Title: Pain and temperature encoding in the human thalamic somatic sensory nucleus (Ventral caudal - Vc): Inhibition-related bursting evoked by somatic stimuli.

Running Title: Thalamic spike trains encoding pain and temperature.

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Number of figures and tables 6 figures and 1 table
Number of pages 38

Key words
somatic sensory nucleus of thalamus; human; mechanoreception, thermal stimuli; pain; thalamus

Acknowledgements
Supported by grants to FAL from the Eli Lilly Corporation and the NIH (NS 383493, NS 40059). We thank L.Rowland for excellent technical assistance.
ABSTRACT

Stimulus-evoked inhibitory events have not been demonstrated in thalamic spike trains encoding of pain and temperature stimuli. We have now tested the hypothesis that the human thalamic response to mechanical and thermal stimuli is characterized by low threshold calcium spike (LTS) associated bursts of high frequency action potentials preceded by prolonged inhibition.

The results included 57 neurons recorded in the human thalamic principal somatic sensory nucleus (Ventral Caudal, Vc) of 24 patients during awake surgery. Neurons were classified by the grading of their response with stimulus intensity into the painful range (graded or non-graded), and the stimulus response (to mechanical, cold, or heat stimuli). Firing rates were analyzed by the response to all stimuli combined (stimuli overall) and to the stimulus characteristic of the stimulus response type (optimal stimulus), e.g. cold stimuli for neurons of the cold stimulus response type. All neuronal categories had clear stimulus-evoked LTS bursting as identified by the criteria for selecting bursts in the spike train, by significant preburst inhibition, and by preburst inter-spike interval not significantly less than 100ms. Stimulus-evoked LTS burst rates were significantly higher for neurons in the cold stimulus response type, independent of the firing rate between bursts. The parameters of preburst inhibition were largely independent of the neuronal category and the stimuli included in the analysis, which suggests inhibitory mechanisms are similar across neuronal types. Therefore, LTS bursting is a substantial, non-linear component of the spontaneous and stimulus-evoked activity of thalamic neurons in awake humans.
INTRODUCTION:

The excitatory responses of neurons in the human thalamic principal somatic sensory nucleus (ventral caudal - Vc) to somatic sensory stimuli are well known (Lee et al. 1999; Lenz et al. 1988b). These responses can be understood in terms of the glutamatergic inputs from afferent fibers to monkey thalamo-cortical projection neurons (Beggs et al. 2003; Dougherty et al. 1996; Dougherty et al. 1998; Ralston and Ralston 1992; Ralston, III and Ralston 1994). These same studies demonstrate that afferent fibers activate inhibitory interneurons and glomeruli, both of which produce inhibitory GABAergic feedback to projection neurons (Beggs et al. 2003; Ralston, III and Ralston 1994). Collaterals of the axons of projection neurons synapse in the thalamic nucleus reticularis, again resulting in GABAergic inhibitory feedback to the projection neurons (Deschenes et al. 1985).

Inhibitory events may contribute to the bursts of action potentials which have been identified in the spontaneous firing of projection neurons in Vc studied in patients with chronic pain or movement disorders (Jeanmonod et al. 1996; Lenz et al. 1994c; Lenz et al. 1998; Radhakrishnan et al. 1999; Zirh et al. 1997). These high frequency action potential bursts crown a voltage dependent calcium conductance which is de-inactivated by prolonged inhibition (low threshold spike - LTS) (Jahnsen and Llinas 1984; Steriade et al. 1990).

We now address the hypothesis that the stimulus-evoked activity of neurons in Vc is characterized by inhibitory events leading to LTS bursting. This hypothesis has been tested by studying the response of single neurons in human Vc to non-painful and painful stimuli of the mechanical, heat and cold submodalities during awake surgical procedures.
in patients with movement disorders. The results demonstrate that while all neuron types have stimulus-evoked LTS bursting, those neurons responding to cold have the highest burst rates. This paper describes analysis of bursting in a group of Vc neurons previously characterized by their firing rates in response to the same stimuli (Lee et al. 1999).

METHODS

These results are based on analysis of spontaneous activity and responses to somatic stimuli from 57 neurons recorded in 24 patients along 30 trajectories through Vc core the region where the neurons had receptive fields to innocuous cutaneous stimuli (Lenz et al. 1988b). The subjects carried diagnoses of essential tremor (15 patients), Parkinson’s tremor (5), intention tremor (2), and dystonia (2) (Watts and Koller 1998). All subjects were taken off medications for the treatment of movement disorders for twenty-four hours prior to the procedure. No patient had either abnormal somatic sensory function as assessed by standard sensory testing (Lenz et al. 1993), or an abnormal MRI.

Thalamic exploration was performed as a stereotactic procedure using the Leksell frame (Lenz et al. 1988a). First, the three-dimensional frame coordinates of the anterior (AC) and posterior commissures (PC) were measured by CT/MRI scan. Physiological corroborations of anatomical loci was then performed under local anesthesia, i.e., subject fully conscious, by using both single neuron recording and microstimulation as previously described (Lee et al. 1999; Lenz et al. 1988a). Both recording and stimulation results were used to generate a map (Figure 1) which was fitted to the sagittal sections of a standard atlas (Schaltenbrand and Bailey 1959).

