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 middle thalamic complex that relays nociceptive inputs to the basolateral amygdala and cingulate cortex, and plays a critical role in the control of awareness. The present study was undertaken to characterize the electroresponsive properties of CMT neurons. Similar to relay neurons found throughout the dorsal thalamus, CMT cells assumed tonic or burst firing modes depending on their membrane potentials. However, they showed little evidence of the $I_{H}$-mediated inward rectification usually displayed by dorsal thalamic relay cells at hyperpolarized membrane potentials. Two subtypes of CMT neurons were identified when comparing their responses to depolarization applied from negative potentials. Some cells generated a low-threshold spike burst followed by tonic firing, whereas others remained silent after the initial burst, irrespective of the amount of depolarizing current injected. Equal proportions of the two cell types were found among neurons retrogradely labeled from the basolateral amygdala. Their morphological properties were heterogeneous but distinct from the classical bushy relay cell type that prevails in most of the dorsal thalamus. We propose that the marginal influence of $I_{H}$ in CMT relative to other dorsal thalamic nuclei has significant network-level consequences. Because $I_{H}$ promotes the genesis of highly coherent delta oscillations in thalamocortical networks during sleep, these oscillations may be weaker or less coherent in CMT. Consequently, delta oscillations would be more easily disrupted by peripheral inputs, providing a potential mechanism for the reported role of CMT in eliciting arousal from sleep or anesthesia.

Keywords: Thalamus, amygdala, pain, defensive behaviors, bursting
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 nicotine elicit arousal from deep anesthesia, as determined using behavioral and electroencephalographic criteria (Alkire et al. 2007; Leung et al. 2014).

Probably related to the above, the CMT nucleus is also involved in nociception. Whereas the caudal CMT participates in the discriminatory/sensory aspect of pain, the rostral CMT was implicated in the affective and motivational aspects of pain (reviewed in Van der Werf et al. 2002). Consistent with this, peripheral nociceptive (but not light touch) stimulation increases the number of CMT neurons expressing c-fos (Kuroda et al. 1995). Conversely, lidocaine injections into the CMT reduce formalin induced pain behavior (McKenna and Melzak 1994). The contributions of the CMT nucleus to the affective aspects of pain are thought to depend on its strong projections to the anterior cingulate cortex (area 24) and the amygdala (Vertes et al. 2012; Chai et al. 2010).

CMT’s projections to the amygdala are of particular significance for the mechanisms underlying the acquisition and expression of conditioned fear responses (reviewed in Duvarcı and Pare 2014). While several thalamic nuclei contribute projections to the basolateral complex and central nucleus of the amygdala (Turner and Herkenham 1991; Vertes et al. 2006, 2012; Vertes and Hoover 2008), 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 (Vertes et
al. 2012), a critical node in the intra-amygdala circuits of conditioned fear (Anglanda-Figueroa and Quirk 2005; Herry et al. 2008; Amano et al. 2011). 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 Llinas 1984a,b), subsequent work revealed considerable variations across nuclei as well as between different subsets of relay cells in the same nucleus (Steriade et al. 1993; Hu et al. 1994; Li et al. 2003; Beatty et al. 2009). 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 basolateral nucleus of the amygdala.
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, 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. Prior to the experiments, rats were habituated to the animal facility and handling for one week.

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 NaH$_2$PO$_4$, 26 NaHCO$_3$, 1 MgCl$_2$, 2 CaCl$_2$, 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 video-microscopy. 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 KCl, 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 the Results section 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 a Digidata 1550 interface (Axon 
Instruments, Foster City, CA) controlled by pClamp 10.3 (Molecular Devices; Sunnyvale, CA).

