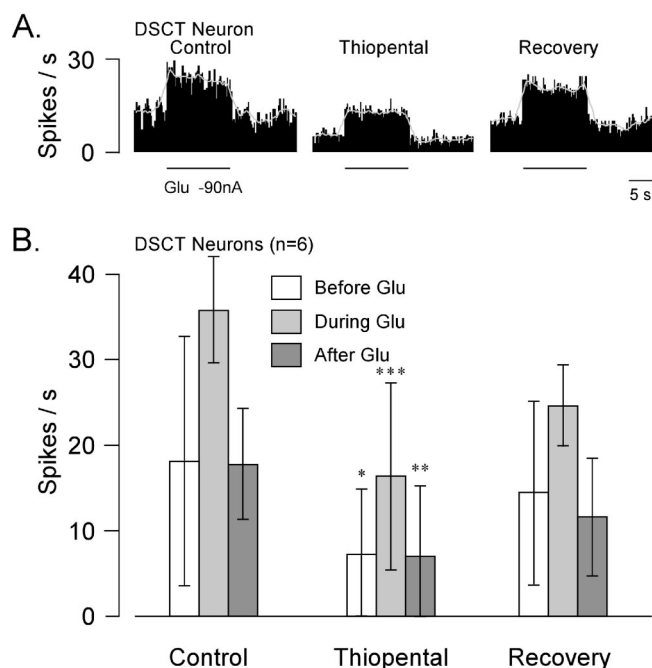


Fig. 7. (A) Effect of thiopental on glutamate-evoked responses of dorsal spinocerebellar tract (DSCT) neurons. The periglutamate responses represent the computer-averaged histogram plot of five consecutive glutamate trials during control, drug-free conditions during quiet wakefulness, during thiopental-induced anesthesia, and following recovery of consciousness 20–30 min after infusion of thiopental. Glutamate was ejected juxtacellularly during the time period indicated by the bar. (B) Summary histograms of spike activity (mean \pm SD) around the glutamate pulses before, during, and after thiopental administration. *Statistically significant differences from quiet wakefulness ($P < 0.05$, paired Student t test). Glu = glutamate.

A total of eight DSCT neurons from three animals (animal no. 1: $n = 3$, animal no. 2: $n = 3$, animal no. 3: $n = 2$) responding to low-intensity stimulation of the sciatic nerve were examined before, during, and after recovery from thiopental-induced anesthesia and are summarized in figure 6B. Overall, 5 min following thiopental infusion, DSCT neuron response magnitude decreased by 23% from 0.71 ± 0.19 to 0.55 ± 0.15 spikes/trial and latency-to-onset increased by 38% from 3.39 ± 0.44 to 4.7 ± 0.86 ms when compared with quiet wakefulness ($P < 0.05$). The sciatic nerve-evoked responses gradually recovered toward control values, but for these eight cells, both the response magnitude and latency-to-onset remained significantly suppressed up to 20 min after injection, a time point when the animals had already regained conscious “quasi-waking” behavior as indicated by relative electroencephalogram desynchronization, occasional eye movements, and reappearance of muscle electromyogram tone accompanied by occasional postural adjustments.

Spinoreticular Neurons. The response of two SRT neurons (animal no. 5) to sciatic nerve stimulation and one SRT neuron (animal no. 7) responding to quadriceps muscle stimulation resembled those responses obtained

for DSCT neurons and consisted of short latency and longer latency components prior to administration of thiopental. Thiopental reduced the response magnitude in all three cells, reaching a peak effect 5 min after infusion. Response recovery occurred within 20 min for each cell. The latency to onset was prolonged in one cell tested with sciatic and quadriceps stimulation.

Thiopental Effects on Glutamate-Evoked Neuronal Activity

Dorsal Spinocerebellar Tract Neurons. Glutamate was applied to six DSCT neurons from four animals (animal no. 6: $n = 3$, animal no. 7: $n = 2$, animal no. 8: $n = 1$) at regular intervals during control periods of quiet wakefulness and then during the induction and recovery from thiopental anesthesia. Glutamate, ejected at currents ranging from 30 to 100 nA over 10–15-s time periods and 2–30-s intervals, readily excited DSCT neurons. An example of a DSCT neuron's computer-averaged response to five consecutive glutamate pulse applications is illustrated in figure 7. In this particular cell, the computer-averaged spontaneous, glutamate-driven, and postglutamate spike activity measured 16.0, 27.0, and 14.3 spikes/s, respectively. During thiopental anesthesia, these values were reduced to 8.3, 11.8, and 7.2 spikes/s, respectively. Twenty minutes following thiopental administration, there was a partial recovery to 10.3, 18.0, and 9.8 spikes/s, respectively.