- Place Figure 1 about here –
Pre-operative Testing:

The protocol for these studies was identical to that for the prior report of responses to somatic stimuli (Lee et al. 1999), and was reviewed and approved annually by the Hopkins Institutional Review Board. All subjects signed an informed consent for these studies. Initial sensory testing included determination of mechanical and thermal thresholds for face and arm. Subjects described these stimuli with verbal descriptors, which were chosen from a questionnaire of ideal descriptors (Lenz et al. 1994a; Torgerson et al. 1988). Painful sensations were also described by a visual analog scale (VAS) pain rating anchored by the verbal statement that ‘zero is no pain and ten is the most intense sensation imaginable’ (Gracely et al. 1978; Gracely et al. 1979; Lenz et al. 1994a).

As in the previous report of the same raw data (Figure 1 in (Lee et al. 1999)), sensory stimuli included: 1) brushing the skin with a camel-hair brush, 2) separate application to a fold of skin of a large arterial clip (painful in 40% of subjects, with a pain VAS of 1.9/10 averaged over all patients (Lee et al. 1999), 3) a medium clip (painful in 75% with VAS of 3.3/10), and 4) a small clip (painful in 90% with VAS of 8.2/10) arterial clip (Chung et al. 1986; Guilbaud et al. 1987; Surmeier et al. 1988). Additionally, mechanical stimulation was carried out with a non-penetrating towel clip with two parallel 4 mm by 5 mm serrated surfaces that could be approximated by 10 reproducible steps on a ratchet.

Thermal stimuli were applied by using a Peltier device (LTS-3, 1-inch square head, Thermal Devices, Golden Valley, MN), which delivered a series of thermal stimuli from an adapting temperature of 33°C to 6°C (painful in 30% with VAS of 1.2/10), 12°C (painful in 5% with VAS of 5/10), 18°C (painful in 0%), and 24°C (painful in 2% with
VAS of 1.3/10), 42°C (painful in 21% with VAS of 1.8/10), 45°C (painful in 42% with VAS of 3.6/10), and 48°C (painful in 78% with VAS of 5.3/10) (Lee et al. 1999).

**Study of Thalamic Activity:**

As the electrode was advanced we applied search stimuli which included gentle stroking, brisk tapping, and manual pinching on the contralateral face and hand and in the projected field for threshold micro-stimulation. Neurons that responded to manual pinching were selected for application of quantitative stimuli more often than for those that responded to stroking or tapping (Lee et al. 1999).

When a neuron was isolated, spontaneous activity was first recorded for a period of 30 to 60 seconds. The center and boundaries of the receptive field were then defined by non-painful, mechanical stimuli. Thereafter, all stimuli applied during pre-operative sensory testing were applied. The timing of the stimulus was indicated by the output of the Peltier for thermal stimuli (duration: heat 10 s individual temperature, cold 20 s) and by a foot pedal for mechanical stimuli (duration: 10 s/individual clip setting). These clip or manual mechanical stimuli are standard for studies of nociception in primates (Bushnell et al. 1993; Casey and Morrow 1983; Lenz et al. 2004; Pollin and Albe-Fessard 1979; Willis et al. 1973; Willis 1985). The application of a clip to a fold of skin takes approximately one second; therefore, the time of onset is judged subjectively by the examiner and signaled by use of a foot pedal.

All stimuli were applied as close as possible to the center of the receptive field while minimizing the overlap between the areas stimulated by the different modalities. The site where the mechanical stimuli were applied varied from stimulus to stimulus but always included the center of the receptive field (Lee et al. 1999). The location of the
thermal stimuli from the Peltier always included the center of the receptive field, and the Peltier was moved between the cold and the hot series of stimuli. All recording and stimulation results were recorded manually and on tape (Model 4000, Vetter Corp, Reberburg PA) for postoperative analysis.

**Analysis of Thalamic Activity:**

The neuronal response to somatic stimuli was categorized by the response to mechanical and thermal stimuli as previously described (Lee et al. 1999). Low Threshold (LT) neurons responded only to non-painful stimuli. Multiple receptive (MR) neurons responded significantly to both brushing and compressive stimuli, but were not graded with intensity into the painful range. Multiple receptive neurons included: 1) MR for neurons responding only to mechanical stimuli, 2) MRC for neurons also responding to cold, and 3) MRH for neurons also responding to heat. Wide Dynamic Range neurons (WDR) responded to brushing and compressive stimuli in a graded fashion to stimuli across the intensive continuum from the non-painful into the painful range (Lee et al. 1999). These neurons included: WDR for neurons responding only to mechanical stimuli, WDRC for those also responding to cold, and WDRH for those also responding to heat. Thus, there were a total of seven neuronal categories: LT, MR, MRC, MRH, WDR, WDRC and WDRH.

Neurons were also classified by two separate dimensions of their response to somatic stimuli. The first was the presence of either graded (e.g. WDR) or non-graded responses (e.g. MR) to stimuli across the intensive continuum into the painful range (neuronal response). The second was by the presence of a response to mechanical stimuli only, or to cold or hot stimuli (stimulus response). Therefore, our classification included
seven neuronal categories, which were sub-divided by two neuronal responses and three stimulus responses, not including the LT category.

