Electroresponsive properties of rat central medial thalamic neurons

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The central medial thalamic (CMT) nucleus is a poorly known component of the intralaminar/midline thalamic complex that plays a critical role in the control of awareness. Indeed, changes in CMT activity appear to precipitate the loss of consciousness that occurs during induction of general anesthesia and at the onset of slow-wave sleep (Baker et al. 2014). Moreover, local CMT injections of minute concentrations of lidocaine elicit arousal from deep anesthesia, as determined using behavioral and electroencephalographic criteria (Alkire et al. 2007; Leung et al. 2014).

The projections of CMT to the amygdala are of particular significance for the mechanisms underlying the acquisition and expression of conditioned fear responses [reviewed in DuvArci and Pare (2014)]. Whereas several thalamic nuclei contribute projections to the basolateral complex and central nucleus of the amygdala (Turner and Herkenham 1991; Vertes and Hoover 2008; Vertes et al. 2006, 2012), most prior studies have focused on the role of the medial geniculate nucleus. Moreover, the rostral CMT stands apart among thalamic nuclei projecting to the amygdala, because its axons end selectively in the basolateral nucleus of the amygdala (BL) (Vertes et al. 2012), a critical node in the intra-amygdala circuits of conditioned fear (Amano et al. 2011; Anglanda-Figueroa and Quirk 2005; Herry et al. 2008). Thus CMT is well positioned to regulate the acquisition and expression of conditioned fear responses.

As a first step in analyzing possible contributions of CMT to emotional expression, the present study aimed to characterize the electroresponsive properties of CMT neurons. Indeed, the transformations performed by a group of neurons on the inputs they receive are critically dependent on their physiological properties. Although early studies emphasized the uniform physiological properties of relay neurons in different dorsal thalamic nuclei (Jahnsen and Linas 1984a, b), subsequent work revealed considerable variations across nuclei, as well as between different subsets of relay cells in the same nucleus (Beatty et al. 2009; Hu et al. 1994; Li et al. 2003; Steriade et al. 1993). Consistent with this, we found that CMT contains two different cell types, which then led us to examine whether they contribute differential projections to the BL.

MATERIALS AND METHODS

Experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and with the approval of the Institutional Animal Care and Use Committee of Rutgers University-Newark (Newark, NJ). We used adult (60–120 days), male Lewis rats (Charles River Laboratories, New Field, NJ), maintained on a 12-h light/dark cycle. The animals were housed three per cage with ad libitum access to food and water. Before the experiments, rats were habituated to the animal facility and handling for 1 wk.

Whole-Cell Patch Recording of CMT Cells In Vitro

Slice preparation. Rats (n = 43) were anesthetized using avertin (300 mg/kg ip), followed by isoflurane. After abolition of all reflexes, they were perfused through the heart with a cold (4°C), modified...
artificial cerebrospinal fluid (aCSF) solution containing the following (in mM): 124 NaCl, 2.5 KCl, 1.25 NaH2PO4, 26 NaHCO3, 1 MgCl2, 2 CaCl2, and 10 glucose (pH 7.2, 300 mOsm). Their brains were then extracted and cut in 250-μm-thick coronal slices with a vibrating microtome, while submerged in the same solution as above. After cutting, slices were transferred to an incubating chamber, where they were allowed to recover for at least 1 h at room temperature in aCSF. The temperature of the chamber was maintained at 34°C for 20 min and then returned to room temperature. Later, slices were transferred one at a time to a recording chamber perfused with oxygenated aCSF at 32°C (6 ml/min).

Electrophysiology. We obtained whole-cell patch-clamp recordings of CMT neurons under visual guidance with differential interference contrast and infrared videomicroscopy. We used micropipettes (5–8 MΩ) pulled from borosilicate glass capillaries and filled with a solution composed of the following (in mM): 130 K-gluconate, 10 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid, 10 KCI, 2 MgCl2, 2 ATP-Mg, and 0.2 GTP-tris(hydroxy-methyl)aminomethane (pH 7.2, 280 mOsm). The liquid junction potential was 10 mV with this solution. Membrane potential (V_m) values reported in RESULTS were not corrected for the junction potential. Current-clamp recordings were obtained via a MultiClamp 700B amplifier (low-pass filter set at 4 kHz) and digitized at 10 kHz using an Axon Digidata 1550 interface (Molecular Devices, Sunnyvale, CA), controlled by pClamp 10.3 (Molecular Devices).