A total of six neurons were examined with juxtacellularly applied glutamate in this fashion, and, overall, thiopental produced comparable results (figure 7B). When correcting for spontaneous activity, the glutamate-evoked responses decreased by 42% from a mean of 17.6 to 9.1 spikes/s ($P < 0.05$). The corrected glutamate-evoked responses partially recovered to 9.1 spikes/s 20 min after thiopental infusion.

Spinoreticular Tract Neurons. Three SRT neurons were also tested with juxtacellular glutamate in two animals (animal no. 5: $n = 1$, animal no. 7: $n = 2$). The effects were consistent with those presented above for DSCT neurons. Thiopental reversibly reduced the spontaneous spike activity, glutamate-driven spike activity, and postglutamate spike activity. The mean values for these three SRT neurons measured 17, 25, and 13 spikes/s and 9, 13, and 6 spikes/s during the peak suppression produced by thiopental, respectively ($P < 0.05$). Two of the three cells were lost before the 20-min period during which recovery would have been measured. The response magnitude of the remaining SRT neuron recovered to 64% of its baseline level before it was lost.

Discussion

The current study used extracellular recording techniques in chronically instrumented animal preparations

to examine the effect of cortical, lumbar sensory, and motor systems before, during, and after the recovery from the anesthetic state induced by the short-acting injectable barbiturate thiopental. Our chronic animal preparation is unique in that it is drug-free, intact, and provides an auspicious view of the activity of these cellular systems concomitantly during near-normal conditions without the confounds associated with acute surgery or other anesthetics that are endemic to "acute" preparations. The change in discharge of these cellular pathways was evaluated around the single injection of a short-acting barbiturate that produces a clinical state of anesthesia. Hence, from a "systems" viewpoint, our studies provide unique insight into the mechanisms of barbiturate anesthesia in humans.

The systemic administration 15 mg/kg thiopental rapidly induced a brief state of anesthesia that was characterized by distinct slow and large-amplitude slow waves in the cortical electroencephalogram, reduced electromyogram activity, and minimal or no eye movements compared with preceding episodes of wakefulness in chronically instrumented cats. The cortical electroencephalogram waveform during thiopental anesthesia was composed waxing and waning oscillations (figs. 3 and 4) reminiscent of natural slow waves that occur during quiet or non-REM sleep. However, similar to natural active or REM sleep, electromyogram activity was markedly suppressed or abolished during thiopental anesthesia. Thus, the anesthetic state induced by systemic administration of thiopental was clearly discernible from either natural sleep state. It was critical to be able to differentiate anesthetic states from natural sleep states since, during active sleep, the spontaneous discharge of both DSCT and SRT neurons undergoes significant modulation.^{14,15,17,18}

Effect of Thiopental on Dorsal Spinocerebellar Tract and Spinoreticular Neurons

In the current study, it was found that the state of thiopental anesthesia was clearly associated with a marked reversible suppression of the spontaneous discharge and peripherally evoked responses of DSCT and SRT neurons. The DSCT principally relays afferent proprioceptive inputs from muscle spindles and Golgi tendon organs important for sensorimotor integration,^{3,4} while the SRT principally relays tactile and nociceptive information to the brain.^{5-8,22} Thus, it is possible that the thiopental-induced suppression of spontaneous discharge in both the DSCT and SRT may have occurred indirectly as a result of the loss of muscle tone, although the finding that peripherally evoked neuronal responses were also suppressed during thiopental anesthesia makes this unlikely. Nevertheless, a major limitation of the current study is that thiopental was administered systemically, and, therefore, the site and mechanism of drug action is not easily resolved. It is conceivable that

spinal presynaptic or postsynaptic inhibitory mechanisms as well as supraspinal mechanisms contributed to the observed suppression of DSCT and SRT neurons during thiopental-induced anesthesia. The reduced rate of DSCT and SRT neurons during thiopental anesthesia compared with wakefulness indicate that a dramatic reduction occurs in the transfer of ongoing sensory transmission to the cerebellum, reticular formation, and higher brain centers during this state.