Post-operative analysis focused on rate indices, e.g. burst rates, and inhibitory indices, e.g. preburst interval. All stimuli of a sub-modality were combined for the analysis, so that all cold stimuli were combined for analysis, for example. LTS bursts were identified by criteria identical to criteria used in studies of awake monkeys and based on intracellular confirmation (Ramcharan et al. 2000b; Ramcharan et al. 2000a). Criteria (50-6-16) to select bursts were as follows: the inter-spike interval (ISI) preceding the first action potential in the burst had a duration > 50 ms, the ISI following the first action potential in the burst had a duration of <6 ms, and all following action potentials were considered as part of the burst if their ISI increased by no more than 2 ms for each succeeding action potential, up to maximum ISI of 16 ms. Bursts of two action potentials were included, consistent with all studies of awake animals (Domich et al. 1986; Glenn and Steriade 1982; Jeanmonod et al. 1996; Lenz et al. 1994c; Radhakrishnan et al. 1999; Ramcharan et al. 2000b).

We also calculated the primary event rate, a well established measure of the firing rate between bursts (Cox and Lewis 1966; Lenz et al. 1994c; McCarley et al. 1983; Reinagel et al. 1999). The primary event rate included all single spikes plus the first spike in each burst. The ratio of burst rate/primary event rate was calculated to determine whether the burst rate was dependent upon background firing. If the difference in burst rates between two neuronal categories was lost when this ratio is calculated, then the difference in bursting was considered to be dependent on the primary event rate.
All firing rate indices were calculated by subtracting the baseline firing value and expressed in units of s\(^{-1}\) (see Results: Spontaneous bursting and inhibitory events). Ratios of rates were calculated without subtracting the baseline value, since they are the result of a non-linear transformation. In some cases differences between the seven neuronal categories (LT, MR, MRC, MRH, WDR, WDRC, and WDRH) were compared by a one way ANOVA. Post hoc testing was carried out by Tukey’s Honestly Significant Difference test (HSD).

Rate indices were also analyzed by a two-way ANOVA by on the neuron type classified by two axes: 1) the presence of graded or non-graded responses to stimuli of different intensity (neuronal response), 2) the response to mechanical, cold or hot stimuli (stimulus response). Post hoc testing of the main effects of neuronal response, stimulus response, and interaction was then carried out (Tukey’s Honestly Significant Difference test - HSD).

Inhibitory events were also analyzed to confirm that selected bursts were associated with LTS since LTS bursts must be preceded by a prolonged inhibition. We first tested whether mean preburst ISIs were significantly different from 100 ms, which is the inhibitory interval consistent with the maximal LTS (Jahnsen and Llinas 1984). The mean for a neuronal category was judged to be different from 100 ms if 100 ms was outside the 95% confidence intervals for the distribution of preburst ISIs (2.7 SEM, Bonferroni corrected experiment-wise estimate of error).

Interpretation of the preburst ISI is not entirely straightforward since a preburst ISI > 50 msec in the selection criteria may be the result either of an inhibitory event or of a primary event rate of less than 20 s\(^{-1}\). We identified the presence of preburst inhibition
by normalizing the preburst inhibition to the primary event rate, a standard measure of firing rates between bursts (ratio of the preburst ISI/inverse of the primary event rate) (Cox and Lewis 1966; Lenz et al. 1994c; McCarley et al. 1983; Reinagel et al. 1999). The mean for a neuronal category was judged to be greater than one if the 95% confidence interval for the distribution of this ratio was greater than one (2.7 SEM, Bonferroni corrected experiment-wise estimate of error). A ratio of significantly greater than one was taken to indicate a preburst inhibition. As in the case of rate indices preburst ISI and the ratio were tested by a one-way ANOVA by neuronal category and a two-way ANOVA by neuronal response and stimulus response. All tests were considered significant for \( P < 0.05 \).

RESULTS

These results consist of quantitative sensory testing on 57 neurons recorded along 30 trajectories in 24 patients, as previously described (Lee et al. 1999). There were fifteen neurons with graded neuronal responses classified as WDR (6 neurons), WDRC (3 neurons), and WDRH (6 neurons). There were twenty-five neurons with non-graded neuronal responses classified as MR (17 neurons), MRC (5 neurons), and MRH (3 neurons). On average, these neurons had their largest response to brushing and a smaller response to mechanical stimuli. There were nine neurons in the LT category which had a response to brush, but not to other mechanical stimuli. In addition, four neurons responded to manual compression and four neurons to tapping (n=4), but not to the quantitative stimuli used in this study.

- Place Figure 2 about here –
Figure 2 shows an example of the firing of a neuron responding to cold and mechanical stimuli (MRC neuron: 05055, see also Figures 2, 4, 6 in (Lee et al. 1999)). This neuron fires in bursts of action potentials during the response to most stimuli. However, there is a good deal of variability in bursting during spontaneous or prestimulus activity and during the response a single sub-modality of stimulation e.g. 18°C and 24°C. Some stimuli, such as heat (42° and 45°C) had very low burst rates, suggesting that some stimuli might fail to evoke bursts consistently. Therefore we examined all stimulus epochs for the presence of bursting.

**Epochs with bursting examined by Neuronal Category and Sub-modality of Stimulation.**

Bursting occurred during the majority of stimulation epochs (30-120s, see Methods section on ‘Study of thalamic … ’) for all neuronal categories during the response to mechanical, cold, and heat stimuli (Table 1). The cold stimuli evoked bursting in a higher proportion of epochs for LT (p=0.008) and MR (p=0.007) than for WDR neurons. There were no other significant differences in the proportions of such epochs. Since bursting was present in the response of all neuronal categories to all stimuli we next looked for quantitative differences in bursting as a function of neuron classification and stimulus sub-modality. Burst rates were not related to stimulus intensity for any sub-modality of stimulation. Therefore, subsequent analyses combined all intensities of stimulation within each sub-modality.

