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CEREBELLAR-RESPONSIVE NEURONS IN THE THALAMIC VENTROANTERIOR–VENTROLATERAL COMPLEX OF RATS: *IN VIVO* ELECTROPHYSIOLOGY

S. F. SAWYER,* S. J. YOUNG,† P. M. GROVES‡ and J. M. TEPPER‡§

*Department of Physiology and Pharmacology, Bowman Gray School of Medicine, Wake Forest University, Medical Center Boulevard, Winston-Salem, NC 27157, U.S.A.

†Department of Psychiatry, School of Medicine, University of California at San Diego, La Jolla, CA 92093, U.S.A.

‡Center for Molecular and Behavioral Neuroscience, Aidekman Research Center, Rutgers, The State University of New Jersey, 197 University Avenue, Newark, NJ 07102, U.S.A.

Abstract—*In vivo* intracellular recordings were obtained from identified thalamocortical neurons in the ventroanterior–ventrolateral complex in urethane-anesthetized rats. This thalamic nucleus has few interneurons. Neurons that responded to cerebellar stimulation were injected intracellularly with horseradish peroxidase or biocytin and examined with light and electron microscopy (see companion paper). Intrinsic membrane properties and voltage-dependent rhythmic activity of cerebellar-responsive ventroanterior ventrolateral neurons were similar to those described previously for thalamic neurons. Thus, in addition to conventional "fast" Na^+-dependent spikes, rat ventroanterior-ventrolateral neurons had "slow" Ca^{2+}-mediated low-threshold spikes and membrane conductances that supported rhythmic oscillations. Two modes of spontaneous activity were observed: (i) a tonic firing pattern that consisted of irregularly occurring fast spikes that predominated when the membrane potential was more positive than about -60 mV, and (ii) a rhythmic firing pattern, observed when the membrane potential was more negative than about -65 mV, composed of periodic (4–8 Hz) membrane hyperpolarizations and ramp depolarizations that often produced a low-threshold spike and a burst of fast spikes. In some neurons, spontaneous fast prepotentials were also observed, often with a relatively constant rate (up to 70 Hz).

Cerebellar stimulation elicited excitatory postsynaptic potentials that in some cases appeared to be all-or-none and were similar in form to fast prepotentials. Stimulation of ipsilateral motor cortex elicited a short-latency antidromic response followed by a monosynaptic excitatory postsynaptic potential, which had a slower rise time than excitatory postsynaptic potentials evoked from cerebellum, suggesting that cortical inputs were electrotonically distal to cerebellar inputs. In the presence of moderate membrane hyperpolarization, the cortically evoked excitatory postsynaptic potential was followed by a long-lasting hyperpolarization (100–400 ms duration), a rebound depolarization and one or two cycles resembling spontaneous rhythmic activity. Membrane conductance was increased during the initial component of the long hyperpolarization, much of which was probably due to an inhibitory postsynaptic potential. In contrast, membrane conductance was unchanged or slightly decreased during the latter three-quarters of the long hyperpolarization. The amplitude of this component of the long hyperpolarization usually decreased when the membrane was hyperpolarized with intracellular current injection. Thus, both disfacilitation and an inhibitory postsynaptic potential may have contributed to the latter portion of the cortically-evoked long hyperpolarization. The cortically-evoked inhibitory postsynaptic potentials likely originated predominantly from feedforward activation of GABAergic neurons in the thalamic reticular nuclei. The disfacilitation probably resulted from activation of inhibitory circuits intrinsic to the cortex and/or corticothalamic circuits that transiently reduced tonic cortical excitatory drive onto thalamic neurons. The possibility that disfacilitation occurred following experimentally-induced synchronous activation of cortical circuitry suggests that the cerebral cortex provides a significant degree of tonic excitatory drive onto neurons in the ventroanterior ventrolateral complex of rats.

We conclude that the synaptic and intrinsic membrane properties of thalamic neurons in the ventroanterior–ventrolateral complex of rats are fundamentally similar to previously described properties of thalamocortical neurons of other species (e.g. felines) in related nuclei that possess GABAergic interneurons, in spite of the scarcity of GABAergic interneurons in rat ventroanterior–ventrolateral complex. Furthermore, rhythmic disfacilitation of cortical inputs to thalamus may contribute to the maintenance of the rhythmic activity in thalamocortical circuits that is prominent during different behavioral states.

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§To whom correspondence should be addressed.
Abbreviations: AHP, afterhyperpolarization; dLGN, dorsal lateral geniculate nucleus; EEG, electroencephalogram; EGTA, ethyleneglycoltetra-acetate; EPSP, excitatory postsynaptic potential; FPP, fast prepotential; HRP, horseradish peroxidase; IPSP, inhibitory postsynaptic potential; LTS, low-threshold spike; TRN, thalamic reticular nucleus; VAL, ventroanterior ventrolateral complex.

The cerebellar nuclei exert synaptic influences on "upper motor neurons" in primary motor cortex via dorsal thalamus.[109] The primary thalamic nucleus in the cerebello-thalamo-cortical pathway in rats is the ventroanterior–ventrolateral (VAL) complex. As in corresponding thalamic nuclei of higher order mammals, the VAL receives a topographically organized input from the cerebellar nuclei and has reciprocal connections with motor cortex.[4,12,26,108] The thalamic link in this pathway has traditionally been considered to be a single-synapse, short-latency "relay" or "gate", depending on the operational mode of the thalamus. For example, afferent impulses from the cerebellar nuclei are conveyed to cortex with a high degree of fidelity when the thalamic neuron firing pattern is tonic and irregular, as during electroencephalogram (EEG) desynchronization. This mode is associated with sustained membrane depolarization.[35] In contrast, transfer of subcortical afferent impulse activity to cortex is significantly altered when the membrane potential of thalamic neurons oscillates rhythmically between periods of burst activity and hyperpolarization, such as during EEG synchronization.[28,51,88,94] Although consideration of thalamic nuclei as a relay/gate station during wake/sleep cycles is of heuristic value, this view is a well-appreciated oversimplification. In fact, several anatomical substrates exist for thalamic integration and processing of subcortical inputs. For example, the neurons in the thalamic reticular nucleus (TRN) are GABAergic[21,38] and project topographically to dorsal thalamus.[12] The TRN receives excitatory inputs from axon collaterals of corticothalamic and thalamocortical neurons[1,95] that originate from reciprocally connected ipsilateral cortical and thalamic areas, thereby engaging the TRN in topographically-aligned feedforward and feedback inhibitory circuits with dorsal thalamus.[12,83] Thalamic interneurons, which are driven by excitatory cortical and subcortical afferents, provide an additional means for GABAergic feedforward inhibition.[20,46,56,57,104]

The cerebellar-recipient ventral tier nuclei in dorsal thalamus of felines and primates possess an extensive GABAergic interneuronal synaptic network[46,77,84] that works in conjunction with afferents from the TRN. In stark contrast, rodent VAL has few GABAergic interneurons,[60,68,79,81] with the TRN being the only substantial GABAergic input to the VAL in rats, except for a restricted ventral territory that is innervated by the basal ganglia.[22] The intrinsic membrane properties and synaptically-evoked responses of thalamic projection neurons have been well characterized in nuclei with abundant numbers of GABAergic interneurons,[49,94] including motor-related thalamic nuclei in the cat.[19,23,25,66,78] The paucity of interneuronal GABAergic circuitry in rat VAL provides an impetus to examine the electrophysiological properties of neurons in this nucleus. Functional comparisons with neurons in

corresponding nuclei of higher order mammals, and with neurons in other thalamic nuclei in rodents that possess interneurons, should provide insight regarding the contribution of interneurons to integrative synaptic processing by the thalamus. The aim of the present study was to describe the *in vivo* electrophysiological properties of neurons in the VAL of rats and to examine the consequences of the absence of GABAergic interneurons on the processing of cortical and subcortical synaptic inputs by VAL thalamocortical neurons.