To characterize the electroresponsive properties of recorded cells, we applied a graded series of hyperpolarizing and depolarizing current pulses (10 pA increments, 500 ms in duration) from rest and two additional prepulse potentials (i.e., −70 and −55 mV).

Retrograde Tracing

In some experiments, to target our whole-cell recordings selectively to CMT neurons projecting to BL, a subset of 16 rats received bilateral infusions (0.3 μl/side) of the retrograde tracer cholera toxin subunit B (CTB; Alexa Fluor 594; Life Technologies, Thermo Fisher Scientific, Grand Island, NY). The tracer was pressure injected using a micro-syringe aimed to the following stereotaxic coordinates (relative interface [Molecular Devices, Sunnyvale, CA], controlled by pClamp 10.3 (Molecular Devices).

To characterize the electroresponsive properties of recorded cells, we applied a graded series of hyperpolarizing and depolarizing current pulses (10 pA increments, 500 ms in duration) from rest and two additional prepulse potentials (i.e., −70 and −55 mV).

Morphological Identification

To study the morphology of recorded neurons, in a subset of experiments, 0.5% biocytin was added to the pipette solution. Biocytin diffused into the cells as their electroresponsive properties were recorded. After termination of the recordings and adequate time for biocytin to diffuse throughout the neuron’s dendrites, the slices were removed from the chamber and fixed for >12 h in 4% paraformaldehyde in 0.1 M PB saline (pH 7.4). Following four, 10-min washes in PB at 4°C, slices were incubated for 12 h at 20°C in solution containing 0.5% Triton and 1% A and B solutions of an ABC kit (Vector Laboratories, Burlingame, CA) in PB. The following day, the slices were washed in PB (4 × 10 min); biocytin was visualized by incubating the sections in 0.1 M PB solution consisting of diaminobenzidine tetrahydrochloride (0.05%; Sigma, St. Louis, MO), 2.5 mM nickel ammonium sulfate (Thermo Fisher Scientific), and NaPH4 (1%; Sigma) for 5–10 min. The sections were then washed in PB (5 × 10 min), mounted on gelatin-coated slides, and air dried. Then, sections

![Fig. 1. Two types of CMT neurons. Voltage responses of 2 central medial thalamic (CMT) neurons [A: single low-threshold bursting (SLB) cell; B: tonically firing (TF) neuron] to a graded series of hyperpolarizing or depolarizing current pulses applied from −55 mV (A1 and B1) or −70 mV (A2 and B2). A1 and B1: insets show the initial bursting response elicited by depolarization from −70 mV with an expanded time base. Voltage and time calibrations provided in between A1 and B1 apply to all panels except for the insets, where a different time calibration was used, as indicated.](image-url)
SLB, single low-threshold bursting; TF, tonically firing; AP, action potential; Amp, amplitude; LTS, low-threshold spike; Max., maximum; Freq., frequency; AHP, after-hyperpolarization.

### Analyses and Statistics

Input resistance was calculated from the linear portion of current-voltage plots. The amplitude of after-hyperpolarizations (AHPs) was measured in two conditions: 1) at the offset of a large (90 pA), depolarizing current pulse, applied from a prepulse potential of −70 mV, and 2) after individual spikes elicited by just-above-threshold depolarizations from a prepulse potential of −55 mV. In the latter case, we computed the difference between the AHP peak and the spike threshold, for which its identification was rendered unambiguous by differentiating the $V_m$ values.

All data are reported as averages ± SE. All statistical tests were two sided. In all cases, all available cells, trials, and subjects were included in the statistical analyses, as appropriate. No subjects or cells were excluded. To access the significance of differences in physiological properties between cell types, we used Student’s t-tests for independent samples with a significance threshold of $P = 0.05$. Because some of the parameters compared did not meet the assumptions of this test, we also used a Mann-Whitney U-test and obtained the same results in all cases. Thus for simplicity, we only report the results of the t-tests.