- Place Table 1 about here -

**Bursting activity: Stimuli overall.**

The primary event rate, the burst rate, and the burst rate/primary event rate were first analyzed by the response to all stimuli combined (stimuli overall) in a two-way
ANOVA by neuronal response and stimulus response (see Methods: Analysis of Thalamic … ). The primary event rates in response to stimuli overall were significantly related to stimulus response due to higher rates for neurons responding to cold than heat or to mechanical stimuli only (Figure 3C, left). There was significant interaction due to MRC neurons having higher primary event rates than WDRC neurons. The primary event rate, the burst rate, and the burst rate/primary event rate varied significantly with stimulus response, but did not vary significantly with neuronal response.

- Place Figure 3 about here –

The burst rate (Figure 3, middle column) varied significantly with stimulus response due to higher rates for neurons responding to cold than those responding to heat or to mechanical stimuli only (Figure 3B). The interaction term was significant, although significant differences were not found for any pair of neuronal categories on post hoc analysis (3C, middle column).

To determine whether the burst rate was dependent upon background firing, the ratio of burst rate/primary event rate was calculated (see Methods: Analysis of Thalamic … ). The burst rate/primary event rate in response to overall stimuli showed a significant main effect of stimulus response, due to a higher ratio for neurons responding to cold than for those responding to heat or only to mechanical stimuli. Neither the neuronal response nor interaction was significant. Thus the burst rate was significantly higher among neurons responding to cold, independent of the primary event rate.

These results suggest that bursting was preferentially related to neural elements subserving cold. Those neural elements might have been the fibers transmitting cold to the thalamus, or the inhibitory connections of neurons responding to cold. To test
whether the afferent fiber was a critical determinant of bursting we next analyzed the response of neurons to their optimal stimulus, e.g. cold for MRC neurons.

**Bursting activity: Optimal stimulus response.**

The optimal stimulus was defined as brushing for low threshold neurons (LT), and as non-painful and painful intensities of heat for MRH and WDRH neuronal categories, or cold for MRC and WDRC neuronal categories, or mechanical stimuli only for MR and WDR neuronal categories (Lee et al. 1999). The primary event rate in response to optimal stimuli was significantly related to stimulus response due to a significantly higher rate for neurons responding to cold than for those responding to heat stimuli or to mechanical stimuli only. Neither the main effect for neuronal response (Figure 4, left upper panel) nor to interaction was significant.

Burst rate in response to optimal stimuli was significantly related to stimulus response due to rates for neurons responding to cold being higher than for those responding to heat or to mechanical stimuli only (Figure 4B, middle column). Neither the neuronal response (Figure 4, middle column – lower panel) nor the interaction term was significant (not shown).

The burst rate/primary event rate in response to optimal stimuli was significantly related to stimulus response, due to a higher ratio was higher for neurons responding to cold than for those responding to heat or to mechanical stimuli only (Figure 4C, right column). Neither the main effect of neuronal response (Figure 4, right column – upper panel), nor to the interaction term was significant. Therefore, burst indices for the optimal stimulus and stimuli overall were significantly related to stimulus response. This effect was due to higher burst indices among cells responding to cold, which is consistent with a
mechanism involving afferent fibers subserving cold. To test this mechanism we next analyzed bursting in response to all stimuli except the optimal stimulus so that the effect of the afferent fiber on the stimulus response is eliminated.

**Bursting activity: Response to all stimuli except the optimal stimulus:**

For all stimuli except the optimal stimulus the primary event rate was significantly related to stimulus response ($F=12.3$, df=2, $p<0.001$), but not to neuronal response ($F=3.7$, df=1, $p=0.057$) with significant interaction ($F=9.3$, df=2, $p<0.001$). Post hoc tests showed that MRC neurons (24.4/s) had a significantly higher primary event rate than MR (7.9/s) and MRH neurons (3.7/s, $p<0.001$). WDRH neurons (14.1/s) had a significantly higher primary event rate than WDR neurons (-3.4/s, $p<0.001$).

The burst rate for all stimuli except optimal was significantly related to the interaction term ($F=4.4$, $p=0.01$) but not to the stimulus response ($F=1.6$, $p=0.20$) or neuronal response ($F=0.1$, $p=0.72$). The burst rates were as follows: MRC 0.34/s, MR 0.34, WDRH 0.29, WDRC 0.23, WDR 0.14, and MRH -0.12). The burst rate for MRC neurons was significantly higher than for MRH neurons ($p=0.02$).

Burst/primary event rate was significantly related to stimulus response ($F=7.6$, $p<0.001$), but to neither neuronal response ($F=3.5$, $p=0.064$) nor the interaction term ($F=1.4$, $p=0.248$). Post hoc testing for stimulus type showed that the burst/primary event rate was significantly higher for neurons responding to cold (0.06, $p=0.003$) or mechanical stimuli only (0.05, $p=0.018$) than for those responding to heat (0.03). Higher burst rates that were observed among cells responding to cold persisted even if the optimal response was excluded from stimuli overall. This suggests that the bursting
behavior of neurons responding to cold is not due to the afferent fiber transmitting cold stimuli, but rather due to the inhibitory connections of these neurons.

