State-Dependent GABAergic Inhibition of Sciatic Nerve-Evoked Responses of Dorsal Spinocerebellar Tract Neurons

Niwat Taepavarapruk, Shelly A. McErlane, Angela Chan, Sylvia Chow, Liz Fabian, and Peter J. Soja
Faculty of Pharmaceutical Sciences, Division of Pharmacology and Toxicology, The University of British Columbia, Vancouver V6T 1Z3, Canada

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Taepavarapruk, Niwat, Shelly A. McErlane, Angela Chan, Sylvia Chow, Liz Fabian, and Peter J. Soja. State-dependent GABAergic inhibition of sciatic nerve-evoked responses of dorsal spinocerebellar tract neurons. J Neurophysiol 92: 1479–1490, 2004. First published April 21, 2004; 10.1152/jn.01108.2003. Peripheral nerve-evoked potentials recorded in the cerebellum 35 yr ago inferred that sensory transmission via the dorsal spinocerebellar tract (DSCT) is reduced occasionally and only during eye movements of active sleep compared with wakefulness or quiet sleep. A reduction or withdrawal of primary afferent input and/or ongoing inhibition of individual lumbar DSCT neurons may underlie this occurrence. This study distinguished between these possibilities by examining whether peripheral nerve-evoked responses recorded from individual DSCT neurons are suppressed specifically during active sleep, and if so, whether GABA mediates this phenomenon. Synaptic responses to threshold stimuli applied to the sciatic nerve were characterized by a single spike response at short latency and/or a longer latency burst of action potentials. During the state of quiet wakefulness, response magnitude did not differ from that observed during quiet sleep. During active sleep, short and long latency responses were suppressed by 26 and 14%, respectively, and returned to pre-active sleep levels following awakening from active sleep. Sciatic nerve-evoked early and late responses were further analyzed in a paired fashion around computer-tagged eye movement events that hallmark the state of active sleep. Response magnitude was suppressed by 14.4 and 11.5%, respectively, during eye movement events of active sleep. The GABA_A antagonist bicuculline, applied juxtacellularly by microiontophoresis, abolished response suppression during non–eye movement periods and eye movement events of active sleep. In conclusion, synaptic transmission via DSCT neurons is inhibited by GABA tonically during non–eye movement periods and phasically during eye movement events of active sleep.

INTRODUCTION

Recent chronic unit recording studies of individual upper lumbar sensory projection neurons comprising the dorsal spinocerebellar tract (DSCT) have indicated that these cells are subjected to inhibition specifically during the behavioral state of active sleep as opposed to other states, such as wakefulness or quiet sleep (Soja et al. 1996, 2001a; Taepavarapruk et al. 2002). In particular, the spontaneous and glutamate-evoked responses of DSCT neurons are markedly reduced during active sleep and are mediated by the “fast” inhibitory amino acid neurotransmitters GABA and glycine. Evoked potential studies conducted in the 1960s revealed that peripheral nerve-evoked responses recorded in the cerebellum were indeed suppressed only during intense eye movements of active sleep (Carli et al. 1966, 1967a,b). These findings suggest that the magnitude of low-threshold, peripherally evoked responses of DSCT neurons may also depend on behavioral state.

In this study, we investigated this issue for the first time by applying low-intensity stimuli to the sciatic nerve in the intact chronic cat preparation and quantifying the evoked responses of individual DSCT neurons as a function of sleep and wakefulness. We were also interested in determining if modulation of peripheral nerve-evoked responses was specifically related to the presence of eye movement events that hallmark the state of active sleep, in view of earlier studies that employed simple field potential recordings and indicated sensory transmission was suppressed in association with eye movements (Carli et al. 1966, 1967a,b).

Finally, if peripheral nerve-evoked responses of DSCT neurons were indeed suppressed during active sleep, we wished to determine if GABA, a classical inhibitory neurotransmitter in the spinal cord, underlies this phenomenon, and if so, whether it does specifically during non–eye movement periods and/or eye movement events of active sleep.

The rationale employed here was that direct activation of the axons of primary afferent neurons would bypass their normal transduction mechanisms and impulse transmission from peripheral receptors. If the response magnitude of DSCT neurons evoked by stimuli applied directly to the axons of primary afferent neurons decreases during active sleep, this would indicate that state-specific inhibition was occurring at the level of the spinal cord at synapses located between primary afferents and the recorded cells. Moreover, juxtacellular administration of appropriate inhibitory neurotransmitter antagonists near the recorded DSCT neurons should block this state-dependent inhibition. Such findings would support the theory that a neural pathway from higher brain centers is engaged to dampen ascending sensory inflow as opposed to a withdrawal of afferent input or disfacilitation of DSCT neurons arising as a consequence of motor atonia that is present during active sleep.

Here we report that synchronous synaptic activation of DSCT neurons by primary afferents is subjected to GABAergic inhibitory influences during tonic non–eye movement periods and phasic eye movement events, the latter of which hallmark the behavioral state of active sleep.
METHODS

Surgical procedures

All experimental procedures reported herein were performed on a total of three adult intact, unanesthetized cats and complied with (inter)national and institutional (University of British Columbia Animal Care Committee) statutes. Under deep gaseous anesthesia (45–60% N₂O in 1.5–2.5% halothane/oxygen mixture), the animals were implanted with head- and lumbar-restraining devices. Electrodes were implanted into the frontal sinus [electroencephalogram (EEG)], lateral geniculate nucleus of the thalamus [ponto-geniculo-occipital (PGO) wave activity], the orbital plate [electro-oculogram (EOG)], and neck muscles (EMG). Through the use of these electrodes, each animal’s behavioral states of wakefulness and sleep could be determined. A complete recovery over a 3-mo period was required before any recordings from DSCT neurons were performed. Procedures for surgical implantation, the gradual adaptation to painless head and lumbar restraint during the latter half of the 3-mo recovery period, identification of sleep/wake states, and antidromic identification of spinal projection neurons in the chronic intact cat have been previously described in detail (Soja et al. 1995).