EXPERIMENTAL PROCEDURES

Animal surgery

Male Sprague–Dawley rats (Charles River) weighing 200–300 g were anesthetized with urethane (1.3 g/kg, i.p.), secured on a stereotaxic apparatus and prepared for intracellular recording as described previously.[98] All wound margins and points of contact were coated with 5% lidocaine ointment. The scalp was removed and small burr holes drilled to permit placement of stimulating electrodes into the left primary motor cortex (i.e. the lateral agranular frontal cortical field; A 2.5 mm from bregma, L 2.2 mm from midline, V 1.5 mm ventral to the surface of the cortex; coordinates from atlas of Paxinos and Watson[70]) and the right cerebellar nuclei (A −3.5 mm from lambda, L 2.5 mm, V 5.0 mm, and A −4.3 mm, L 2.8 mm, V 5.0 mm). The stimulating electrodes were affixed to the cranium with cyanoacrylate glue and dental cement, and an opening (centered at A 4.9 mm from lambda, L 2.2) was drilled above the left thalamus for recording. The cerebral cortex was left intact. The stereotaxic apparatus and recording assembly were placed on a Micro G air isolation table to minimize vibration. The atlanto-occipital membrane was punctured to allow drainage of cerebrospinal fluid and the animal was suspended via C2 and tail clamps to reduce vascular- and respiratory-induced pulsations. A well of dental cement was formed around the recording hole, and after insertion of the recording electrode into the brain the well was filled with a mixture of paraffin and paraffin oil to prevent tissue drying and to increase the stability of intracellular recordings. Body temperature was maintained at 37 ± 1°C with a solid state heating pad. All animals were treated in strict accordance with the U.S. Public Health Service *Guide for the Care and Use of Laboratory Animals*.

Recording and stimulation

Micropipettes for intracellular recordings were made from 2.0 mm (o.d.) capillary glass (WPI) using a Narishige PE-2 pipette puller. For horseradish peroxidase (HRP)-filled microelectrodes, the tips were broken to approximately 0.2–0.6 μm under microscopic control. The micropipettes had *in vivo* impedances ranging from 50 to 100 MΩ and were filled with one of the following solutions: (i) 5% HRP (Sigma Type VI) in 0.5 M potassium methylsulfate and 0.05 M Tris buffer (pH 7.6); (ii) 3% biocytin in 1 M potassium acetate; or (iii) 3 M potassium acetate and 0.2 M EGTA. Intracellular recordings were performed with either a WPI M-707A or NeuroData IR-183 active bridge amplifier, monitored with a Tektronix 565 or 5113 dual beam oscilloscope, and digitized on-line by a Nicolet 2090 or 4094C digital oscilloscope.

Extracellular stimulating currents were monophasic rectangular wave pulses (0.1–2.0 mA, 20–300 μs) delivered through bipolar enamel-coated stainless steel wires (250 μm tip separation) with a Grass S-88 stimulator and a Grass SIU-5 stimulus isolation unit or a Winston A-65 timer and SC-100 constant current stimulator/isolator.

Electrophysiological characterization and intracellular labeling

The bridge balance and capacity compensation were adjusted prior to and immediately after impalement, and subsequently checked extracellularly. Non-linearities of the HRP-filled recording microelectrodes often hampered precise determination of membrane potential during current injection, but this difficulty was largely overcome by the use of biocytin-filled microelectrodes.[37]

Following impalement and stabilization of thalamic neurons, the cerebellar nuclei and motor cortex were stimulated to evoke synaptic and antidromic responses. Responses were characterized as antidromic (from cortical stimulation) based on constant latency, absence of underlying synaptic potentials, and collision with spontaneous and intracellularly-evoked spikes.[29] In many neurons, intrinsic membrane properties were investigated using a variety of paradigms involving intracellular current injection. If stable recordings persisted throughout the electrophysiological characterization and the neuron responded to cerebellar stimulation, HRP or biocytin was microiontophoretically injected into the neuron with 2–7 nA depolarizing current pulses (300 ms duration pulses with a 50% duty cycle) for 3–10 min. Only one neuron was injected per animal.

Alterations in neuronal input resistance were examined during cortically-evoked responses by injection of hyperpolarizing pulses (0.1–0.2 nA, 15–25 ms duration) over the time course of the cortically-evoked response. Changes in membrane potential due to the injection of current were used to calculate input resistance. Since membrane potential often changed substantially during the current pulse due to the time-varying aspects of the cortically-evoked response, it was necessary to compute the membrane potential deflection relative to an interpolated membrane potential that was derived from values before and after the current pulse.

The membrane time constant and neuron electrotonic length of VAL neurons were estimated from analysis of membrane potential transients in response to small intracellular hyperpolarizing current pulses using a computer program (Oscilloscope) written and kindly provided by Dr C. J. Wilson. Peeled exponentials of membrane potential transients were used to calculate cable parameters of neurons from the relation $L_N = \pi/[(\tau_0/\tau_1) - 1]^{-1/2}$, where L_N is electrotonic length of the neuron; τ_0, the slow time constant, provides an estimate of τ_m, the passive membrane constant; and τ_1, is the fast (equalizing) time constant.[76]

Tissue preparation and anatomical analysis

Details about tissue processing for light and electron microscopy are presented in the companion paper.[80] Tissue sections containing intracellularly stained neurons were examined with a Leitz OrthoLux II light microscope using ×50 and ×100 oil immersion objective lenses. The location of stimulating electrodes in motor cortex and the cerebellar nuclei was confirmed histologically in frozen sections counterstained with Neutral Red.

Statistics

Statistical analyses utilized Student's *t*-test. Data are summarized in the text as mean ± S.E.M.

RESULTS

Spontaneous activity and intrinsic membrane properties of ventroanterior–ventrolateral neurons

Database. Stable intracellular recordings were obtained from 93 neurons. Intracellular recordings from the VAL were obtained exclusively from thalamocortical neurons, based on electrophysiological properties and antidromic activation from cortical

stimulation (see below) and, for intracellularly stained neurons, light microscopic examination. Intracellular injection of biocytin, which crosses gap junctions,[31] was never observed to label more than one neuron. A photomicrograph of an intracellularly stained VAL neuron that responded to cerebellar stimulation with a monosynaptic excitatory postsynaptic potential (EPSP) is shown in Fig. 1. The axon of this neuron was traced into motor cortex. A full description of the anatomy of the intracellularly stained neurons is presented in the companion paper.[80]

Two operational firing modes. VAL neurons displayed two distinct patterns of spontaneous spike activity that are common to thalamic relay neurons: a "tonic firing mode", characterized by irregular firing of conventional Na^+-dependent[47] "fast" action potentials (1.0–1.7 ms duration; 50–70 mV amplitude) (Fig. 2), and a "rhythmic firing mode", in which oscillations in membrane potential often resulted in periodic (4–8 Hz) all-or-none "slow" action potentials (20–40 ms; 20–35 mV) upon which one or more fast action potentials often rode (Fig. 3). The slow action potentials were separated by a long-lasting (100–250 ms) hyperpolarization that gradually subsided to a ramp membrane depolarization. As described below, slow action potentials appeared to be identical to the Ca^{2+}-dependent low-threshold spikes (LTSs) described by Llinás and Jahnsen,[47] and we adopt this terminology.