**Inhibitory events: Ratio of Preburst ISI/inverse of the primary event rate.**

The presumed LTS bursting activity described above assumes the presence of a preburst inhibition (Jahnsen and Llinas 1984). We attempted to distinguish prolonged preburst ISIs due to a pause/inhibition before the burst from prolonged preburst ISIs reflecting a slow firing rate, i.e. all interburst ISIs are prolonged. The interburst ISI is the inverse of the primary event rate (see Methods: Analysis of Thalamic …). Therefore if we calculate the ratio of the preburst ISI/inverse of the primary event rate, then a preburst pause/inhibition will result in a ratio of greater than 1. In Figure 5A, the error bars of mean ± 2.7 SEM indicate the 95% confidence limits for the ratio (Bonferroni corrected experiment-wise estimate of error). The ratio for stimuli overall of all neuronal categories was significantly greater than 1, as indicated by error bars above the dashed horizontal line. Therefore, all neuronal categories had significant preburst inhibition in response to stimuli overall (Figure 5A - left). In the case of the optimal response (Figure 5A - right) all neuronal categories had significant preburst inhibition, except MRH neurons.

- Place Figure 5 about here –

This ratio was significantly different between neuronal categories for stimuli overall (Figure 5A – left column). Post hoc testing revealed that the ratio was significantly larger for WDRH than any other neuron type except MRC. The ratio for optimal stimuli was significantly dependent on neuronal categories (Figure 5A, right).
Post hoc testing showed that LT and MR neurons had a significantly larger preburst inhibition than WDR neurons.

The two-way ANOVA for preburst interval of stimuli overall showed significance by interaction but not by stimulus response or neuronal response. Post hoc analysis (Figure 5B, left) revealed that the ratio for WDRH neurons was significantly higher than for the MRH neurons. The two-way ANOVA for optimal stimuli showed no significant main effects, but significant interaction (Figure 5B, right, $F=5.3$, $df=2$, $p=0.006$). The post hoc testing on interaction found that MR neurons had significantly ($p=0.017$) higher ratios than WDR neurons, consistent with the one-way ANOVA. WDRH had a significantly higher ratio than WDR neurons ($p=0.040$). Therefore, WDR neurons consistently had the least pronounced preburst inhibition.

**Inhibitory Events: Preburst ISI.**

Maximal LTS burst amplitude occurs with the duration of preburst inhibition of 100msec (Jahnsen and Llinas 1984). The preburst ISIs were not significantly less that 100 msec in the case of any neuronal category for stimuli overall, since the error bars overlap or are above the 100msec horizontal dashed line in that panel (see above and Methods: Analysis of Thalamic …). Preburst ISIs were greater than 50 msec, since error bars were above the lowest dashed horizontal line for all neuronal categories except MRH. Therefore, optimal stimuli preburst ISIs were not significantly less than 100msec but were significantly greater than 50 msec in the case of all categories except the MRH neurons.
The preburst ISIs were not significantly different among different neuronal categories (one-way ANOVA) for either stimuli overall (Figure 6A, left) or optimal stimuli (Figure 6A, right).

The two-way ANOVA for stimuli overall which showed significant effects by only by neuronal response. Post hoc testing revealed that preburst ISIs were significantly longer for the graded response type than non-graded response type (Figure 6B – left). The two-way ANOVA for optimal stimuli showed no significant effects.

By many measures WDR neurons the weakest preburst inhibition, i.e. smallest ratio, and the longest preburst ISI, suggesting that their firing is dominated slow primary event rates.

For stimuli overall and optimal stimuli the different neuronal categories had significant preburst inhibition, and preburst ISIs which were not significantly less than 100 ms. The only exception is in the optimal response for the MRH, that might be explained by the high variance and small sample size (see Figure 6A). These results demonstrate that the preburst ISI is not likely an artifact of the burst selection criteria (preburst ISI > 50 ms), but is consistent with a maximal LTS (Jahnsen and Llinas 1984).

Spontaneous bursting and inhibitory events.

Spontaneous firing was studied as a measure of the baseline firing pattern which commonly displayed bursts for these neurons (Table 1). The two-way ANOVAs for primary event rate or burst rate showed no significance. The burst/primary event rates during spontaneous period showed significance by stimulus response (F=3.6, p=0.037), but not to neither neuronal response (F=0.4, p=0.544) nor interaction (F=1.9, p=0.165). The post hoc test for stimulus response showed that neurons responding to cold had
significantly higher burst/primary event rates (0.06) than those responding to heat (0.02, p=0.048). Thus, spontaneous bursting in neurons responding to cold was higher than those responding to heat, suggesting that the burst rate of neurons responding to heat, but not those responding to cold, was dependent upon the primary event rate.

The ratio of preburst ISI/inverse of primary event rates for spontaneous activity were significantly greater than 1 for all neuron types, indicating significant spontaneous preburst inhibition. Preburst ISIs during spontaneous period were not significantly less than 100 msec for any neuronal category. For spontaneous activity the two-way ANOVA showed no significant effects by either the ratio of Preburst ISI/inverse of the primary event rate or Preburst ISI.