Sciatic nerve stimulation

A cuff electrode was implanted on the sciatic nerve during a separate surgical procedure performed under gaseous anesthesia ~3 wk before recording sessions commenced (Soja et al. 1995). Electrode leads were tunneled subcutaneously and externalized to a 10-pin connector plug affixed to the lumbar restraint (see Fig. 1 in Soja et al. 1995). During neuronal recording sessions, single shock stimuli (0.05 ms duration, 1.0 Hz interval) were applied at current intensities sufficient (~1.0 T) to synaptically activate DSCT neurons as evidenced by recording short or longer latency action potential(s).

Behavioral state and sleep cycles

Each preparation readily cycled between sleep and wakefulness during each experimental recording session that typically lasted 5–6 h. Recording sessions were performed over 4 consecutive days (Soja et al. 1996). DSCT neuron activity was recorded over multiple sleep cycles. Each sleep cycle consisted of the following states: wakefulness, quiet sleep, active sleep, and awakening from active sleep. Electrophysiological criteria for determining states of wakefulness and sleep were identical to those reported in detail previously (Soja et al. 1995, 1996, 2001a,b; Taepavarapruk et al. 2002).

FIG. 1. Methods used to analyze sciatic nerve-evoked spike activity from a dorsal spino cerebellar tract (DSCT) neuron during wakefulness and sleep. A: behavioral state characterized by 1st, 2nd, 3rd, and 4th traces (electroencephalogram (EEG), electro-oculogram (EOG), ponto-geniculo-occipital (PGO), and EMG). DSCT neuron spike waveform activity (5th trace) and stimulus trigger (6th trace) were continuously recorded and streamed into a computer program (Spike 2). B: time expanded 50-s epochs of wakefulness (W), quiet sleep (QS), active sleep (AS), and awakening from active sleep (AW) taken from the compressed record in A showing 100 consecutive stimulus presentations to sciatic nerve used in compiling peristimulus time histograms (PSTHs). Sciatic nerve-evoked (0.04 ms, 1.0 Hz, 250 μA) DSCT neuron activity, consisting of short “early” and longer “late” latency responses, was converted to transistor-transistor logic (TTL) pulses. C: superimposed oscilloscope traces depict the response (shaded areas) of this neuron to sciatic nerve stimulation. D: cumulative PSTHs (0.5-ms bin width) constructed from 75 sciatic nerve stimuli during each state in B showing 100 consecutive stimulus presentations to sciatic nerve used in compiling peristimulus time histograms (PSTHs). Sciatic nerve-evoked (0.04 ms, 1.0 Hz, 250 μA) DSCT neuron activity, consisting of short “early” and longer “late” latency responses, was converted to transistor-transistor logic (TTL) pulses. C: superimposed oscilloscope traces depict the response (shaded areas) of this neuron to sciatic nerve stimulation. D: cumulative PSTHs (0.5-ms bin width) constructed from 75 sciatic nerve stimuli during each state in B. Stimulus artifacts are truncated at time bin 0. Numbers over each PSTH indicate average number of spikes per trial for early and late responses (shaded areas). Early and late response magnitudes were reduced during active sleep by ~20 and 39%, respectively, compared with responses obtained during preceding episode of wakefulness.
**Antidromic identification and recording procedures**

Low-intensity search stimuli (0.05 ms, 0.5 Hz, 100–300 μA) were applied to a removable stimulating microelectrode that was stereotaxically positioned within the anterior cerebellar lobule during each recording session to “backfire” DSCT neurons using conventional criteria, i.e., constant latency, collision between spontaneous and antidromically propagated spikes, and high-frequency following (Soja et al. 1995, 1996). All DSCT neurons reported herein satisfied these criteria for antidromicity. Antidromic search stimuli did not cause any indications of arousal, EEG desynchronization, or interfere with the animal’s normal cycling between sleep and wakefulness.

All behavioral state data (EEG, EOG, PGO, and EMG activities) were recorded continuously and digitized “on-line” (sampling rate: 0.2 kHz) into separate waveform channels using a Pentium 4 computer equipped with commercially available software (Spike 2, version 4.01, Cambridge Electronic Design, Cambridge, UK) and hardware (1401-plus, Cambridge Electronic Design). DSCT neuron activity was recorded extracellularly (1,000×, band-pass: 0.3 kHz, −10 kHz) using an AC-coupled amplifier (model 1800, AM-Systems, Carlsborg, WA).