The tonic mode predominated when the steady state membrane potential of the neuron was more depolarized than about −60 mV. This firing mode was characterized by a roughly linear relation between firing rate and the extent of membrane depolarization, with little evidence of spike frequency adaptation below ∼100 spikes/s (Fig. 2B, C). In contrast, rhythmic firing occurred when the neuron was hyperpolarized beyond about −65 mV, and firing rates of fast spikes were not linearly related to membrane depolarization. Many but not all VAL neurons could be converted to spontaneous rhythmic firing with intracellular injection of hyperpolarizing current.

Characterization of low-threshold spikes. Similar to neurons in other thalamic nuclei,[16,25,40,44,47,64] LTSs had regenerative properties with a longer time course and different ionic basis than those of conventional fast action potentials. For example, LTSs occurred in an all-or-none fashion with a threshold of about −65 mV, compared to a more depolarized threshold of about −50 to −55 mV for fast spikes (Fig. 3A). Once triggered, the conductance underlying the LTS inactivated, requiring sustained membrane hyperpolarization for de-inactivation (Fig. 3C). The ionic basis of LTS generation was an increase in intracellular levels of Ca^{2+} ions, presumably by means of Ca^{2+} influx via voltage-dependent channels,[13,17,33,96] since spontaneous and evoked LTSs were blocked within minutes of impalement with microelectrodes that
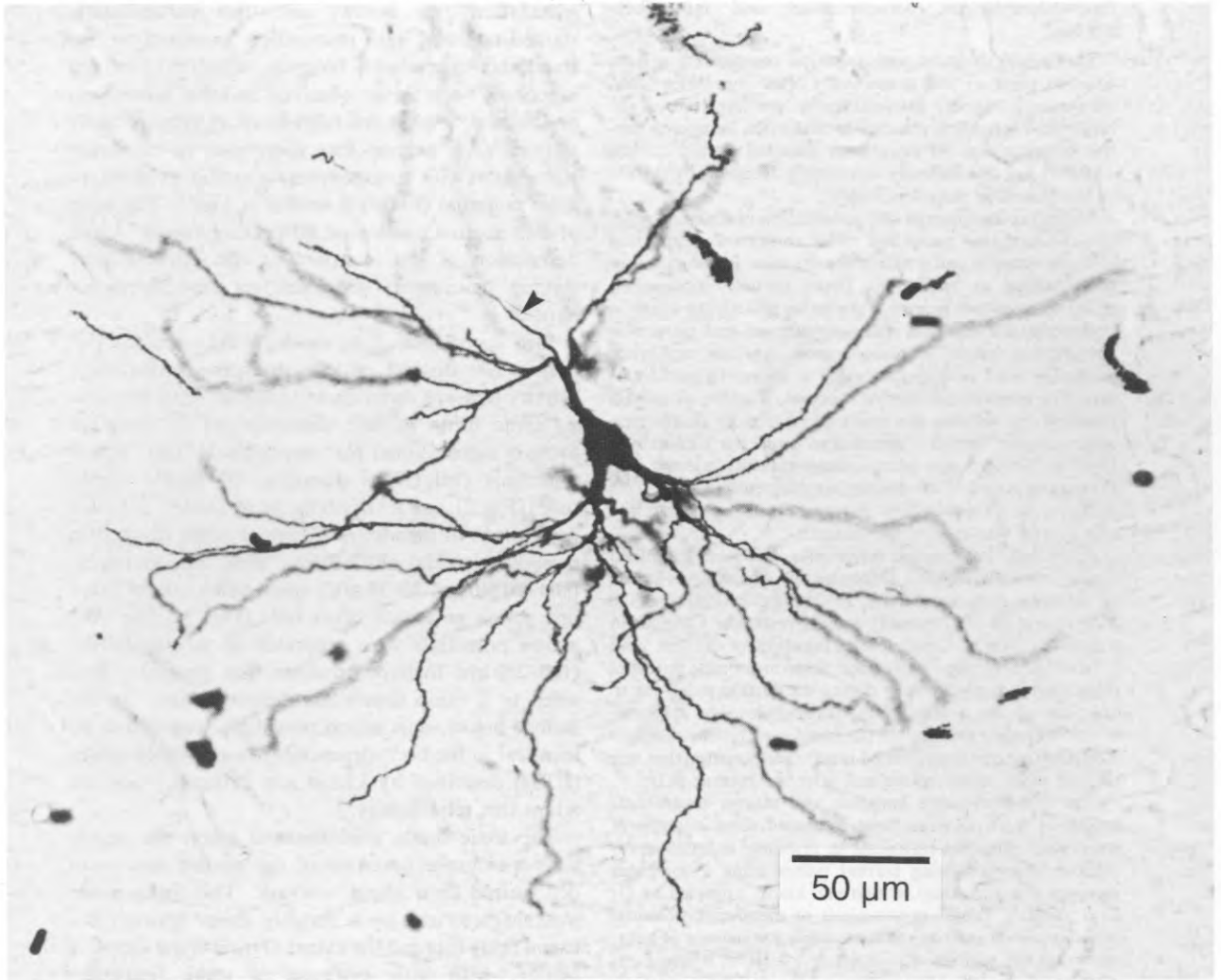
Fig. 1. Photomicrograph of an intracellularly labeled cerebellar-responsive VAL neuron. The labeled axon (arrowhead) coursed laterally and emitted collaterals in the thalamic reticular nucleus. The axon was traced into deep layers of motor cortex. This neuron's dense dendritic field, tufted branching pattern and dendrites covered with spinous appendages was typical of thalamocortical neurons in VAL. Dorsal is up, lateral is left.

contained 0.2 M of the Ca^{2+} chelator EGTA (Fig. 3B).

Cable properties. The input resistance of thalamic neurons ranged from 9 to 40 MΩ. Most VAL neurons exhibited a small degree of outward rectification during membrane hyperpolarization, as illustrated in Fig. 4B. Electrotonic properties were calculated for three VAL neurons by peeling exponentials of voltage transients to current pulse injections. The first two peeled exponentials yielded slow (τ_0) and fast (τ_1) time constants. The membrane time constant ($\tau_m \cong \tau_0$) was 22.3 ± 6.4 ms, with a fast transient of 1.37 ± 0.13 ms. The amplitudes of the slow (E_0) and fast (E_1) exponentials were 0.64 ± 0.09 and 0.79 ± 0.24, respectively. The neuronal electrotonic length (L_N) was 0.79 ± 0.07. The cable properties of a representative VAL neuron are shown in Fig. 4C.