DISCUSSION

These results demonstrate that all neuronal categories have LTS bursts evoked by multiple somatic stimuli, based on standard selection criteria (Table 1). The ratio of preburst ISI/inverse of the primary event rate demonstrates that the preburst ISIs were the result of significant preburst inhibition, and not to slow primary event rates. The preburst ISIs were not significantly shorter than 100 msec, consistent with maximal LTS amplitude, but were significantly longer that the 50msec minimum preburst ISI required by the burst selection criteria. Therefore, these results do not reflect an artifact of the burst selection criteria. Altogether, these results are strong evidence for the presence of stimulus-evoked inhibition leading to LTS bursts during both spontaneous activity and the excitatory response to peripheral stimuli of thalamic neurons in awake humans.

Methodological Considerations.
Different criteria have been used to identify LTS bursts in thalamic ‘relay’ nuclei in both different species (Domich et al. 1986; Guido et al. 1992; Lu et al. 1992; Ramcharan et al. 2000b), and awake humans with neurological disorders (Jeanmonod et al. 1996; Lenz et al. 1994c; Lenz et al. 1998; Radhakrishnan et al. 1999; Zirh et al. 1997). Except in the case of humans, all of the criteria mentioned above have been validated by intracellular confirmation (Deschenes et al. 1984; Domich et al. 1986; Guido et al. 1992; Lu et al. 1992; Ramcharan et al. 2000b). We have adopted selection criteria which have been validated by intracellular recordings and applied in studies of awake old world primates (Ramcharan et al. 2000b; Ramcharan et al. 2000a). The ability of these criteria to select LTS bursts in humans demonstrated by the presence of significant preburst inhibition of a duration (approximately 100 ms) that is consistent with maximal (see Figures 5 and 6).

**Afferent mechanisms mediating thalamic bursting evoked by somatic stimuli.**

The present study demonstrates that neurons responding to cold stimuli have higher rates of stimulus-evoked LTS bursting (Figures 3 and 4). The higher stimulus-evoked burst rates among neurons responding to cold was independent of the background firing rate since they persisted after burst rates were normalized to the primary event rate. Another possibility is that bursting among thalamic neurons responding to cold is the result of cold-evoked bursting in the periphery, which is transmitted to the thalamus.

Thalamic neuronal responses to cool, heat and painful stimuli may originate in Aδ cool fibers, Aδ- or C-mechano-heat fibers and be transmitted to spinothalamic tract (STT) neurons in the dorsal horn (Apkarian and Hodge 1989a; Apkarian and Hodge 1989b; Craig 1990; Craig et al. 1994; Dostrovsky and Craig 1996; Ferrington et al. 1987;
Kumazawa et al. 1975). The axons of these neurons project to ventral posterior nucleus (Apkarian and Hodge 1989b; Ferrington et al. 1987; Mehler 1962) and, perhaps, to the posterior part of ventral medial nucleus, i.e. VMpo (Craig et al. 1994; Dostrovsky and Craig 1996) cf (Graziano and Jones 2004; Lenz et al. 2004; Willis, Jr. et al. 2001). Another possibility is that Vc neurons responding to cold might receive inputs from type 1 slowly adapting mechanoreceptors which are activated by cold (Burton et al. 1972; Duclaux and Kenshalo 1972; Hensel and Zotterman 1951; Iggo and Muir 1969). The activity of these fibers is transmitted through the dorsal column nuclei to the ventral posterior thalamus (Burton et al. 1970; Willis and Coggeshall 1991). The present report of bursting activity in thalamic neurons responding to cold stimuli is reminiscent of the response of cold receptors to cold stimuli (Iggo 1969; Kenshalo and Duclaux 1977). Cool responsive neurons in the ventral posterior nucleus also respond to bursting activity at high frequency (ISIs of 2-4 ms) during the cooling phase following a heat stimulus (Martin, III and Manning 1971). However, transmission of thalamic bursting from the periphery is in doubt because STT neurons responding to cold do not show the bursting, unlike the primary afferents and the thalamic neurons (Iggo and Ramsey 1976; Poulos 1975). The activity of these STT neurons may reflect their response to multiple primary afferents firing out of phase. Therefore, it seems unlikely that thalamic bursting is the result of transmission of bursting activity from the periphery. The present evidence for stimulus-evoked LTS bursting in Vc argues for a mechanism based on thalamic circuitry rather than transmission of bursting activity to the thalamus via afferent pathways.

**Thalamic circuitry related to stimulus-evoked inhibitory events.**
In primate species, afferent axons terminate on excitatory amino acid (EAA) receptors based on both anatomic and electrophysiologic criteria (Dougherty et al. 1996; Jones 1983; Sherman and Guillery 2001). Axons in the monkey dorsal column pathway form triadic structures in the ventral posterior nucleus by terminating separately on the dendrite of a GABAergic interneuron and the dendrite of a thalamic projection neuron (Ralston, III and Ralston 1994). That GABAergic dendrite then forms an inhibitory synapse on the same projection neuron. Therefore, the afferent evoked EPSP in the projection neuron is immediately followed by an IPSP produced by input from the GABAergic interneuron (Ralston, III and Ralston 1994). This arrangement shortens the afferent evoked EPSP and so provides short latency inhibitory feedback to excitatory somatic sensory input. Conversely, STT terminals commonly end in simple axo-dendritic terminations (Ralston, III and Ralston 1994), which are clustered together on the dendrite.