**DSCT neuron spike acquisition procedures**

The signal-to-noise ratio of DSCT neurons exceeded 4:1 (Soja et al. 1995). The action potential height and shape of each DSCT neuron was constantly monitored on a storage oscilloscope, and the stability of the response was verified throughout the duration of each cell’s entire recording time. Substantial ROM memory allocation was required to capture all behavioral state signals and DSCT action potential activity. Hence, spike sorting routines available in Spike 2 were not utilized or required. Instead, a spike processor (model DI130, Digitimer, Hertfordshire, UK) was used to discriminate “on-line” continuous spike activity recorded from DSCT neurons across sleep and waking states (Fig. 1). DSCT neuronal spikes crossing a voltage level discriminator were converted to transistor transistor logic (TTL) pulses, time-stamped, and saved in a separate Spike 2 channel (Figs. 1B and 2B). Saccadic (EOG) and vertical (VOR) eye movements were detected using a trigger TTL pulse from the EOG waveform activity crossing voltage window levels that were preset to correspond to baseline EOG (Fig. 2A, EOG trace) were saved in new channels in the Spike 2 file for each cell. These markers, in turn, were used to create a gate corresponding to the onset and offset of active sleep-related eye movement events or eye movement events during wakefulness. A minimum duration of 250 ms was used as a default for these eye movement events. Eye movement durations were summed and the eye movement density (i.e., summed eye movement event time/total active sleep time) determined across all recording experiments.

**PSTH analyses**

Computer-generated PSTHs were used to quantitatively assess the responsiveness of DSCT neurons to low-intensity stimulation of the sciatic nerve. Primary and secondary PSTH analysis methods were employed in this study. The primary PSTH analysis method was used to determine if DSCT neuron responsiveness was dependent on behavioral state. Here, PSTHs were constructed using 75–200 consecutive sciatic nerve-evoked responses obtained during wakefulness, quiet sleep, active sleep, and awakening from active sleep. Responses during active sleep included those occurring during non–eye movement periods as well as eye movement events. TTL pulses corresponding to DSCT neuron action potentials were cumulatively summed and plotted in 0.5-ms binwidths against the stimulus TTL pulse at time 0. A Spike 2 script was used to determine the average latency-onset (ms) and response magnitude (spikes/stimulus) from user-defined epochs corresponding to early (2–4.5 ms) and/or late (6–200 ms) synaptic responses (see Results).

A secondary PSTH analysis routine and Spike 2 script was used to quantify sciatic nerve-evoked activity for each DSCT neuron specifically around eye movement events of active sleep. Eye movements were identified and gated as an event (Fig. 2A). Stimulus and response TTL markers “during” eye movement events were tagged and matched with markers “before” and “after” each eye movement event (Fig. 2A). A trio of PSTHs, (0.5 ms binwidth) corresponding to before, during, and after eye movement events were constructed from all corresponding “before,” “during,” and “after” TTL markers (Fig. 2D). Each of the three PSTHs during active sleep comprised the same number of nonconsecutive trials across the entire state of active sleep. For comparison, equivalent trial PSTHs were also constructed during wakefulness, quiet sleep, and awakening from active sleep. The average latency-to-onset (ms) and response magnitude (spikes/stimulus) were calculated over user-defined epochs corresponding to early and late responses.

**Eye movement events**

Analyses were performed off-line with data acquisition software (Spike 2, Cambridge Electronic Design) and analysis subroutine scripts that were specially developed in-house for Spike 2 software. These scripts are available from P. J. Soja on request. The behavioral state of active sleep is comprised of hallmark phasic periods where eye movement events of variable length and intensity occur and tonic periods where no eye movement events are present (referred to as the non–eye movement periods of active sleep). Briefly, off-line analyses employed a Spike 2 script that demarcated eye movement episodes from the EOG waveform activity. Marker pulses depicting peaks and troughs of EOG waveform activity crossing voltage window levels that were preset to correspond to baseline EOG (Fig. 2A, EOG trace) were saved in new channels in the Spike 2 file for each cell. These markers, in turn, were used to create a gate corresponding to the onset and offset of active sleep-related eye movement events or eye movement events during wakefulness. A minimum duration of 250 ms was used as a default for these eye movement events. Eye movement durations were summed and the eye movement density (i.e., summed eye movement event time/total active sleep time) determined across all recording experiments.

**Microiontophoresis procedures**

In several experiments, the single barrel recording microelectrode in the spinal cord was removed and replaced with a commercial multibarrel glass micropipette (Carbostar-4, Kation Scientific, Minneapolis, MN) for extracellular unit recording and simultaneous juxtacel lar drug microiontophoresis (see Fig. 1A, magnified box enclosure in Taepavarapruk et al. 2002). The main carbon fiber-containing recording barrel was surrounded by barrels filled with the inhibitory amino acid agonist GABA (0.5 M, pH 3.5), the inhibitory amino acid antagonist bicuculline methiodide (Curtis et al. 1971; Knjovic et al. 1977) (BIC: 0.02 M, pH 4.0), or sodium chloride (NaCl: 4 M). All drugs used for microiontophoresis were dissolved in distilled water and were obtained from Sigma-Aldrich. Drugs were ejected using positive currents. Negative currents (<10 nA) were continuously applied to each drug barrel to minimize spontaneous leakage. Automatic current neutralization procedures were performed using a drug barrel containing 4 M NaCl during drug microiontophoresis (Taepavarapruk et al. 2002).

In these experiments, a two sleep cycle paradigm was employed whereby the DSCT neuronal response magnitude to sciatic nerve stimulation was first determined during active sleep (non–eye movement and eye movement portions) and compared with preceding episodes of wakefulness, quiet sleep, and awakening from active sleep. The response magnitude was tested in the second sleep cycle while the GABA_A receptor antagonist, bicuculline (Curtis et al. 1971; Knjovic et al. 1977), was continuously ejected. Bicuculline was microiontophoresically released in quantities sufficient to block GABA-evoked responses during the state of wakefulness or quiet sleep prior to active sleep in this second sleep cycle. Once this pharmacological control was achieved, the bicuculline ejection was maintained throughout the subsequent
episode of active sleep during the second (test-bicuculine) sleep cycle. We have previously utilized multiple control and test sleep cycle microiontophoretic paradigms for determining the neurotransmitter basis for the inhibition of spontaneous spike activity of DSCT neurons during active sleep (Taepavarapruk et al. 2002).