Synaptic responses of ventroanterior–ventrolateral neurons

Cortically-evoked responses. Electrical stimulation of ipsilateral motor cortex produced a characteristic response that consisted of a short-latency action potential, an EPSP, and a voltage-dependent late hyperpolarization/ramp depolarization sequence.

The initial component of cortically-evoked responses was often a short-latency (1.22 ± 0.09 ms; $N = 53$) antidromic spike (Fig. 5A) that was extinguished by collision with spontaneous or intracellularly evoked spikes. Antidromic invasion was often observed only when neurons were depolarized with current injection, and was blocked by injection of hyperpolarizing current. On rare occasions, an "M" spike[27] was evident when the full antidromic spike was absent, suggestive of a failure of the antidromic
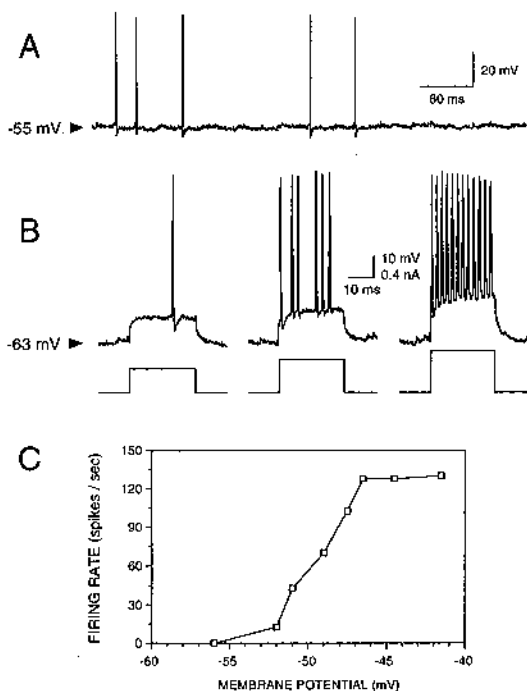
Fig. 2. Tonic firing mode of VAL thalamic neurons. (A) Irregular spike activity in the absence of any prominent oscillations in membrane potential. This firing mode predominated when VAL neurons were relatively depolarized (membrane potential more positive than about −60 mV). (B) Depolarizing current pulses evoked fast spikes with a threshold of about −54 mV in this neuron. (C) The relation between membrane potential and firing rate for a VAL neuron. Based on traces in B and other traces not shown. The firing rate of this neuron was linearly related to membrane potential with no spike frequency adaptation up to 130 spikes/s. Same neuron in A–C. Micropipette electrolyte was 1 M potassium acetate and 3% biocytin.

spike to invade the initial segment or cell body. This phenomenon likely contributed to the relatively low proportion of VAL neurons (57%) that were antidromically driven from cortical stimulation, in spite of anatomical evidence for a predominance of thalamocortical neurons in the VAL.[79-81,105]

At a slightly longer onset latency (4.41 ± 0.80 ms; $N = 11$), a slowing rising EPSP was often recorded following cortical stimulation (Fig. 5A, B). The amplitude of the EPSP was increased with injection of hyperpolarizing current (Fig. 5A), with a maximal amplitude of 5–8 mV in the absence of current injection. The EPSP was monosynaptic, as demonstrated by its invariant latency when the intensity of cortical stimulation was increased (Fig. 5B).

The cortically-evoked EPSP was often followed by a long-lasting hyperpolarization (100-400 ms duration; up to 25 mV in amplitude) that eventually gave way to a rebound depolarization (Fig. 5C, D). This cortically-evoked hyperpolarization will be referred to as a "long after hyperpolarization (AHP)". An LTS was usually generated on the tail end of the long AHP, producing a sequence that resembled spon-

taneous rhythmic activity, but which subsided after one or two cycles (Fig. 5D). The occurrence of an antidromic spike or prominent EPSP was not necessary for the production of the long AHP, and an LTS was not necessary for the generation of rhythmic sequences.
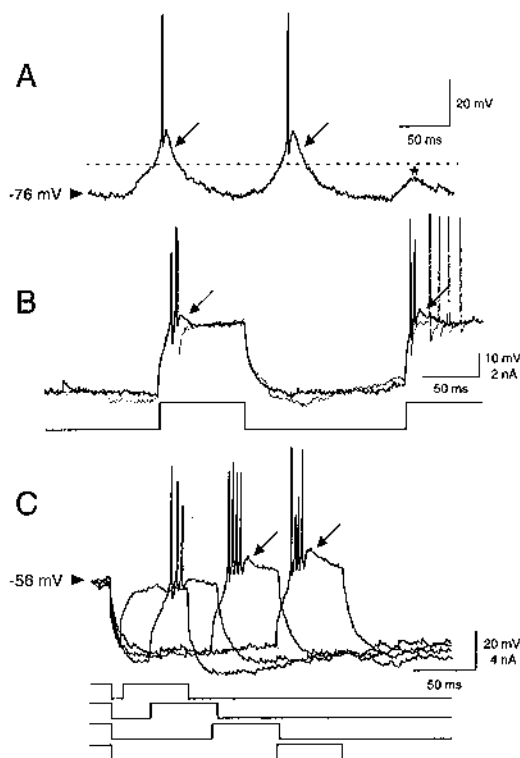