Thalamic projection neurons also receive inhibitory GABAergic non-triadic synapses, arising from thalamic nucleus reticularis and intrinsic inhibitory interneurons. Cortico-thalamic axons commonly send a branch to neurons of the thalamic reticular nucleus that project back to thalamic projection neurons, either directly or indirectly (Bourassa et al. 1995; Darian-Smith et al. 1999; Deschenes et al. 1994). Therefore, there are many possible explanations of inhibitory events and the associated LTS bursting evoked by somatic sensory pathways afferent to the thalamus.

In comparison to other neuron types, those responding to cold have higher rates of stimulus-evoked LTS bursts, regardless of the stimuli analyzed. Therefore, it seems unlikely that burst firing is related directly to the afferent fiber transmitting cold. It is
more likely that the increased bursting is the result of the properties and inhibitory connections of neurons responding to cold. These stimulus-evoked inhibitory events may result from afferent connections to the inhibitory circuitry described above (Jones 1985; Sherman and Guillery 2001; Steriade et al. 1997).

Although neurons responding to cold have more stimulus-evoked LTS bursts, our inhibitory indices (preburst ISIs and the preburst ISI/inverse of the primary event rate) are not significantly different among neuronal categories, regardless of the stimuli analyzed. Therefore, increased bursting in neurons responding to cold may be the result of differences in the numbers of afferent-activated inhibitory events, the size of which is similar across neuronal categories in Vc.

Whatever the mechanism of this stimulus-related LTS bursting, the resulting spike trains include long pauses followed by brief, intense bursts of action potentials which cannot be described by a linear model (Bendat and Piersol 1976). Previous studies have documented the presence of bursting, non-linear, transformations of sensory signals in the forebrain visual system (Guido et al. 1995; Livingstone et al. 1996; Martinez-Conde et al. 2002). In addition, descending control of eye movements related to visual stimulation can transform sensory input by evoking LTS bursts (Martinez-Conde et al. 2002; Ramcharan et al. 2001). In the present data, the spike trains of WDR neurons, transmitting sensory aspect of pain (Price et al. 2003; Price and Dubner 1977), have the smallest preburst inhibition. This suggests that preburst ISIs are strongly influenced by interburst firing rates.

It is not clear how bursting in the present data relates to the assumption of linearity of thalamic pain and temperature transmission that is explicit in primate
thalamic stimulus-response functions (Bushnell et al. 1993; Kenshalo, Jr. et al. 1980; Lee et al. 1999). The same assumption is implicit in the graded mechanical stimulus-response function that defines WDR neurons in the dorsal horn (Kumazawa and Perl 1978; Maixner et al. 1986; Willis et al. 1973), thalamus (Bushnell and Duncan 1987; Lenz et al. 1994b; Morrow and Casey 1992), and cortex (Kenshalo, Jr. and Isensee 1983; Price et al. 2003). Stimulus-evoked LTS bursting may be related to non-linear, binary processes in the primate thalamus and cortex (Bornhovd et al. 2002; Coghill et al. 1999; Lenz et al. 2004) which contribute to attentional or cognitive aspects of pain (Becker et al. 1993; Bornhovd et al. 2002; Zaslansky et al. 1995).


Table 1 Proportion (percentage) stimulus epochs with identified bursts by neuronal categories and stimulus sub-modality.

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<th>MRT</th>
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<td>5/11</td>
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<td>15/18</td>
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<tr>
<td></td>
<td>(91%)</td>
<td>(94)</td>
<td>(91)</td>
<td>(90)</td>
<td>(45)</td>
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<td>(83)</td>
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<tr>
<td>heat</td>
<td>28/32</td>
<td>39/46</td>
<td>12/12</td>
<td>6/7</td>
<td>10/12</td>
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<td>(88)</td>
<td>(85)</td>
<td>(100)</td>
<td>(86)</td>
<td>(83)</td>
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<td>(67)</td>
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<td>compress</td>
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<td>76/85</td>
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<td>SA</td>
<td>9/11</td>
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<td>(93)</td>
<td>(83)</td>
<td>(77)</td>
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SA, spontaneous activity
**FIGURE LEGENDS.**

**Figure 1: Map of receptive and projected fields for trajectories in the regions of the Vc in a single patient (85.095)**  Panel A. positions of the trajectories relative to nuclear boundaries as estimated radiologically from the position of the anterior commissure-posterior commissure (AC-PC) line. The AC-PC line is indicated by the approximately horizontal solid line; the trajectories are shown by the pair of solid lines oblique to that line.

The anterior border of the area where most neurons had deep or cutaneous receptive fields, approximately vertical dashed line, was defined as the anterior border of the core of Vc, i.e. neuron 3. The posterior border of the core of Vc (approximately vertical solid line) was defined as the most posterior neuron with a cutaneous receptive field, i.e. neuron 25. The most inferior neuron with a receptive field, i.e. neuron 57 in Panel B, was used to determine the inferior border of the core of Vc as indicated by the approximately horizontal dashed line in Panel B. Neuron 48 was studied during quantitative somatic stimuli and found to have a non-graded neuronal response of the heat stimulus response type, i.e. MRH. The scale applies to A and B.