**Statistical analyses**

Values are reported as means ± SE in the text and figures. Each recorded DSCT neuron served as its own control. The magnitude and latency-to-onset of DSCT neuron responses to sciatic nerve stimulation determined from each PSTH were assessed as a function of behavioral state. Most of the population data reported herein were not normally distributed. Therefore, parameters of response magnitude and response latency were universally compared during active sleep with eye movement events, active sleep with non–eye movement periods, wakefulness, quiet sleep, and awakening from active sleep using a nonparametric Friedman repeated-measures ANOVA on ranks to determine if a statistical difference was present. Then, to isolate the group or groups that differed from the others, a multiple comparison procedure was performed (Student–Newman–Keuls method). Relative percent changes in response magnitude during non–eye movement periods of active sleep, early and late response magnitude decreased by −27 and −17%, respectively, compared with non–eye movement periods of active sleep.

![Diagram of Methods](http://jn.physiology.org/)

**FIG. 2.** Methods used to analyze sciatic nerve-evoked spike activity from a DSCT neuron around eye movement events (EM) of active sleep. A: 8-s epoch of the active sleep episode around an eye movement event is represented by the 1st, 2nd, and 7th traces (EEG, EOG, and EMG), and DSCT neuron waveform activity was continuously recorded and streamed into a computer program (Spike 2). A subroutine was used to demarcate onset and offset of EOG events from TTL pulses formed from EOG crossings at preset voltage levels (dotted horizontal lines in A). TTL pulses corresponding to sciatic nerve stimuli (0.04 ms, 1.0 Hz, 300 μA) and evoked DSCT neuron responses are shown in the bottom 2 traces. Stimulus and response markers “during” eye movement events were tagged and matched with markers “before” and “after” each eye movement event (4th–6th traces from top). B: time-expanded view of 5 consecutive overlaid traces of a DSCT neuron’s response to sciatic nerve stimulation around an individual eye movement event of active sleep. Fidelity of the early response was attenuated during this particular eye movement event (1 response occurred per 5 stimulus trials vs. 4 and 5 responses per 5 trials before and after the eye movement event, respectively). C: cumulative PSTHs of DSCT neuron activity over the entire period of active sleep around all detected eye movement events. During eye movement periods of active sleep, early and late response magnitude decreased by −27 and −17%, respectively, compared with non–eye movement periods of active sleep.
RESULTS

General characteristics of DSCT neurons

Experiments were performed on a total of 60 antidromically identified DSCT neurons obtained from three cats, all of which displayed spontaneous spike activity during the state of wakefulness. Fifty of these neurons were examined using a single barrel micropipette, and the remaining 10 neurons were recorded using a multibarrel micropipette. The antidromic latency for all 60 cells measured 3.28 ± 0.10 (SE) ms (range, 2.5–6.5 ms). The estimated average axonal conduction velocity measured 79.5 ± 1.72 m/s (range, 38.5–101.2 m/s). These observations are consistent with those of previous studies of DSCT neurons recorded across sleep and wakefulness (Soja et al. 1996, 2001a; Taepavarapruk et al. 2002, 2003b).

DSCT neuron responses to sciatic nerve stimulation during wakefulness

During the control state of wakefulness, threshold single shock stimuli applied to the sciatic nerve (0.05 ms, 0.2–0.5 mA, 1.0 Hz) produced a single action potential or a train of action potentials in 50 DSCT neurons tested. The nature of the response varied among DSCT neurons. In 18 of these neurons, a single spike response was recorded with a group mean latency of 3.27 ± 0.15 ms. In 40 other DSCT neurons, a burst of two to six action potentials occurred at a mean latency of 23.7 ± 2.23 ms. In our population of 50 DSCT neurons, 8 DSCT neurons displayed both short latency and longer latency spike responses.

Modulation of sciatic nerve responses during wakefulness and sleep

The primary PSTH analysis method was used to determine if DSCT neuronal responsiveness to sciatic nerve stimulation depended on behavioral state. PSTHs were constructed using an average of 125 ± 10 consecutive sciatic nerve stimuli presented during wakefulness, quiet sleep, active sleep, and awakening from active sleep. The response magnitude and onset latency were analyzed and compared as a function of state from these PSTHs. The “early” responses in 18 of 50 DSCT neurons did not differ in their group mean (±SE) magnitude between the states of wakefulness (1.07 ± 0.07 spikes/trial), quiet sleep (0.98 ± 0.06 spikes/trial), or awakening from active sleep (1.01 ± 0.09 spikes/trial; P > 0.05, repeated-measures ANOVA). Overall, for these same 18 neurons, the short latency response during active sleep (0.79 ± 0.09 spikes/trial) decreased by 26% compared with the control state of wakefulness (P < 0.05, repeated-measures ANOVA, Student-Newman-Keuls method). An example of a DSCT neuron responding to consecutive sciatic nerve stimuli during sleep and wakefulness is presented in Fig. 1. In contrast to response magnitude, the latency of the early response did not significantly differ between wakefulness (3.27 ± 0.15 ms), quiet sleep (3.37 ± 0.23 ms), active sleep (3.43 ± 0.24 ms), or awakening from active sleep (3.48 ± 0.23 ms; P > 0.05, repeated-measures ANOVA).