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

**Retrograde tracing**

In some experiments, in order to selectively target our whole-cell recordings to CMT 
neurons projecting to BL, a subset of 16 rats received bilateral infusions (0.3 µl per side) of the 
retrograde tracer Cholera toxin subunit B (CTB; Alexa Fluor conjugate-488; Life Technologies, 
Grand Island, NY). The tracer was pressure injected using a micro-syringe aimed to the 
following stereotaxic coordinates (in mm and relative to bregma; AP -2.2, ML 5 mm, DV -9 
mm). One to two weeks later, brain slices were prepared as above. CTB positive CMT neurons 
were visualized with a Zeiss microscope (Axioscope). An additional two rats received CTB 
infusions in BL but were only used for histological purposes. One to two weeks after the tracer 
infusions, under deep anesthesia (as above), these rats were perfused through the heart with 
saline (250 ml) followed by a fixative containing 4% paraformaldehyde in 0.15 M phosphate 
buffer (pH 7.4).
**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 hours in 4% paraformaldehyde in 0.1 M phosphate buffer saline (pH 7.4). Following four 10 min washes in PB, sections were incubated for 12 h at 20°C in solution containing 0.5% triton, 1% A and B solutions of an ABC kit (Vector, 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), 2.5 mM nickel ammonium sulfate (Fisher), 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 were cleared using solutions of progressively higher alcohol concentrations (from 20%-100%) followed by 10 min submersion in xylene. The sections were coverslipped with Entellan (Merck) for examination and reconstruction under a bright field microscope. Labeled neurons were observed with a Nikon Eclipse E800 microscope using a ×40 objective and photographed.

**Analyses and statistics.**

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

All data are reported as average ± SEM. 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 recordings 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 basolateral nucleus of the amygdala. 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 ± SEMs 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; top panel of Fig. 1A1, B1), they exhibited markedly different firing patterns when depolarized from a hyperpolarized potential (-70 mV and beyond; top panels of Fig. 1A2, B2). Half of the cells (29/60 cells, 48%), hereafter termed single low-threshold bursting cells (SLB), 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, top). 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**, top panel).

By contrast, in the second type of CMT cells (31/60, 52%), positive current pulses from
pre-pulse potentials negative to rest elicited an initial high frequency spike burst (3.6 ± 0.3 action
potentials) followed by tonic firing (**Fig. 1B2**, top panel). In these cells (hereafter termed
tonically firing –TF– neurons), the tonic firing that followed the initial low-threshold spike 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, 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,
elicted by rheobase depolarization from a hyperpolarized state, did not differ between cell types.
Similarly, maximal intra-burst firing frequency, as measured by the shortest inter-spike 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 pre-pulse potential of -70 mV and (2) after individual
spikes elicited by just above threshold depolarizations from a pre-pulse potential of -55 mV (see
details in 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 a 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 low-threshold spike burst.

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 nucleus

To determine whether TF and SLB cells both project to the basolateral amygdala, one to two weeks prior to the electrophysiological experiments, 16 rats received bilateral infusions of the retrograde tracer CTB (conjugated to Alexa-fluor 488) in the basolateral nucleus of the amygdala. Figure 2A shows a representative example of 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 Pape 1996; Biel et al. 2009). To examine the possibility that this peculiarity of CMT cells resulted from our recording techniques, we compared them to 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 figure 3 (A, VPM; B, CMT), 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 pre-pulse 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 3-fold lower input resistance than CMT cells (VPM: 161.0 ± 27.13 MΩ, n=12; CMT: 499.9 ± 32.0 MΩ, n=60; 8.17, p<0.001). Nevertheless, sag ratios differed significantly between CMT and VPM neurons (CMT, 0.98 ± 0.01, n=60; VPM, 0.90 ± 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 membrane potential 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. Also, the maximal intra-burst spike frequency was significantly lower for CMT than VPM cells. Note that all these differences remained significant when we compared VPM cells to 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 were recovered (6 SLB and 5 TF). For comparison, we also labeled four VPM cells.