### RESULTS

#### Database

We studied the electroresponsive properties of CMT neurons using visually guided whole-cell, patch-clamp recordings in coronal slices maintained in vitro. We obtained stable record-
ings from 85 CMT cells with resting potentials negative to −50 mV and overshooting action potentials. Of these, 11 were morphologically identified with biocytin, and 12 were positively identified as projection neurons using retrograde transport of CTB from the BL. For comparison, we also obtained recordings from 12 relay neurons in the nearby ventroposteromedial (VPM) thalamic nucleus, four of which were morphologically identified. Because prior immunohistochemical studies have revealed that the rat dorsal thalamus contains very few GABAergic local-circuit cells [reviewed in Jones (2007)], all recorded cells are presumed to be relay cells. Below, unless otherwise stated, all values are reported as averages ± SE, and all statistical tests are Bonferroni-corrected unpaired t-tests.

Two CMT Cell Types

Two CMT cell types were distinguished based on the voltage dependence of their firing patterns (Fig. 1). They were routinely observed in slices from the same subjects. Although both types displayed tonic firing when depolarized from a depolarized potential (−55 mV; Fig. 1, A1 and B1), they exhibited markedly different firing patterns when depolarized from a hyperpolarized potential (−70 mV and beyond; Fig. 1, A2 and B2). One-half of the cells (29/60 cells, 48%), hereafter termed single low-threshold bursting (SLB) cells, displayed a single low-threshold spike (LTS), crowned by a burst of 4.1 ± 0.3 action potentials in response to suprathreshold depolarizing current steps (Fig. 1A2). However, after the initial high-frequency spike burst, SLB cells did not fire, even when challenged with high-amplitude depolarizing current pulses (up to 200 pA; Fig. 1A2).

By contrast, in the second type of CMT cells (31/60, 52%), positive current pulses from prepulse potentials negative to rest elicited an initial high-frequency spike burst (3.6 ± 0.3 action potentials), followed by tonic firing (Fig. 1B2). In these cells [hereafter termed tonically firing (TF) neurons], the tonic firing that followed the initial LTS burst augmented in frequency with positive current pulses of increasing amplitude.

The two types of CMT cells differed in other ways. First, TF neurons had a markedly higher input resistance than SLB cells (TF cells, 653.2 ± 40.2 MΩ, n = 31; SLB, 366.1 ± 36.2 MΩ, n = 29; t = −5.307, P < 0.001). Moreover, TF neurons also had a significantly lower rheobase than SLB cells (TF cells, 26.45 ± 2.84 pA, n = 31; SLB, 61.4 ± 6.58 pA, n = 29; t = 4.88, P < 0.001). Yet, as detailed in Table 1, the two cell types had a similar resting potential and displayed comparable action-potential thresholds, amplitudes, and durations. Moreover, all rebound LTS characteristics were similar for both cell types. For instance, LTS amplitudes and latencies, elicited by rheobase depolarization from a hyperpolarized state, did not differ between cell types. Similarly, maximal intraburst firing frequency, as measured by the shortest interspike interval in the burst, was also consistent between cell types.

AHP amplitudes were measured in two conditions: 1) at the offset of a large (90 pA) depolarizing current pulse applied from a prepulse potential of −70 mV and 2) after individual spikes elicited by just-above threshold depolarizations from a prepulse potential of −55 mV (see details in MATERIALS AND METHODS; Table 1). For the condition that concerns us most, from −70 mV when the firing pattern of the two cell types is so different, AHP amplitudes were higher for SLB than TF cells, although not significantly different (P = 0.1). This is remarkable, given that the input resistance of SLB cells is much lower than that of TF cells and that TF cells fired tonically for the duration of the current pulses, whereas SLB cells only fire a LTS burst.

**Fig. 3.** Comparison between ventroposteromedial (VPM) and CMT neurons. A: response of VPM (left) and CMT (right) neurons to hyperpolarizing current pulses (VPM, 100 pA; CM, 50 and 100 pA) applied from −55 mV. Inset: voltage responses were scaled so that the amplitude of the early nadir matched (VPM, thin line, 100 pA; CM, thick line, 50 pA). B and C: voltage response of 2 different VPM cells (left and right) to current pulses (inset in C1) applied from −55 mV (B1 and C1) or −70 mV (B2 and C2). Except for the differing steady currents (required to set the prepulse potential at −55 and 70 mV), the same current steps were applied in B1 and C1 and B2 and C2.
Finally, only one spontaneously firing cell was observed in each group (TF: 1/31 cells; SLB: 1/29 cells). This finding is in contrast to the high proportions of spontaneously active cells found in the paraventricular thalamic nucleus, another midline thalamic nucleus (Zhang et al. 2009). The two CMT cell types were observed in nearly equal proportions in the rostral and caudal sectors of the CMT nucleus (rostral, 22 SLB and 24 TF neurons; caudal, 7 SLB and 7 TF neurons; Fisher exact test, \( P = 0.76 \)).