The “late” responses were also analyzed during sleep and waking states. The group mean magnitude of the late response in 40 of 50 DSCT neurons did not differ between the states of wakefulness (3.01 ± 0.32 spikes/trial), quiet sleep (2.91 ± 0.29 spikes/trial), or awakening from active sleep (2.92 ± 0.32 spikes/trial; P > 0.05, repeated-measures ANOVA, Student-Newman-Keuls method). Overall, the long latency response during active sleep (2.59 ± 0.31 spikes/trial) decreased ~14% compared with the control state of wakefulness (P < 0.05, repeated-measures ANOVA, Student-Newman-Keuls method). The onset of the “late” response did not differ significantly (P > 0.05) between wakefulness (23.7 ± 2.23 ms), quiet sleep (24.3 ± 2.17 ms), active sleep (23.7 ± 1.93 ms), or awakening from active sleep (23.8 ± 3.31 ms).

Hence, experimentally evoked monosynaptic and polysynaptic transmission between primary afferent terminals and identified DSCT neurons is significantly decreased specifically during the state of active sleep.

General characteristics of eye movement events during active sleep

Eye movement events hallmark the state of active sleep. The mean number of eye movement events, individual event duration, and cumulative eye movement event time across a total of 65 active sleep episodes measured 15.3 ± 1.3 events, 2.3 ± 0.1 s, and 40.4 ± 7.4 s, respectively. The mean total active sleep duration measured 449.2 ± 63.8 s. Finally, the density of eye movement events detected during the state of active sleep in this study measured 9.6% of this behavioral state.

Modulation of sciatic nerve-evoked responses of DSCT neurons during eye movement events of active sleep

Since the density of eye movement events during active sleep amounted to ~10% of this entire state, any suppression of synaptic transmission between primary afferents and the recorded DSCT neurons reported above using primary PSTH analyses may have been masked by the proportionally larger number of responses averaged during non–eye movement portions of the active sleep state. To investigate this possibility, we analyzed the response magnitude and latency-to-onset data of early and late responses derived from PSTHs of the same sciatic nerve-evoked DSCT neurons before, during, and after individual eye movement events of active sleep using secondary subroutines developed in-house. An average number of 41 ± 6 stimulus trials was used to construct these cumulative PSTHs across each state and around eye movement events. PSTHs were compared on an equal trial basis with PSTHs constructed from data in the preceding states of wakefulness, quiet sleep, or awakening from active sleep.

The group mean (±SE) magnitude of the early sciatic responses did not differ between the states of wakefulness, quiet sleep, or awakening from active sleep (Fig. 3A; P > 0.05, repeated-measures ANOVA). However, during the non–eye movement events of active sleep, the group mean response magnitude decreased by 25.8% before and 24.3% after eye movement events (Fig. 3A; P < 0.05, repeated-measures ANOVA, Student-Newman-Keuls method). During eye movement events of active sleep, the early sciatic nerve-evoked response magnitude decreased further by 14.4% (Fig. 3A; P < 0.05, repeated-measures ANOVA, Student-Newman-Keuls method), which corresponds to an overall 36.4% decrease in
the response magnitude compared with response magnitude during the preceding state of wakefulness (Fig. 3A; \( P < 0.05 \), repeated-measures ANOVA, Student-Newman-Keuls method).

The population plot in Fig. 4 indicates that the early response in 8 of 18 (44.4%) DSCT neurons underwent \( >25\% \) suppression in response magnitude, and none were facilitated before eye movement events of active sleep. During the eye movement events of active sleep, the early response underwent \( >25\% \) suppression response magnitude in 12 of 18 (66.7%) of the DSCT neurons (Fig. 4). The dashed curve in Fig. 4 indicates that the suppression of the early response increased further during eye movement periods of active sleep for the entire population of 18 neurons. The latency-to-onset of the early response did not differ across the sleep wake cycle or around eye movement events of active sleep (Fig. 3B; \( P > 0.05 \), repeated-measures ANOVA).

The magnitude of the late response was affected in the same manner as the early response. That is, the response magnitude of 40 DSCT neurons did not differ between wakefulness, quiet sleep, or awakening from active sleep (Fig. 3A; \( P > 0.05 \), repeated-measures ANOVA).

**FIG. 3.** Histograms depicting the response magnitude of the early and late response recorded from DSCT neurons during wakefulness (W), quiet sleep (QS), active sleep (AS), and awakening from active sleep (AW). Response magnitude (A) and latency (B) was further analyzed around eye movement events (EM) of active sleep, i.e., before, during, and after EM events. Each histogram bar represents the group mean \( \pm SE \). *Significant differences compared with the control state of wakefulness. **Significant difference from responses obtained before or after EM events (\( P < 0.05 \), repeated-measures ANOVA, Student-Newman-Keuls method).

**FIG. 4.** Histograms depicting the change in response magnitude of sciatic nerve-evoked spike activity recorded from Clarke’s Column DSCT neurons before eye movement events (black bars) and during eye movement events (gray bars) of active sleep compared with wakefulness. Negative values on the abscissa represent suppression; positive values represent facilitation. Note that response magnitude of spike activity evoked by sciatic nerve stimuli was suppressed in the majority of DSCT neurons examined. Curves indicate distribution of these data about their mean before (solid curve) and during (dashed curve) the eye movement portions of active sleep. Note that during eye movement events, both the early and late responses as a population, were further suppressed.
repeated-measures ANOVA), but was reduced by 11.5 and 8.6% before and after eye movement events of active sleep, respectively (Fig. 3A; $P < 0.05$, repeated-measures ANOVA, Student-Newman-Keuls method). Similar to the early response, the late response magnitude underwent an additional 11.5% suppression during eye movement events of active sleep (Fig. 3A; $P < 0.05$, repeated-measures ANOVA, Student-Newman-Keuls method). This corresponds to an overall 21.6% decrease in the magnitude of the late response during eye movements of active sleep relative to wakefulness.