Paralleling the physiological differences between CMT and VPM neurons, their morphology was also strikingly different (Fig. 4). All the VPM cells 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 (Von Kolliker 1896; Turner et al. 1997; reviewed in Jones 2007). 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 first branching point, average dendritic length, and dendritic 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 (inter-varicose 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 the insets of figure 4B1 and 4C1.
DISCUSSION

The present study was undertaken to characterize the electroresponsive properties of CMT neurons that project to the basolateral nucleus of the amygdala. 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 (Padilla-Coreano et al. 2012; Do-Monte et al. 2015). 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, $K_{v4}$ and $K_{v1}$ 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 membrane potential (Jahnsen and Llinas 1984a,b). Whereas depolarizing current pulses elicited tonic firing from depolarized membrane potentials, they evoked high
frequency spike bursts from membrane potentials 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 low-
threshold spike 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 low-threshold spike burst. The fact that SLB
cells could fire tonically at high rates when depolarized from positive but not negative potentials
suggest that the differential expression of a Ca$^{2+}$-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 Ca$^{2+}$ influx caused by the initial low-threshold spike 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 low-threshold spike bursts were higher in SLB than TF neurons, although
not significantly so ($p = 0.1$). 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 low-threshold spike burst, this trend is a 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 half 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 basolateral
nucleus of the amygdala. 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 (Von Kolliker 1896; Turner et al. 1997; reviewed in Jones 2007; however
see Deschenes 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.

Relative to other thalamic relay cells, CMT neurons exhibit unusual physiological properties

Whereas most dorsal thalamic relay neurons show a pronounced, $I_{\text{H}}$-mediated (reviewed
in Pape 1996; Biel et al. 2009), 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 ~5 times lower than in
control recordings from VPM cells. While this property of CMT cells might reflect a lower
overall HCN channel density than seen in relay cells from other thalamic nuclei, it might also
depend on the expression of different HCN sub-units, as these determine the kinetics and voltage-dependence of $I_{\text{H}}$.

What is the significance of this unusual property of CMT neurons? Besides its influence on the biophysical properties of single cells, the expression of $I_{\text{H}}$, or in this case, the lack of, has network-level consequences, which may be relevant to the hypothesized involvement of CMT in the control of awareness (Alkire et al. 2007; Baker et al. 2014; Leung et al. 2014). In other thalamic nuclei, the $I_{\text{H}}$-mediated inward rectification was shown to play a major role in the genesis of intrinsic delta frequency oscillations (McCormick and Pape 1990; Soltesz et al. 1991; McCormick and Huguenard 1992). Specifically, the Ca$^{2+}$ influx associated with low-threshold spike bursts activates Ca$^{2+}$-dependent potassium conductances, causing a membrane hyperpolarization that activates $I_{\text{H}}$. In turn, the depolarization produced by $I_{\text{H}}$ triggers another low-threshold spike burst, initiating the next oscillatory cycle. Blocking $I_{\text{H}}$ abolishes the intrinsic delta oscillations (McCormick and Huguenard 1992). While 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 (CurroDossi 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 $I_{\text{H}}$ 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 previously reported (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 versus other dorsal thalamic nuclei in naturally sleeping subjects to determine if it is consistent with the aforementioned speculations.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

ITJ and DP designed the study. ITJ, RY and NZG conducted the experiments. ITJ and DP did the data analysis. DP wrote the manuscript.
REFERENCES


FIGURE LEGENDS

Figure 1. Two types of CMT neurons. Voltage responses of two CMT neurons (A, SLB cell; B, TF neuron) to graded series of hyperpolarizing or depolarizing current pulses applied from -55 mV (A1, B1) or -70 mV (A2, B2). Insets in A2 and B2 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.

Figure 2. Both types of CMT neurons project to the amygdala. (A) CTB injection site in the BL nucleus of the amygdala. (B) CMT neurons retrogradely labeled from the BL nucleus at a low (B1) and high (B2) magnification. (C) Same BL-projecting CMT neuron and recording pipette (asterisks) as seen with differential interference microscopy (C1) and fluorescence (C2). (D) Examples of BL-projecting SLB (D1) and TF (D2) neurons. Both panels show voltage responses to 100 pA depolarizing current pulses applied from -70 mV.