To assess the postsynaptic excitability of DSCT and SRT neurons, glutamate was microiontophoretically applied to evoke responses in individual DSCT and SRT neurons during control, drug-free conditions, and then again after the administration of an anesthetic dose of thiopental. This technique has previously been used to detect the postsynaptic inhibition induced by juxtacellular application inhibitory amino acids such as glycine and γ -aminobutyric acid to DSCT and SRT neurons.¹⁶ Glutamate-evoked DSCT and SRT neuron responses were suppressed after the administration of thiopental, which suggests that postsynaptic excitability of these neurons is decreased during thiopental-induced anesthesia. A recent study reported that thiopental (1.5 mg/kg, intravenous) administered to the torso circulation of isoflurane-anesthetized goats significantly depressed dorsal horn neuronal responses to noxious stimulation, whereas application of the same dose to the isolated cerebral circulation was without effect.³³ Other evidence along these lines suggests that barbiturates do, in fact, exert a postsynaptic inhibition that leads to a shunting of afferent input to identified motoneurons in the cat lumbar spinal cord.³⁴ On the basis of these findings, we suggest that thiopental exerts a direct suppressive action on DSCT and SRT neurons in the spinal cord.

Although few comparable data exist whereby the actions of thiopental or other barbiturates have been examined on sensory tract neuron excitability, the spontaneous activity of DSCT and SRT neurons recorded from anesthetized cats is reported to be much lower than that found for awake cats, which may indicate a suppressive action of anesthesia and acute surgery on the activity on these neurons.^{5-7,15,16,35,36} Perhaps most relevant to the current report is the study by Hori *et al.*,³⁷ who reported that low doses of pentobarbital enhanced and larger doses suppressed the spontaneous and pinch-evoked responses of spinothalamic tract-like neurons in acutely decerebrate monkeys. Similar effects of low-dose barbiturate on unidentified neurons in the lumbar segments of awake cats have also been reported.^{38,39} In contrast, no increase in the responses of DSCT neurons *per se* to a low dose (5 mg/kg) of thiopental or in DSCT or SRT neurons to peripheral stimuli during recovery from thiopental anesthesia, when "lower" *in vivo* concentrations would be expected, was observed. However, the enhanced excitability of spinal cord dorsal horn neurons after low-dose pentobarbital administration in the afore-

mentioned studies has been attributed to the lifting of a presumed tonic descending inhibition of nociceptor-driven inputs³⁷⁻⁴⁰ and thus, in theory, would be mediated through a supraspinal rather than a spinal site of drug action.

Functional Considerations

The chronic preparation used in the current study provided a unique opportunity to assess synaptic transmission before, during, and following recovery from barbiturate anesthesia through two distinct sensory pathways, concurrently with motoneuron and cortical cell activities.¹³ In contrast to pain sensitivity, it has been found that impairment of reflex coordination lasts for several hours after administration of a single dose of thiopental in humans.^{1,2} The prolonged thiopental-induced suppression of the excitability of DSCT neurons, which provide proprioceptive input, may partly underlie this side effect. Indeed, thiopental exerted a relatively greater and longer effect on the excitability of sensory tract (DSCT and SRT) neurons and antigravity motoneurons subserving the neck musculature than cortical neurons mediating the slow, large-amplitude waves characteristic of the state of anesthesia.^{31,32} This difference was predicted based on the reports in the literature where "hangover effects" related to sensorimotor deficits occur in patients recovering from short periods of barbiturate anesthesia.^{1,2} The motor suppression is likely to affect both α and γ motoneurons and could occur on these cells through presynaptic and postsynaptic inhibitory processes as discussed for DSCT and SRT neurons. The current studies form the basis for assessing other injectable (e.g., propofol) or inhalational (e.g., isoflurane) anesthetic agents for their time-dependent actions on the excitability of sensory tract neurons, motoneurons, and cortical neurons in an intact preparation devoid of the usual confounding factors associated with this kind of work.

Finally, the current study also serves as an important reminder that future investigations, which focus on the control of sensory transmission *via* DSCT,¹⁷ SRT, or other afferent pathways, should be cognizant of the depression that occurs in these systems when using anesthetic agents during the surgical preparation of the cord prior to unit recording studies. Any experimental procedure that is performed with the intent of investigating inhibition on such cells may likely be already at a disadvantage for detecting a clear measure of such manipulations. Examples of this kind include studies where opioids⁴¹⁻⁴³ or cholinergic agents⁴⁴ are microinjected intracerebrally and the excitability of lumbar cord neurons subsequently measured. Such studies have the potential to generate equivocal results leading to controversies in the literature if the effects of anesthetic agents on the recorded cells are not considered or adequately controlled for.¹⁶

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