Figure 4 indicates that the late response in 15 of 40 (37.5%) DSCT neurons underwent $>25\%$ suppression in response magnitude, while 2 of 40 (5%) cells underwent $>25\%$ facilitation before eye movement events of active sleep. During the eye movement events of active sleep, response magnitude decreased $>25\%$ in 23 of 40 (57.5%) of the DSCT neurons. Only one neuron (2.5%) underwent $>25\%$ facilitation during eye movement events (Fig. 4). The dashed curve in Fig. 4 indicates that the suppression of the late response increased during eye movement periods of active sleep for the entire population of 40 neurons. The latency-to-onset of the late response did not differ across the sleep wake cycle or around eye movement events of active sleep.

Overall, the results indicate that peripheral nerve-evoked activity of identified DSCT neurons is significantly attenuated during eye movement events of active sleep compared with non–eye movement periods of active sleep. To investigate if the extent of eye movement–related suppression was related to the amount of suppression of the sciatic nerve-evoked activity that occurs during non–eye movement active sleep relative to wakefulness, these two ratios were plotted against each other (Fig. 5). There was no significant relationship (correlation coefficient: $-0.23$; $P > 0.05$, Spearman rank-order correlation) identified between these two parameters. Thus, in this study, it is unlikely that ongoing inhibition during non–eye movement portions of active sleep masks the neuronal sup-

FIG. 5. Scatter plot showing change in sciatic nerve-evoked response magnitude ($○$, early response; ●, late response) of DSCT neurons during non–eye movement active sleep relative to wakefulness vs. change in response magnitude during eye movement events relative to non–eye movement active sleep. No significant relationship between the 2 parameters was identified ($P > 0.05$, Spearman rank-order correlation), indicating that ongoing neuronal suppression during the non–eye movement parts of active sleep did not mask the effect of eye movement events on sciatic nerve-evoked activity.

press that DSCT neurons undergo during eye movement events of active sleep.

Active sleep-related inhibition of sciatic nerve-evoked responses of DSCT neurons: sensitivity to bicuculline

The GABA$_A$ receptor antagonist bicuculline was assessed for its ability to block the suppression of DSCT neuron activity around eye movements during active sleep. This was accomplished in 10 DSCT neurons that responded to sciatic nerve stimulation; 4 of these responded with a short (early) latency response, and the remaining 6 responded with a longer (late) latency response. The experimental paradigm taken in these particular experiments also required monitoring each cell’s activity over a minimum of at least two consecutive sleep cycles. The first sleep cycle served as a control to establish baseline levels of active sleep–specific neuronal suppression. The second sleep cycle served as a test to assess active sleep-related suppression of DSCT neuron activity in the presence of the sustained release of bicuculline. During the second sleep cycle, bicuculline ejection currents (67.8 $\pm$ 7.7 nA, 15.4 $\pm$ 2.7 min, $n = 10$ neurons) were first adjusted for each neuron by confirming the drug’s ability to block the suppressive actions of GABA pulsed at regular 10 s intervals (46.2 $\pm$ 9.2 nA, $n = 10$ neurons) on sciatic nerve-evoked responses. Once this was confirmed (Fig. 6), the ejection current applied to the bicuculline barrel for each cell was maintained into and throughout the state of active sleep and subsequent awakening from active sleep.

During the first control sleep cycle, the early response magnitude in four DSCT neurons did not differ between the states of wakefulness, quiet sleep, and awakening from active sleep (Fig. 7A; $P > 0.05$, repeated-measures ANOVA). How-
ever, compared with the state of wakefulness, the group mean magnitude of the early response during active sleep decreased by 32.3, 44.3, and 28.7% before, during, and after eye movement events, respectively (Fig. 7A; \( P < 0.05 \), repeated-measures ANOVA, Student-Newman-Keuls method). As shown in Fig. 7A, the 12% additional suppression observed during the eye movement events of active sleep was statistically significant from the response magnitude before eye movement events of active sleep (\( P < 0.05 \), repeated-measures ANOVA, Student-Newman-Keuls method). During the second sleep cycle and in the presence of bicuculline (65.5 ± 7.8 nA, 15.4 ± 3.7 min), suppression of the early response in the same four DSCT neurons was abolished during active sleep (Fig. 7A; \( P > 0.05 \), repeated-measures ANOVA).

In contrast to response magnitude, the latency of the early response for the four DSCT neurons did not significantly differ between any of the behavioral states in the first versus second sleep cycles (Fig. 7B; \( P > 0.05 \), repeated-measures ANOVA).

Similarly, the magnitude of the late response recorded from six other DSCT neurons decreased in the first (control) sleep cycle by 27.8, 41.5, and 23.7% before, during, and after eye movement events, respectively (Fig. 7A; \( P < 0.05 \), repeated-measures ANOVA, Student-Newman-Keuls method). The additional 13.7% suppression of the late response observed during the eye movement events of active sleep was also statistically significant from that observed before eye movement events of active sleep (Fig. 7A; \( P < 0.05 \), repeated-measures ANOVA, Student-Newman-Keuls method). During the second sleep cycle, in the presence of bicuculline (69.3 ± 12.2 nA, 15.3 ± 4.0 min), suppression of the late response in the same six DSCT neurons was also abolished (Fig. 7A; \( P > 0.05 \), repeated-measures ANOVA).