**Physiological Properties of CMT Neurons Projecting to the BL**

To determine whether TF and SLB cells both project to the basolateral amygdala, 1–2 wk before the electrophysiological experiments, 16 rats received bilateral infusions of the retrograde tracer CTB (conjugated to Alexa Fluor 594) in the BL. Figure 2A shows a representative example of the CTB infusion site in the basolateral amygdala. CTB injections in the basolateral amygdala resulted in prominent retrograde labeling in CMT (Fig. 2B). Under visual control, we targeted our recording pipettes to CTB-labeled (Fig. 2C; \( n = 12 \)) or unlabeled (\( n = 13 \)) CMT neurons. However, the incidence of TF and SLB cells was similar in both subsets of cells (labeled, 6 TF and 6 SLB; unlabeled, 6 TF and 7 SLB), suggesting that both cell types contribute projections to the basolateral amygdala.

**Comparison between the Physiological Properties of CMT and VPM Neurons**

Surprisingly, TF and SLB cells showed very little evidence of the time- and voltage-dependent inward rectification (Fig. 1) that is characteristic of relay cells in the dorsal thalamus [for instance, see McCormick and Pape (1990); Timofeev and Steriade (1996)]. Typically, this property manifests itself as a marked depolarizing sag in the voltage response of relay cells to prolonged hyperpolarizing current pulses and reflects the activation of a hyperpolarization-activated mixed cationic conductance, termed \( I_H \) [reviewed in Biel et al. (2009); Pape (1996)]. To examine the possibility that this peculiarity of CMT cells resulted from our recording techniques, we compared them with neurons of a prototypical dorsal thalamic relay nucleus, VPM (\( n = 12 \)). It should be noted that VPM is just lateral to CMT, allowing us to record the two types of cells in the same slices and rats.

As shown in Fig. 3, A and B (VPM and CMT, respectively), these experiments revealed that the near absence of depolarizing sag in CMT cells was not due to our recording methods, as all VPM neurons displayed this phenomenon. To quantify this in two cell types, we applied \(-100 \) pA pulses, lasting 500 ms from a prepulse potential of \(-55 \) mV, and measured the difference between the voltage responses at its maximum and just before the pulse offset. A sag ratio was then computed by expressing the latter in percent of the maximal response. Note that this approach likely underestimated the differences in sag ratio between the two cell types, as VPM had, on average, a threefold lower input resistance than CMT cells (VPM: \(161.0 \pm 27.1 \, \Omega\), \( n = 12 \); CMT: \(499.9 \pm 32.0 \, \Omega\), \( n = 60 \); \( t = 8.17, P < 0.001 \)). Nevertheless, sag ratios differed significantly between CMT and VPM neurons (CMT, \(0.98 \pm 0.01 \), \( n = 60 \); VPM, \(0.90 \pm 0.01 \), \( P < 0.001 \)). Stated otherwise, the time-dependent attenuation of the voltage response to hyperpolarizing current pulses (the sag) was five times lower in CMT than VPM neurons.

CMT and VPM types differed in many other ways. As detailed in Table 2, CMT neurons had a significantly more negative resting \( V_m \) than VPM neurons. Moreover, CMT neurons diverged substantially from VPM cells in most firing parameters. Indeed, CMT neurons exhibited a significantly more depolarized action-potential threshold, as well as lower action-potential amplitudes and durations. Furthermore, the maximal intraburst spike frequency was significantly lower for CMT than VPM cells. Note that all of these differences remained significant when we compared VPM cells with the two types of CMT neurons separately.

**Morphology of CMT Neurons**

Last, we studied the morphological properties of CMT neurons. To this end, 0.5% biocytin was added to the pipette solution. No special protocol was required to label the cells, because biocytin diffused into the cells as we studied their electroresponsive properties. A total of 11 CMT neurons was recovered (6 SLB and 5 TF). For comparison, we also labeled four VPM cells.