Fig. 3. Rhythmic firing mode of VAL thalamic neurons. (A) The rhythmic firing mode was observed when VAL neurons were relatively hyperpolarized (membrane potential more negative than −65 mV). In this example, spontaneous oscillations in membrane potential occurred with a period of 125 ms, with each cycle of membrane hyperpolarization followed by a ramp depolarization. All-or-none LTSs (arrows) were usually elicited during the depolarization phase of spontaneous membrane potential oscillations, and fast spikes rode on LTSs. The dashed line indicates the LTS threshold voltage (−64 mV). The depolarization did not always reach threshold for generation of an LTS (asterisk). Micropipette electrolyte was 0.5 M potassium methylsulfate and 5% HRP. (B) Blockade of LTS with a micropipette that contained 0.2 M EGTA. Within the first minute of impalement (thicker voltage trace), rebound depolarization during offsets of the conditioning hyperpolarizing current injection resulted in LTSs (arrows). Five minutes after impalement (thinner voltage trace) the same current injection parameters failed to elicit an LTS, although membrane potential (−52 mV with no constant current injection) and input resistance (15 MΩ) did not change during impalement. (C) Time course of de-inactivation of LTS. Conditioning hyperpolarizing current was injected into a VAL neuron that had a relatively depolarized membrane potential (−53 mV, positive to LTS threshold, so LTS was inactivated). Depolarizing pulses at various time intervals after the onset of the conditioning hyperpolarization revealed that about 100 ms was necessary for de-inactivation of LTS at this level of membrane hyperpolarization. Arrows point to evoked LTSs. Different neurons in A–C. Amplitudes of fast spikes in A–C were truncated by digitization of analog signals.
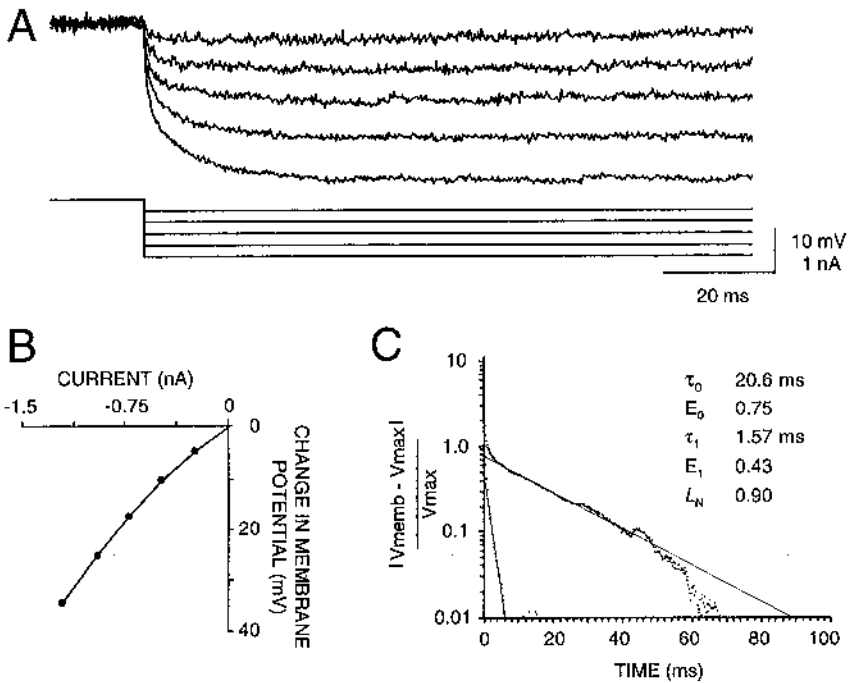
Fig. 4. Calculation of input resistance and cable properties for a representative neuron in the VAL. (A) Membrane voltage transients (top traces) in response to injection of hyperpolarizing current (bottom traces). (B) $I-V$ plot of traces shown in A. Input resistance increased from 21.3 to 28.6 MΩ with increases in membrane hyperpolarization. This small degree of outward rectification was typical of most VAL neurons. (C) Cable parameters derived from peeled exponentials of membrane potential transient for a voltage trace from A (fourth trace from top in A). This VAL neuron was electrotonically compact, with a calculated electrotonic length (L_N) of 0.90. The slow time constant, τ_0, provides an estimate of the τ_m, the passive membrane constant; τ_1 is the fast (equalizing) time constant; E_0 and E_1 are the amplitudes of the peeled exponentials τ_0 and τ_1, respectively.

The amplitude of the cortically-evoked long AHP in VAL neurons was dependent upon membrane potential in two ways. First, a certain level of hyperpolarization was usually necessary for cortical stimulation to elicit a prominent long AHP (and rebound depolarization), as shown in Fig. 5C. Second, as the neuron was further hyperpolarized with constant current injection, the long AHP, while still prominent, was often reduced in amplitude (Fig. 6A, B).

To monitor changes in neuronal input resistance in response to cortical stimulation, small hyperpolarizing current pulses (12–25 ms, 0.1–0.2 nA) were injected into some cerebellar-responsive neurons before and during cortically-induced responses. Care was taken to select neurons with relatively linear current–voltage ($I-V$) relations for this analysis. Two components of the long AHP were revealed based on changes in membrane input resistance: an early component (the initial 30 ms) during which input resistance was decreased by 25%, and a latter component in which input resistance was unchanged or slightly increased (Fig. 6C).

Cerebellar-evoked responses. Stimulation of the cerebellar nuclei produced a short-latency monosynaptic EPSP (2.12 ± 0.10 ms onset latency; $N = 54$) from which fast spikes arose. These EPSPs usually

exhibited an all-or-nothing character, and their amplitude often (but not always) increased with membrane hyperpolarization. Cerebellar-evoked EPSPs had a faster rise time (0.76 ± 0.11 ms time to peak, $N = 10$) than did cortically-evoked EPSPs (4.00 ± 0.77 ms, $N = 7$; $t = 4.96$; $P < 0.01$, d.f. = 16). Unlike the cortically-elicited response, stimulation of the cerebellar nuclei did not produce rhythmic activity or a prominent long AHP, although a period of inactivity typically lasting 100–500 ms usually followed the EPSP (Fig. 7D).

Fast prepotentials

Many thalamocortical VAL neurons exhibited all-or-none spontaneous triangular-shaped depolarizing potentials (Fig. 7C). These fast prepotentials (FPPs) had an amplitude of 3–10 mV, with a fast rise and slow decay that resembled EPSPs elicited from cerebellar stimulation (Fig. 7B). Indeed, rise times of FPPs (0.63 ± 0.04 ms time to peak, $N = 145$) and cerebellar-evoked EPSPs (0.76 ± 0.11, $N = 10$) were statistically indistinguishable ($t = 0.82$, $P > 0.05$, d.f. = 154). FPPs were observed over a wide range of membrane potentials, and in some neurons occurred at a relatively constant rate (up to 70 Hz) (Fig. 7C). Single spikes arose from the FPPs when the neuron was depolarized.
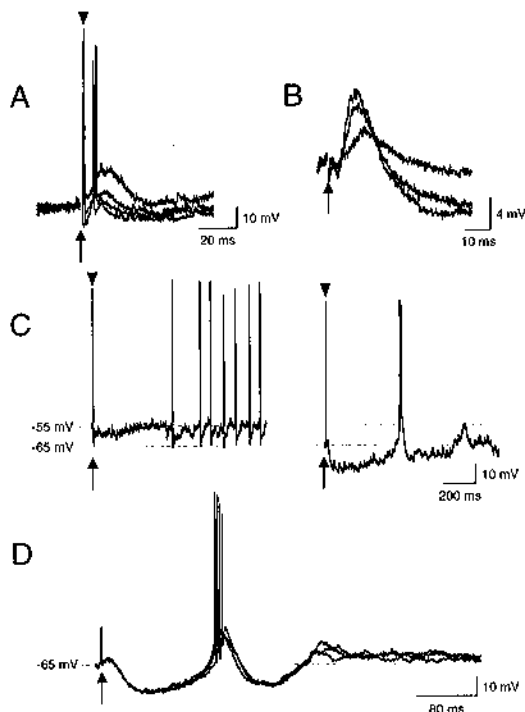
Fig. 5. Cortically-evoked responses in VAL neurons. (A) Short-latency responses to stimulation of the ipsilateral motor cortex. A short-latency antidromic spike (arrowhead) was followed by a monosynaptic EPSP. Four single sweeps at different membrane potentials are superimposed to emphasize differences in EPSP amplitudes. The amplitude of the EPSP increased with increasing levels of membrane hyperpolarization. Membrane potential varied from −58 mV (no intracellular constant current injection; smallest amplitude EPSP) to −70 mV (current injection = −1.5 nA; largest amplitude EPSP). In all panels, arrows point to cortical stimulus artifacts. (B) Power series for EPSPs elicited by cortical stimulation. Increasing the magnitude of cortical stimulation (0.4, 0.7, 1.0 mA) increased the amplitude of the EPSP without altering the onset latency of the cortically-evoked EPSP, indicative of a monosynaptic synaptic input. Each trace is an average of four sweeps. Membrane potential = −60 mV. (C) Effect of membrane potential on latter components of the cortically-evoked response. Left trace: with membrane potential at −55 mV, cortical stimulation elicited an antidromic spike (arrowhead) followed by a period of 500 ms without action potentials during which there was no prominent change in membrane potential. Rebound activity after 500 ms was confined to "fast" spikes. Right trace: at −65 mV (constant current injection of −0.75 nA), cortical stimulation elicited an antidromic spike, a long AHP and a rebound LTS. (D) Cortical stimulation produced an EPSP, long AHP, rebound LTS with a burst of fast spikes, and a second cycle of hyperpolarization. "Rhythmic activity" dissipated after two cycles. Overlay of three traces. Micropipette electrolyte was 1 M potassium acetate and 3% biocytin for all traces. Different neurons in A–D. Amplitude of fast spikes in A, C and D was truncated by digitization of analog signals.