The latency of the late response did not differ between behavioral state in the first (control) and second (test-bicuculline) sleep cycles (Fig. 7B; \( P > 0.05 \), repeated-measures ANOVA).

These data suggest that the inhibitory neurotransmitter GABA participates in the active sleep-specific suppression of early and late synaptic responses of DSCT neurons evoked by low-intensity sciatic nerve stimulation. GABA mediates the tonic suppression during non–eye movement events as well as the increased phasic suppression during eye movement events of active sleep.

**DISCUSSION**

This study was performed to examine for the first time the response characteristics of DSCT neurons to low-intensity stimulation of the sciatic nerve in chronic unanesthetized cats during electrophysiologically confirmed behavioral states of wakefulness, quiet sleep, and active sleep. The discussion will focus on the response profile of DSCT neurons to peripheral afferents, reduced responsiveness of DSCT neurons during...
active sleep, the effect of bicuculline on such inhibitions, and the functional significance of state-dependent GABAergic control of DSCT neurons.

Nature of sciatic nerve-evoked responses of DSCT neurons in the chronic preparation

In this study, sciatic nerve stimulation resulted in the synaptic activation of DSCT neurons that was characterized by short latency (early) and long latency (late) spike discharges. The early spike response can be attributed to a monosynaptic linkage of large diameter group I primary afferents with recorded DSCT neurons (Eccles et al. 1961). Indeed, intracellular records published from earlier studies indicate that the latencies of peripheral nerve-evoked DSCT neuronal action potentials crowning monosynaptic excitatory postsynaptic potentials (EPSPs) are similar to the latencies of our spikes recorded extracellularly in response to sciatic nerve stimuli (Curtis et al. 1986; Eccles et al. 1961; Eide et al. 1969; Xi et al. 1997). The late response is likely due to polysynaptic inputs from a variety of receptors via cutaneous and muscular afferent fibers and corresponds closely in terms of onset latency to the type III responses of earlier investigations performed in anesthetized acute animal preparations (Eccles et al. 1961; Eide et al. 1969; Mann 1973; Osborn and Poppele 1983, 1988; Walmsley 1989).

The silent period that often precedes polysynaptic responses is reminiscent of studies performed by earlier investigators where a similar pause occurs in spike activity of DSCT neurons following peripheral nerve stimulation. This pause has been attributed to inhibitory postsynaptic potentials (IPSPs) set up by local circuit or extrasegmental interneurons (Knox et al. 1977).

Despite the similarities in peripheral nerve-evoked response patterns of DSCT neurons between earlier studies using acute anesthetized animal preparations and ours, important differences also exist. Most importantly, the neural distortion caused by depressant actions of anesthetics is absent in this study. Indeed, barbiturate anesthetics significantly reduce response magnitude and increase the latency-to-onset of early responses in DSCT neurons by 23 and 28%, respectively (Soja et al. 2002). Moreover, as shown in this report, the response magnitude is clearly dependent on behavioral state.

State-dependent suppression of sciatic nerve-evoked responses

In the mid-1960s, Carli et al. (1966, 1967a,b) first suggested that sensory transmission was reduced during active sleep. They reported that hindlimb nerve-evoked responses recorded as a population or as field potentials from ipsilateral T12 ventrolateral quadrants or the cerebellum were occasionally reduced in magnitude during phasic rapid eye movement (REM) episodes of active sleep as opposed to other states such as wakefulness, quiet sleep, or tonic non-REM periods of active sleep (Carli et al. 1966, 1967a,b). These investigators were unable to unequivocally determine if the modulation of evoked responses during active sleep was due to changes occurring specifically in the input from dorsal spinocerebellar tract neurons, per se, and/or other ascending pathways. The data reported herein, using chronic single unit recording methodologies, clearly indicate that identified upper lumbar DSCT neurons constitute one ascending second order sensory pathway that contributes to the population response observed in earlier studies. The suppression of DSCT neuron responses to sciatic nerve stimulation we observed is different from earlier evoked potential studies in that evoked DSCT neuron activity undergoes phasic inhibition not only during eye movement episodes but also sustained tonic suppression throughout the non–eye movement portions of active sleep.

Any distinct change in the transfer of sensory input from primary afferents to the recorded neurons is masked if data during phasic eye movement periods are analyzed together with data from non–eye movement periods. The results presented clearly emphasize this point. For example, when the primary PSTH analysis method was used, which by default, included non–eye movement and eye movement periods, there was an overall 26% suppression of the early response. Similarly, when the secondary methods of PSTH analysis were used that selectively parcelled out responses during non–eye movement periods of active sleep, nearly the exact same amount (25.8%) of suppression occurred. However, when response magnitude was analyzed around, i.e., before, during, and after eye movements in a paired fashion, further significant suppression (14.4%) of synaptic transmission occurred specifically during eye movement events of active sleep. This finding is characteristic for the vast majority of DSCT neurons recorded in this study and does not seem to be related to the degree of ongoing tonic suppression that is also present during non–eye movement portions of active sleep.

The source and neural pathway(s) conveying descending influences inhibiting DSCT neurons during non–eye movement and eye movement events of active sleep is not completely understood. The spinal inhibitory neurons recruited by sciatic nerve stimulation that have been suspected of mediating the pause that often precedes the late response of DSCT neurons recorded in the acute preparation (Knox et al. 1977) are unlikely to play a significant causative role since the latency of the late response did not vary as a function of state or after bicuculline administration.