In paralleling the physiological differences between CMT and VPM neurons, their morphology was also strikingly different (Fig. 4). All of the VPM cells that we recovered had a bushy appearance (Fig. 4D), conforming to classical descriptions of the dominant type of relay neurons observed in most dorsal thalamic nuclei [reviewed in Jones (2007); also see Turner et al. (1997); Von Kölliker (1896)]. In contrast, the morphology of CMT cells was extremely diverse, even among SLB (Fig. 4B) or TF (Fig. 4C) neurons. Soma shapes were variable (angular, triangular, oval, or round). Dendritic trees could be bipolar or multipolar. Dendritic branching patterns ranged from cells with a few poorly ramifying dendrites to neurons with multiple primary dendrites that branched profusely.

Table 3 summarizes the morphological properties of SLB and TF neurons. No significant difference was found between the two cell types with respect to soma size, number, or diameter of primary dendrites; distance from soma to the first branching point; average dendritic length; and dendritic

**Table 2. CMT vs. VPM**

<table>
<thead>
<tr>
<th>Input Resistance, ( \Omega )</th>
<th>Rheobase, ( \mu A )</th>
<th>Resting Membrane Potential, mV</th>
<th>AP Threshold, mV</th>
<th>AP Amp., mV</th>
<th>AP Duration, ms</th>
<th>Max. Intraburst firing Freq., Hz</th>
<th>Sag Ratio, mV</th>
<th>AHP Amp. (-55) mV</th>
<th>AHP Amp. (-70) mV</th>
<th>AHP Amp. (-70) mV</th>
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<tbody>
<tr>
<td><strong>CMT</strong></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
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<tr>
<td>499.9 ± 32.0</td>
<td>43.1 ± 4.3</td>
<td>-68.6 ± 0.97</td>
<td>-35.9 ± 0.6</td>
<td>55.0 ± 1.2</td>
<td>0.43 ± 0.02</td>
<td>282.6 ± 9.9</td>
<td>0.98 ± 0.002</td>
<td>210.0 ± 0.7</td>
<td>3.9 ± 0.5</td>
<td></td>
</tr>
<tr>
<td><strong>VPM</strong></td>
<td>161.0 ± 27.1</td>
<td>81.7 ± 14.6</td>
<td>-60.4 ± 1.35</td>
<td>-42.4 ± 0.6</td>
<td>73.5 ± 2.2</td>
<td>475.7 ± 29.8</td>
<td>0.90 ± 0.014</td>
<td>142.0 ± 0.4</td>
<td>2.3 ± 1.3</td>
<td></td>
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<tr>
<td>( t )</td>
<td>8.17</td>
<td>1.78</td>
<td>5.0</td>
<td>0.88</td>
<td>6.65</td>
<td>4.48</td>
<td>6.2</td>
<td>5.2</td>
<td>4.98</td>
<td>2.67</td>
</tr>
<tr>
<td>( P )</td>
<td>&lt;0.001</td>
<td>0.01</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.01</td>
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</table>

CMT, central medial thalamic; VPM, ventroposteromedial.
Fig. 4. Morphological properties of CMT neurons. A: scheme showing position of CMT cells labeled with biocytin and depicted in B and C. DG, dentate gyrus; ATN, anterior thalamic nuclei; H, habenula; V, ventricle; LD, laterodorsal thalamic nucleus; VA, ventral anterior thalamic nucleus. B: SLB neurons. B1: photomicrograph showing morphology of a CMT neuron of the SLB subtype. Inset: dendritic segment with varicosities. The depicted region is enclosed in a rectangle in the lower-power photograph. B2–B6: camera lucida drawings of other SLB neurons. The position of the various cells in CMT is indicated in A. C: TF cells. C1: photomicrograph showing morphology of a CMT neuron of the TF subtype. Inset: dendritic segment with varicosities. The depicted region is enclosed in a rectangle in the lower-power photograph. C2–C5: camera lucida drawings of other TF neurons. The position of the various cells in CMT is indicated in A. D: VPM neuron.
branching, as assessed with a linear Sholl analysis (Ristanović et al. 2006). However, one interesting property observed in nearly all CMT cells, but none of the VPM neurons, was the presence of numerous dendritic varicosities (intervaricose segments ranged between 2 and 30 μm) that made their distal dendrites look like axons. Representative examples of such varicose dendritic segments are depicted in Fig. 4, B1 and C1.