DISCUSSION

Neuronal identification

All intracellular recordings obtained in the experiments reported here were presumed to be from thalamocortical relay neurons. This conclusion is drawn from evidence that in the VAL of rats: (i) GABAergic (local circuit) neurons are scarce,[60,68,79,81] (ii) virtually all neurons in the VAL are retrogradely labeled from injections of HRP into motor cortex,[105] (iii) the axons of all intracellularly labeled VAL neurons were traced beyond the boundaries of dorsal thalamus as they coursed in the direction of the cerebral cortex,[80] and (iv) the antidromic activation of most VAL neurons from stimulation of cerebral cortex (this report).

Intrinsic membrane properties of ventroanterior–ventrolateral neurons

Thalamic projection neurons possess membrane conductances (e.g. $I_{Na+, transient}$, $I_{K+, delayed rectifier}$, I_h, I_{T}, I_A, $I_{K+(Ca2+)}$, I_{K2}, $I_{Na+, persistent}$)[41,49,94] whose voltage-dependent interplay can support the generation of tonic firing and spontaneous delta-like (0.5–4 Hz) rhythmic activity under specific *in vitro* and *in vivo* experimental conditions.[19,52,53] Likewise, this study confirmed that rat VAL neurons have, in addition to conventional Na^+ spike conductances, the Ca^{2+} conductance (I_T)
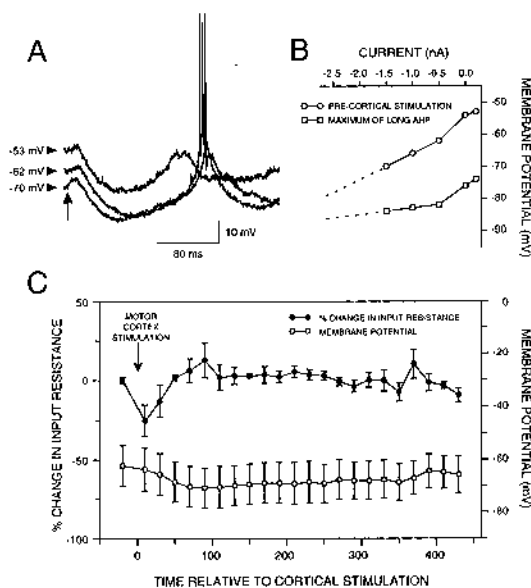

Fig. 6. Evidence that the cortically-evoked long AHP in rat VAL neurons was a combination of an IPSP and cortical disfacilitation. (A) Stimulation of motor cortex (arrow) evoked an EPSP followed by a prominent long AHP. The amplitude of the AHP decreased as the neuron was hyperpolarized with constant current injection (0.0, −1.0, −1.5 nA). (B) *I–V* plot of membrane potential before cortical stimulation and during maximum hyperpolarization of long AHP. The reversal potential of the long AHP was estimated to be about −90 mV (intersection of the two lines), i.e. near the K^+ equilibrium potential. (C) Graphical representation of the time course of membrane potential and input resistance following stimulation of motor cortex for three VAL neurons that had minimal membrane rectification. A decrease in input resistance coincided with the initial component of the long AHP, presumably generated by the GABA$_A$ IPSP. In contrast, during the latter stages of the long AHP, input resistance was slightly elevated or unchanged compared to resting (prestimulation) values.
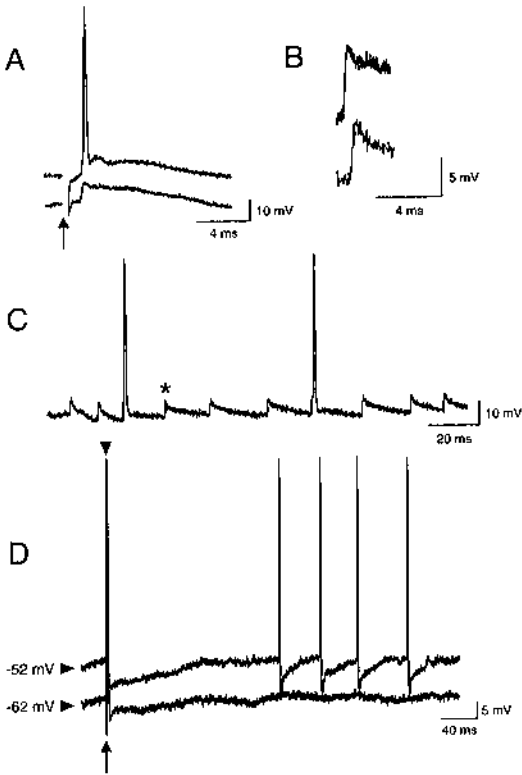
Fig. 7. Cerebellar-evoked responses in VAL neurons. (A) Stimulation of the cerebellar nuclei produced a triangular-shaped EPSP. Top trace shows an orthodromic spike rising from the initial portion of the EPSP. Lower trace illustrates the fast rise time of the EPSP and the protracted decline in depolarization to baseline. Membrane potential was −60 and −64 mV for top and bottom traces, respectively. (B) Similarity in form of a cerebellar-evoked EPSP (top) and a spontaneous FPP (bottom), both of which had fast rise times and a triangular shape. The EPSP is from the bottom trace in A; the FPP is from trace in C (asterisk). (C) Spontaneous FPPs, which in this VAL neuron occurred regularly at about 65 Hz. Fast spikes arose from two of the FPPs. (D) Absence of a prominent hyperpolarization following stimulation of contralateral cerebellar nuclei. With a membrane potential at −52 mV, cerebellar stimulation elicited an orthodromic spike that was followed by a small amplitude hyperpolarization. There was no long AHP at either membrane potential. Micropipette electrolyte was 1 M potassium acetate and 3% biocytin for all traces. Arrow points to stimulus artifact. Different neurons in A, C and D.

that underlies LTSs. Membrane responses to current injection in some neurons suggested the presence of I_A, as indicated by a delayed repolarization from offset of hyperpolarizing current,[41] and $I_{Na+, persistent}$, as indicated by a slow ramp depolarization during sustained depolarization[41] (data not shown). Hence, VAL neurons likely have intrinsic membrane properties comparable to the more fully characterized neurons in the rat dorsal lateral geniculate nucleus (dLGN), cat ventral lateral nucleus and other thalamic nuclei.[49,94]

Neurons in rat VAL exhibited voltage-dependent modes of spontaneous activity that were similar to previous descriptions of thalamic activity *in vivo*.[94]