Figure 3. Comparison between 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; CMT, thick line, 50 pA). (B-C) Voltage response of two different VPM cells (left and right) to current pulses (inset in C1) applied from -55 mV (B1, C1) or -70 mV (B2, C2). Except for the differing steady currents (required to set the pre-pulse potential at -55 and 70 mV), the same current steps were applied in panels 1 and 2.
Figure 4. Morphological properties of CMT neurons. (A) Scheme showing position of CMT cells labeled with biocytin and depicted in panels B-C. (B) SLB neurons. (B1) Photomicrograph showing morphology of a CMT neuron of the SLB subtype. Inset shows dendritic segment with varicosities. The depicted region is enclosed in a rectangle in the lower power photograph. (B2-6) Camera lucida drawings of other SLB neurons. The position of the various cells in CMT is indicated in panel A. (C) TF cells. (C1) Photomicrograph showing morphology of a CMT neuron of the TF subtype. Inset shows dendritic segment with varicosities. The depicted region is enclosed in a rectangle in the lower power photograph. (C2-5) Camera lucida drawings of other TF neurons. The position of the various cells in CMT is indicated in panel A. (D) VPM neuron.
### Table 1: SLB vs. TF cells

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<th>Rheobase (pA)</th>
<th>Resting membrane potential</th>
<th>AP Threshold (mV)</th>
<th>AP Amp. (mV)</th>
<th>AP Duration (ms)</th>
<th>LTS Amp. (mV)</th>
<th>Max. Intra-burst Freq. (Hz)</th>
<th>AHP Amp - 55mV (mV)</th>
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<td>1.33</td>
<td>0.182</td>
<td>0.49</td>
<td>2.68</td>
<td>0.47</td>
<td>1.92</td>
<td>1.68</td>
</tr>
<tr>
<td>p-value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.57</td>
<td>0.19</td>
<td>0.85</td>
<td>0.62</td>
<td>0.01</td>
<td>0.63</td>
<td>0.06</td>
<td>0.10</td>
</tr>
</tbody>
</table>

### Table 2: CMT vs. VPM

<table>
<thead>
<tr>
<th></th>
<th>Input Resistance (MΩ)</th>
<th>Rheobase (pA)</th>
<th>Resting membrane potential</th>
<th>AP Threshold (mV)</th>
<th>AP Amp. (mV)</th>
<th>AP Duration (ms)</th>
<th>Max. Intra-burst firing Freq. (Hz)</th>
<th>Sag Ratio</th>
<th>AHP Amp - 55mV (mV)</th>
<th>AHP Amp - 70mV (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMT</td>
<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>21.0±0.7</td>
<td>3.9±0.5</td>
</tr>
<tr>
<td>VPM</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>0.34±0.02</td>
<td>475.7±29.8</td>
<td>0.90±0.014</td>
<td>14.2±0.4</td>
<td>2.3±1.3</td>
</tr>
<tr>
<td>t-value</td>
<td>8.17</td>
<td>1.78</td>
<td>5.0</td>
<td>6.88</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-value</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;0.001</td>
</tr>
</tbody>
</table>

### Table 3: Morphology Characteristics of CMT Cell Types

<table>
<thead>
<tr>
<th></th>
<th>Soma</th>
<th>Primary Dendrites</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Max. Soma Diameter, µm</td>
<td>Min. Soma Diameter, µm</td>
<td>Number</td>
</tr>
<tr>
<td>SLB</td>
<td>24.8±0.94</td>
<td>16.5±0.83</td>
<td>5.2±1.1</td>
</tr>
<tr>
<td>TF</td>
<td>32.0±2.85</td>
<td>19.5±3.67</td>
<td>4.8±1.11</td>
</tr>
<tr>
<td>t-value</td>
<td>1.19</td>
<td>0.06</td>
<td>1.40</td>
</tr>
<tr>
<td>p-value</td>
<td>0.26</td>
<td>0.96</td>
<td>0.19</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Diameter at 40 µm from soma</th>
<th>Distance to first branching from soma, µm</th>
<th>Average Dendritic Length, µm</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLB</td>
<td>2.39±0.15</td>
<td>24.1±4.85</td>
<td>292.6±21.5</td>
</tr>
<tr>
<td>TF</td>
<td>2.11±0.17</td>
<td>24.9±6.44</td>
<td>252.3±25.6</td>
</tr>
<tr>
<td>t-value</td>
<td>1.22</td>
<td>0.87</td>
<td>0.39</td>
</tr>
<tr>
<td>p-value</td>
<td>0.26</td>
<td>0.96</td>
<td>0.19</td>
</tr>
</tbody>
</table>