### DISCUSSION

The present study was undertaken to characterize the electroresponsive properties of CMT neurons that project to the BL. The interest of this question stems from prior tracing studies indicating that CMT sends a strong and specific projection to the basolateral amygdala (Vertes et al. 2012) and recent findings indicating that midline thalamic nuclei regulate the storage and expression of classically conditioned fear memories via their projections to the amygdala (Do-Monte et al. 2015; Padilla-Coreano et al. 2012). We found that CMT contains two physiological cell types, both of which contribute projections to the basolateral amygdala. Although there was morphological heterogeneity among CMT neurons, they did not correlate with differences in their electroresponsive properties. Below, we consider the significance of these findings in light of prior work on the physiology of the thalamus and amygdala.

### CMT Contains Two Distinct Physiological Cell Types

Two prior studies used patch recordings to study CMT neurons (Kanyshkova et al. 2011; Lioudyno et al. 2013). However, they focused on the influence of specific voltage-dependent channel subtypes—Kv4 and Kv1 channels, respectively—not on the temporal dynamics of current-evoked spiking. Thus the present study completes and extends these earlier investigations by showing that CMT contains two types of neurons endowed with different electroresponsive properties.

Like most relay neurons of the dorsal thalamus, CMT cells exhibited two firing modes, depending on their Vm (Jahnsen and Linas 1984a, b). Whereas depolarizing current pulses elicited tonic firing from depolarized Vm, they evoked high frequency spike bursts from Vm negative to ~55 mV. The difference between the two CMT cell types emerged when we examined their activity after this initial spike burst: one group of cells (SLB neurons) remained silent thereafter, even when challenged with high-amplitude depolarizing current pulses, whereas others (TF neurons) then fired tonically, their discharge rates increasing with the amount of depolarizing current injected. This difference between SLB and TF cells was observed, despite that fact that the two cell types had similar action-potential thresholds, amplitudes, and durations, as well as resting potential values. Given that both cell types project to the basolateral amygdala and that one of them (SLB) only fires at stimulus onset and the other (TF) for the duration of the stimulus, these results suggest that CMT neurons relay both phasic and sustained nociceptive information to the basolateral amygdala.

At present, it is unclear why SLB cells are so “reluctant” to fire after the initial LTS burst. Although SLB cells had a much lower input resistance (~370 MΩ) than TF cells (~660 MΩ), higher positive current injections that depolarized SLB cells beyond spike threshold still could not make them fire after the LTS burst. The fact that SLB cells could fire tonically at high rates when depolarized from positive but not negative potentials suggests that the differential expression of a Ca2+-dependent potassium conductance, possibly at the level of the initial axonal segment, is responsible for the difference between the two cell types. Under this model, the Ca2+ influx caused by the initial LTS burst would cause a strong and long-lasting hyperpolarization at the spike initiation zone, which could not be compensated for by our somatic current injections. Consistent with this explanation, AHP amplitudes following LTS bursts were higher in SLB than TF neurons, although not significantly (P = 0.1). However, given that the input resistance of SLB cells was much lower than that of TF cells and that TF cells fired tonically for the duration of the current pulses, whereas SLB cells only fire a LTS burst, this trend is remarkable and consistent with our hypothesis.

### CMT Neurons Do Not Exhibit the Typical Morphology of Relay Neurons in Other Thalamic Nuclei

The two types of CMT neurons each accounted for nearly one-half of the recordings and had a similar prevalence at rostral and caudal levels of the CMT nucleus. Moreover, their incidence was similar among neurons retrogradely labeled following CTB injections in the BL. The morphology of CMT neurons was heterogeneous, with as much variability within as between the SLB and TF subtypes. Surprisingly, none of the CMT cells had morphological properties that conformed to the classical bushy profile that is prevalent in most dorsal thalamic nuclei [Turner et al. (1997); Von Kölliker (1896); reviewed in Jones (2007); however, see Deschênes et al. (1996)]. Another distinctive feature of CMT cells was the presence of numerous dendritic varicosities, which made distal dendrites resemble axons forming en passant synapses. However, this feature was seen in both TF and SLB neurons.