The voltage-dependent switch from tonic to rhythmic firing *in vivo* by thalamic projection neurons is driven largely by phasic synaptic inputs from the TRN[11,49,91,94,103] in conjunction with altered activity from a variety of thalamic afferents originating in the brainstem[50,54] and possibly cerebral cortex,[55] although cortex is not necessary for thalamic spindle generation.[59,61,91] For example, neurons in the TRN have intrinsic pacemaker properties,[6,7,62,92] and protracted burst firing of these neurons has been correlated with periods of inhibition in recipient dorsal thalamic nuclei.[83,94] A current hypothesis that is in accord with the anatomy of the VAL is that periodic burst firing by TRN neurons produces a barrage of inhibitory postsynaptic potentials (IPSPs)[99,100] that hyperpolarize VAL neurons, resulting in the de-inactivation of I_T and the engagement of a cascade of membrane conductances that support membrane potential oscillations and rhythmic firing.[39-41,53,94]

Cable properties of ventroanterior–ventrolateral neurons

The time constant (τ_m) and electrotonic length (L_N) of neurons in the VAL were similar to previously reported values for relay neurons in other thalamic nuclei.[9,16] These values can only be taken as approximations due to violations of the equivalent cylinder model.[36,43,76] With this caveat in mind, the values none the less indicate that VAL neurons integrate synaptic inputs over a relatively long time frame and are electrotonically compact. Thus, changes in membrane potential arising from synaptic inputs and intrinsic membrane conductances at distal dendrites would be expected to have significant effects at the cell body.

Cortically-evoked synaptic responses

Monosynaptic excitatory postsynaptic potential. Cortically-evoked EPSPs had a slower rise time than cerebellar-evoked EPSPs, suggesting that cortical synaptic inputs were electrotonically distal to cerebellar inputs. Similar inferences have been made for neurons in the cat ventrolateral nucleus.[102] Although a temporal dispersion of conduction times for corticothalamic axons could also contribute to the slower rise time,[23] anatomical studies have shown that most putatively identified corticothalamic axons do indeed synapse onto rat VAL neuron dendrites distal to inputs from the cerebellum.[79,80] Similar synaptic arrangements of cortical inputs onto thalamocortical neurons are found in other thalamic nuclei of many mammalian species.[42]

Long after hyperpolarization. When VAL neurons were slightly hyperpolarized, cortical stimulation elicited a long AHP that persisted for 100–400 ms, followed by a rebound ramp depolarization that often generated an LTS. This response profile has been described since the 1960s,[3] although the origin of the synaptically-induced long AHP has been a source of controversy.[2] More recently, it has been determined that spontaneous and synaptically-

evoked long AHPs of neurons in many thalamic nuclei have multiple components involving activation of GABA receptors. Both $GABA_A$ and $GABA_B$ receptors are present in moderate levels in rat VAL.[8,10,87] The initial component of the long AHP is probably mediated primarily by $GABA_A$ receptors, since it entails an increase in membrane conductance (Fig. 6C), is reversed by intracellular injection of Cl^- and is blocked by $GABA_A$ receptor blockers.[15,34,69,78] As mentioned in the Introduction, there are two known sources of GABAergic inputs to thalamic neurons that could mediate IPSPs from stimulation of cortex: an intrinsic source, the GABAergic thalamic interneurons, which form dendrodendritic and axodendritic synapses with thalamic projection neurons, and an extrinsic source, the GABAergic neurons in the TRN. Considering the scarcity of interneurons in rat VAL, the TRN is more likely the mediator of any GABAergic contribution to cortically-evoked long AHPs in this nucleus. The TRN sends a GABAergic projection[21,38] that terminates on thalamocortical neurons,[18,58,65,67,71] and stimulation of the TRN elicits a $GABA_A$ IPSP[99,100] and a less pronounced $GABA_B$ IPSP[101] in rat thalamic neurons. This GABAergic circuit is driven by monosynaptic excitatory axon collaterals from corticothalamic and thalamocortical neurons.[1] With respect to IPSPs arising from cortical stimulation, the corticothalamic pathway constitutes feedforward inhibition and the thalamocortical pathway a feedback inhibition (via antidromic activation of thalamocortical neurons). The feedforward pathway is probably the more significant contributor to a cortically-evoked IPSP, since there are substantially more corticothalamic neurons than thalamocortical neurons.[82]

The latter two-thirds of the synaptically evoked long AHP has been the subject of numerous *in vitro* studies of neurons in the dLGN nucleus of rats. Unlike VAL, the rodent dLGN has a substantial population of GABAergic interneurons.[30,79] For *in vitro* preparations in which the visual sector of the TRN has been excised, a long AHP can be elicited in presumed thalamocortical neurons in the dLGN from stimulation of the optic tract. The latter component of the long AHP (latency of 20–40 ms) is blocked by $GABA_B$ receptor antagonists and is due to an increase in K^+ conductance.[15,34,86] Thus, at least in the dLGN, this component of the AHP appears to result largely from feedforward activation of GABAergic interneurons. In addition, there is evidence from *in vitro* and *in vivo* experiments that a variety of K^+ conductances intrinsic to thalamic relay neurons also contribute to the latter portion of the long AHP.[49,94]

If the cortically-evoked long AHP observed in VAL neurons in the present study resulted predominantly or exclusively from GABAergic synaptic drive (from the TRN) and activation of intrinsic conductances, a decrease in input resistance would be expected during the long AHP. For example, the *in vitro* input resistance of rat dLGN neurons decreases by 75% and 13–45% following stimulation of the optic tract during the early and late portions, respectively, of the long AHP that is mediated by interneurons.[15,34] In contrast, in this *in vivo* study, whereas the input resistance of VAL neurons during the early part of the cortically-evoked long AHP was substantially reduced, input resistance did not change or was slightly increased during the latter portions of the cortically-evoked long AHP. This suggests that an additional mechanism was involved in the generation of the cortically-evoked long AHP.

In addition to the GABAergic and intrinsic membrane responses, a cortical disfacilitation could contribute to the hyperpolarization during the latter two-thirds of the long AHP for rats under urethane anesthesia. Motor cortex provides a massive, excitatory projection to the VAL[12,105] and cortical neurons maintain spike activity under the urethane anesthesia used in the present study.[5,48,75] Electrical stimulation of motor cortex not only activates corticothalamic neurons but also elicits a synchronous engagement of cortical and thalamic inhibitory circuits that reduces spike activity for hundreds of milliseconds.[14,72,107] Reverberating activity in corticothalamic circuits following cortical stimulation may also contribute to the generation of inhibition in cortex. Hence, following the initial direct excitation of corticothalamic neurons resulting from cortical stimulation, VAL neurons would be expected to experience a period of reduced tonic depolarizing input from cortex, resulting in a hyperpolarization and a concomitant increase in membrane resistance as a consequence of the membrane rectification of these neurons. The net change in input resistance during the latter components of the long AHP would therefore be a balance of increased input resistance from disfacilitation and decreased input resistance from the presumed activation of GABAergic synapses from the TRN and intrinsic membrane conductances. Stimulation of the corticothalamic tract does not elicit disfacilitation in *in vitro* studies of thalamic neurons (e.g. Ref. 55), since the reverberatory cortical inhibitory circuits have been excised in this preparation. A significant component of hyperpolarization following cortical stimulation has been attributed to disfacilitation for thalamic[44] and striatal[106] neurons in urethane-anesthetized rats, and acute decortication produces hyperpolarization and increased input resistance in thalamic neurons of urethane-anesthetized cats.[19] Furthermore, the prominence of cortical disfacilitation in *in vivo* studies may depend on the anesthetic used, e.g. urethane vs barbiturates.[44,97]