Lumbar motoneurons are known to be tonically hyperpolarized during non–eye movement periods of active sleep by a process of glycine-mediated postsynaptic inhibition (Chase et al. 1989; Soja et al. 1991). The amplitude of monosynaptic EPSPs intracellularly recorded from motoneurons decreases during non–eye movement periods of active sleep compared with quiet sleep. During eye movement events, the EPSP amplitude is further decreased (Chandler et al. 1980; Morales and Chase 1981). During eye movement events of active sleep, the membrane potential activity of motoneurons is also characterized by the presence of complex phasic hyperpolarizations which have yet to be characterized pharmacologically (Chase and Morales 1982; Morales and Chase 1981). It is possible that certain brain stem nuclei may relay descending influences to spinal neurons during eye movement events. In this regard, reticulospinal neurons backfired from the L3 spinal cord segment display firing rates reciprocal to DSCT neurons during sleep and wakefulness, being 10-fold higher during non–eye movement events of active sleep compared with preceding episodes of wakefulness or quiet sleep. During eye movements of active sleep, reticulospinal neuron discharge...
is increased another twofold (Soja et al. 2003; Taepavarapruk et al. 2003a; see also Wyzinski et al. 1978).

Irrespective of exactly how reticulospinal neurons are coupled to recorded cells (i.e., direct inhibitory connections and/or excitatory connections with inhibitory interneurons that, in turn, impinge on target neurons or afferent terminals), sciatric nerve-evoked monosynaptic responses such as the early responses recorded from DSCT neurons in this study or monosynaptic EPSPs recorded from motoneurons in the previous studies (Chandler et al. 1980; Morales and Chase 1981), undergo increased phasic suppression during eye movement events. Perhaps parallel interneuronal circuits are recruited by reticulospinal neurons to suppress sensory inflow via the DSCT as well as motor outflow. Such a divergence of reticulospinal inhibitory pathways would potentially obtund ascending sensory inputs and movements that might compromise the continuance of the active sleep state. However, further studies are warranted to investigate this possibility.

Another key issue our data addresses is whether active sleep-specific suppression of the sciatric nerve-evoked responses in DSCT neurons can be adequately explained by withdrawal of afferent input, i.e., disfacilitation, that is secondary to motor atonia and the hyperpolarization of motoneurons (Chase et al. 1989; Morales and Chase 1981; Soja et al. 1991). Disfacilitation is unlikely to account for the reduced synaptic responses in this study because sciatric nerve stimulation bypasses peripheral sensory receptors. Moreover, the magnitude of the monitored incoming volley in several experiments where the sciatric nerve-evoked field potential was recorded in Clarke’s column (unpublished data) did not change during the sleep/wake cycle. Hence, active sleep-related inhibition directed toward the early monosynaptic response likely occurs in the spinal cord between the central terminals of large diameter primary afferents and the recorded DSCT neurons. The site of active sleep-specific suppression for the late responses may occur at presynaptic afferent terminals, and/or postsynaptically on last-order interneurons conveying the late responses to DSCT neurons, and even the recorded cells themselves.

State-dependent inhibition of DSCT neurons by GABA

Recently, we reported that the GABA_A receptor antagonist bicuculline and the glycine antagonist strychnine blocked the active sleep-specific suppression of spontaneous spike activity, as well as the excitatory amino acid-driven responses of DSCT neurons, suggesting that GABA and glycine pre- and/or postsynaptically inhibit sensory inflow via the DSCT (Taepavarapruk et al. 2002). We hypothesized that supraspinal systems are activated during active sleep and inhibit, via GABAergic or glycinergic neurons, second-order DSCT neurons, and even the recorded cells themselves.

Functional significance of state-dependent inhibition of DSCT neurons

Malfunctioning GABA control mechanisms may adversely influence DSCT neurons in certain sleep disorders. For example, benzodiazepines effectively minimize the sensory dysesthesias associated with restless legs syndrome (RLS) and sensorimotor effects accompanying REM behavior disorder (RBD). Patients with RLS experience an imperative desire to move their lower legs because of paraesthesias or dysesthesias associated with restless legs syndrome (RLS) and sensorimotor effects which occur at rest and this often delays sleep onset. Periodic limb movements often accompany RLS symptoms and worsen during active sleep in RLS patients (Patel et al. 1997; Tenkwalder 1998). Ineffective muscular atonia mechanisms and/or excess facilitatory drives onto motoneurons are thought to underlie the varying degrees of sensorimotor activation in RBD (Chase and Morales 1990; Schenck and Mahowald 2002). Taken together, impaired glycine and GABA-mediated inhibi-
tion that normally occurs in motor (Chase et al. 1989; Soja et al. 1991) and ascending sensory pathways such as Clarke’s column DSCT neurons (Soja et al. 1993, 1996, 2001a;b; Taepavarapruk et al. 2002) may contribute to these clinical syndromes. Future electrophysiological studies are warranted to investigate the state-dependent regulation of GABAergic (and glycineric) pathways inhibiting sensory inflow to higher brain centers under natural conditions and conditions emulating RLS and RBD.

CONCLUSION

The results of this study suggest that, during active sleep, the mono- and polysynaptic activation of DSCT neurons is subjected to GABAergic inhibition. Tonic inhibition occurs during the non–eye movement portions of active sleep that is further phasically enhanced during the eye movement events that are characteristic of this behavioral state.

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Present address of N. Taepavarapruk: Department of Physiology, Faculty of Medical Sciences, Naresuan University, Amphure Muang, Phitsanulok, Thailand 65000.

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