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**Table 3. Morphology characteristics of CMT cell types**

<table>
<thead>
<tr>
<th></th>
<th>SLB</th>
<th>TF</th>
<th>t</th>
<th>P</th>
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<tbody>
<tr>
<td>Max. Soma Diameter, μm</td>
<td>24.8 ± 0.94</td>
<td>32.0 ± 2.85</td>
<td>1.19</td>
<td>0.26</td>
</tr>
<tr>
<td>Min. Soma Diameter, μm</td>
<td>16.58 ± 0.83</td>
<td>19.3 ± 3.67</td>
<td>0.06</td>
<td>0.96</td>
</tr>
<tr>
<td>Number</td>
<td>5.2 ± 1.1</td>
<td>4.8 ± 1.1</td>
<td>1.40</td>
<td>0.19</td>
</tr>
<tr>
<td>Diameter at 40 μm from Soma</td>
<td>2.39 ± 0.15</td>
<td>2.11 ± 0.17</td>
<td>0.23</td>
<td>0.83</td>
</tr>
<tr>
<td>Distance to First Branching from Soma, μm</td>
<td>24.1 ± 4.85</td>
<td>24.9 ± 6.44</td>
<td>0.87</td>
<td>0.39</td>
</tr>
<tr>
<td>Average Dendritic Length, μm</td>
<td>292.6 ± 21.5</td>
<td>252.3 ± 25.6</td>
<td>1.22</td>
<td>0.26</td>
</tr>
<tr>
<td>Schoenen Ramification Index</td>
<td>5.3 ± 1.7</td>
<td>4.1 ± 2.1</td>
<td>0.15</td>
<td>0.85</td>
</tr>
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Min., minimum.
Relative to Other Thalamic Relay Cells, CMT Neurons Exhibit Unusual Physiological Properties

Whereas most dorsal thalamic relay neurons show a pronounced, I_{H}-mediated [reviewed in Biel et al. (2009); Pape (1996)] time- and voltage-dependent inward rectification in the hyperpolarizing direction (McCormick and Pape 1990; Timofeev and Steriade 1996), this phenomenon was nearly absent in both types of CMT neurons. Indeed, the depolarizing sag in the voltage response of CMT cells to hyperpolarizing current pulses was approximately five times lower than in control recordings from VPM cells. Whereas this property of CMT cells might reflect a lower overall hyperpolarization-activated cyclic nucleotide-gated (HCN) channel density than seen in relay cells from other thalamic nuclei, it might also depend on the expression of different HCN subunits, as these differ in their voltage and time-dependent activation. I_{H}-mediated inward rectification was shown to play a major role in the genesis of intrinsic delta frequency oscillations (McCormick and Huguenard 1992; McCormick and Pape 1990; Soltész et al. 1991). Specifically, the Ca^{2+} influx associated with LTS bursts activates Ca^{2+}-dependent potassium conductances, causing a membrane hyperpolarization that activates I_{H}. In turn, the depolarization produced by I_{H} triggers another LTS burst, initiating the next oscillatory cycle. The blocking of I_{H} abolishes the intrinsic delta oscillations (McCormick and Huguenard 1992). Whereas relay cells can generate such oscillations in the absence of synaptic transmission, in an intact network, reciprocal corticothalamic connections, as well as the entrainment of reticular thalamic cells, contribute to synchronize the individual oscillations into a coherent population phenomenon (Dossi et al. 1992). As a result, punctual synaptic inputs from the periphery are unlikely to disrupt the oscillation.

By contrast, because of the relatively small influence of I_{H} in CMT neurons, delta oscillations might be absent, less coherent, and/or more susceptible to interference from signals, such as nociceptive inputs impinging onto CMT neurons and its targets. As a result, CMT neurons would be in a unique position to elicit awakening from sleep or anesthesia, as reported previously (Alkire et al. 2007; Baker et al. 2014; Leung et al. 2014). A challenge for future studies will be to contrast the oscillatory activity of neurons in CMT vs. other dorsal thalamic nuclei in naturally sleeping subjects to determine if it is consistent with the aforementioned speculations.

GRANTS

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DISCLOSURES

The authors declare that they have no conflict of interest, financial or otherwise.

AUTHOR CONTRIBUTIONS


REFERENCES


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