In vitro studies of thalamocortical neurons in rat dLGN have shown that the amplitude of the $GABA_B$ component of the synaptically-evoked long AHP is maximal when the membrane potential is between −60 and −75 mV.[15,34,85,86] This response reverses at potentials negative to the K^+ reversal potential, as would be expected for an IPSP that is mediated by an increase in K^+ ions. In contrast, the amplitude of

cortical disfacilitation would be expected to increase
with membrane hyperpolarization, since its reversal
potential would be the same as cortically-evoked
EPSPs, i.e. between 0 and −20 mV. Hence, if the
latter component of the cortically-evoked long AHP
is indeed the combined action of both a $GABA_B$ IPSP
and cortical disfacilitation, then the effects of mem-
brane hyperpolarization on this response are difficult
to predict, since the amplitudes of the IPSP and
disfacilitation would be inversely affected. In our *in
vivo* experiments, injection of hyperpolarizing current
usually reduced the amplitude of the latter two-thirds
of the long AHP. It was not possible to reliably
sustain a hyperpolarization of VAL neurons beyond
−80 mV with intracellular current injection (possibly
due to tonic $GABA_A$ synaptic bombardment from the
TRN with urethane-induced anesthesia[73]), so com-
plete reversal of the long AHP was not accomplished.
However, an extrapolated reversal potential was in
the range of the K^+ equilibrium potential for most
VAL neurons, indicative of a substantive role for a
$GABA_B$ IPSP in mediating this latter component of
the long AHP. In other neurons, the long AHP
exhibited little change in amplitude as a function of
membrane hyperpolarization, a result that is consist-
ent with a summed contribution of an IPSP and
cortical disfacilitation.

Although cortical disfacilitation results from a
non-physiological synchronous activation of cortical
neurons, the presence of this phenomenon has func-
tional implications for thalamocortical interactions.
The existence of stimulation-induced cortical disfacil-
itation indicates that the cerebral cortex provides a
substantial tonic excitatory drive onto thalamic neur-
ons[19,110] that may be critical for effective transmission
of excitatory prethalamic inputs by thalamocortical
neurons,[45] or conversely may "compete" with pretha-
lamic inputs.[23] During synchronized EEG activity,
neurons in thalamic nuclei exhibit periodic oscil-
lations in membrane potential that generate rhythmic
spike activity that is conveyed to, and imposed upon,
the cerebral cortex. In turn, rhythmic activity by
cortex would be expected to elicit alternating episodes
of cortical facilitation and disfacilitation of thalamo-
cortical neurons, thereby serving to reinforce rever-
beratory rhythmic thalamocortical activity driven by
the TRN. Conversely, during spontaneous EEG
desynchronization, disfacilitation would be absent
and the unsynchronized tonic excitatory input by
cortex onto thalamus would promote the sustained
depolarized state of thalamocortical and TRN neur-
ons, which would reinforce the irregular, non-rhyth-
mic firing mode in thalamus.

Cerebellar-evoked synaptic responses

Stimulation of the contralateral cerebellar nuclei
evoked a short-latency, monosynaptic EPSP that was
often followed by a period of reduced spike activity.
EPSPs from cerebellar stimulation exhibited a rapid
rise and a slow decay time that resulted in a sustained

depolarization. Additionally, cerebellar EPSPs ap-
peared to be all-or-none, suggesting a synaptic re-
sponse mediated by one or a few axons. Similar
responses have been observed in cat motor thalamus
in response to cerebellar stimulation, with one poss-
ible difference being that the falling phase of the
slowly-decaying EPSP in cats is often curtailed by an
IPSP.[102] This distinction merits further study, as it
may represent a consequence of feedforward acti-
vation of GABAergic interneurons in the cat,[69] a
response that would be expected to be absent in VAL
of rats.

A poststimulus inhibition usually followed the
cerebellar-evoked EPSP but, in contrast to the case
for cortical stimulation, this inhibition occurred in
the absence of a prominent, protracted voltage-de-
pendent hyperpolarization and did not induce rhyth-
mic firing. A similar response profile to cerebellar
stimulation occurs in the cat ventral lateral nucleus.[93]
There are a number of explanations for the differ-
ences in response profiles to cortical vs cerebellar
stimulation. First, it is possible that cerebellar stimu-
lation, unlike cortical stimulation, does not produce
a prominent disfacilitation, in spite of the fact that
cerebellothalamic neurons are active under urethane
anesthesia.[74] The number of synapses onto VAL
neurons originating from the cerebral cortex far
exceeds those from the cerebellum,[79,80] and it is pre-
sumed that cortex supplies a greater degree of tonic
depolarization. Second, as discussed above, a com-
ponent of the cortically-evoked long AHP likely
results from monosynaptic activation of the GABA-
ergic TRN–thalamus projection. In contrast, the cer-
ebellum does not project to the TRN. Thus, whereas
TRN-induced inhibition of VAL neurons from corti-
cal stimulation would require suprathreshold acti-
vation across only one synapse (cortex→TRN), a
cerebellar-mediated inhibition of VAL neurons
would require suprathreshold activation across two
synapses (cerebellum→VAL→TRN).

Fast prepotentials

Spontaneous and synaptically evoked FPPs were
recorded from many neurons in the VAL, and were
sometimes observed to occur rhythmically at rates
approaching 70 Hz. Similar findings have recently
been reported for neurons in motor thalamus of
urethane-anesthetized rats[74] and cats,[89] and may have
an intrinsic[89] or extrinsic[74,89] origin. Electrical coup-
ling can be ruled out as a source of FPPs due to the
absence of gap junctions in dorsal thalamus[42] and the
lack of dye coupling between neurons following
intracellular injection of Lucifer Yellow[90] or, as ob-
served in the present study, biocytin. Rather, the
similarity of rise times, decay times, amplitudes and
all-or-none character of FPPs and cerebellar-evoked
EPSPs suggest that FPPs in rat VAL neurons may be
spontaneous occurrences of unitary EPSPs,[102] or den-
dritic spikes[24,32,63] driven by impulse activity in cer-
ebellar axons. A role for cerebellothalamic axons in

the generation of FPPs is consistent with the rhythmic, fast firing rates of cerebellothalamic neurons in urethane-anesthetized rats.[74]

CONCLUSION

When comparing the electrophysiological characteristics of neurons in the VAL of rats with neurons in functionally-related thalamic nuclei in other mammalian species, a striking finding is the qualitative similarity in parameters of spontaneous activity, intrinsic membrane properties and synaptic activation from subcortical and cortical afferents, in spite of the lack of interneurons in the VAL. Additional inferences about the functional role of thalamic interneurons, drawn from these electrophysiological data and from ultrastructure anatomical findings, are discussed in the accompanying paper.

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