Panel B. Location of neuronal recordings (ticks to the right of trajectory) and stimulation sites (ticks to the left of the trajectory) and the trajectory (P2) relative to the anterior, posterior, and inferior borders of the core of Vc. Neurons with receptive fields are indicated by long ticks; those without are indicated by short ticks. Scale is as indicated.
Panel C. The projected field and receptive field figurines are shown for each site number in B where a neuron was recorded or stimulation was carried out or both. The threshold (in microamperes) in indicated below the projected field.

**Abbreviations:** AC-PC - anterior commissure-posterior commissure line, NR – No response, Vc - Ventral caudal nucleus (Schaltenbrand and Walker 1982) corresponding to monkey ventral posterior (Hirai and Jones 1989), and Vcpor (Vc portae), corresponding to monkey Pulvinar oralis (Hirai and Jones 1989), Vim - Ventral intermediate (cerebellar relay nucleus), Vop – ventral oral posterior (pallidal relay nucleus). Nuclei relevant to the discussion but not shown in B are Vcpc - Vc parvocellularis corresponding to monkey ventral posterior inferior (lateral to the part of Vc below ACPC in B) (Hirai and Jones 1989), and VMpo – Posterior part of the ventral medial nucleus medial to the inferior aspect of Vcpor in B ((Blomqvist et al. 2000) cf (Graziano and Jones 2004; Lenz et al. 2004; Willis, Jr. et al. 2001)).

**Figure 2: Spike trains recorded from a neuron classified in the MRC group.**

Panel A. The single neuron recording corresponding to the spike train segment in Panel B - 18°C indicated by the line above the *.

Panel B shows the discriminated spike train during the response to different stimuli as labeled. The filled circles above the tracing in A and the spike train in B indicate the first spike in a burst meeting selection criteria. The scale in B is so small that bursts like the second burst (dot) in Panel A can appear as a single spike in the corresponding segment of Panel B - 18°C (line above the *). Bursts like the first and
third bar in Panel A can appear but as thick, vertical lines and not multiple single spikes in the corresponding segment in Panel B - 18ºC. Stimuli are indicated above the spike train as output of the thermode from the Peltier device for temperature stimuli and square wave signal from the foot pedal for mechanical stimuli.

**Figure 3: Primary event rate (left column), Burst rate (middle column), and Burst rate/primary event rate (right column) for the responses to stimuli overall.** For the primary event rate (left column) the two-way ANOVA was significant for stimulus response (B) and for interaction of neuronal response X stimulus response (C), but not by neuronal response (A). The ANOVA statistics were as follows: neuronal response (F=2.0, df=1, p=0.162), stimulus response (F=13.1, df=2, p<0.001) and interaction (F=8.5, df=2, p<0.001).

Similarly, the burst rate (middle column) showed significance for stimulus response (B), and interaction (C) but not for neuronal response (A). The ANOVA statistics were as follows: neuronal response, (F=0.1, df=1, p=0.811), stimulus response (F=7.7, df=2, p<0.001), and the interaction term (F=3.1, df=2, p=0.045).

The ratio of burst rate/primary event rate (right column) in response to overall stimuli showed significance for stimulus response (B), but for neuronal response (A) or interaction (C). For the ratio the ANOVA statistics were as follows: stimulus response (F=15.7, df=2, p<0.001), neuronal response (F=1.2, df=1, p=0.275), interaction (F=2.4, df=2, p=0.093).
Figure 4: Rate indices for responses to optimal stimuli. The primary event rate (left row), burst rate (middle), and ratio (right) in response to optimal stimuli varied significantly only by stimulus response (B). Conventions are as in Figure 3.

Two-way ANOVA statistics were as follows: 1) for primary event rate stimulus response (F=5.7, df=2, p=0.004), neuronal response (F=0.2, df=1, p=0.687), and interaction (F=0.5, df=2, p=0.614), 2) for burst rate: stimulus response (F=15.3, df=2, p<0.001), neuronal response (F=0.0, df=1, p=0.911), and interaction (F=0.1, df=2, p=0.872), 3) for the ratio, stimulus response (F=14.3, df=2, p<0.001), neuronal response (F=0.0, df=1, p>0.932), and interaction (F=0.6, df=2, p=0.56).

Figure 5: Preburst ISI/inverse of the primary event rate ratio for stimuli overall.

Panel A. Ratio of Preburst inhibition/inverse of primary event rate by the seven neuronal categories for Stimuli Overall (left column) and optimal stimuli (right column). The arrows indicate categories for which the firing rates were significantly greater (↑) than other neuron types (↓) in the post hoc analysis. Those categories without an arrow are not significantly different from any other category.

The left column shows the results of analysis of the ratio of preburst ISI/inverse of the primary event rate for stimuli overall. The right column shows that the ratio for optimal stimuli was significantly, which was dependent on neuronal category (A-right panel, one-way ANOVA - F=3.6, df=6, p=0.003).

Panel B. the two-way ANOVA for stimuli overall (left) showed a significant interaction but no significant main effects as follows: stimulus response (F=1.7, df=2,
p<0.0187), and neuronal response (F=0.1, df=1, p<0.715), and interaction (F=12.2, df=2, p<0.001). The two-way ANOVA for optimal stimuli (right) showed significant interaction (B-right panel - F=5.3, df=2, p=0.006), but no significant effects.

**Figure 6: Preburst ISI by NEURONAL CATEGORY (A) and neuronal response (B).**

Panel A. the preburst ISI for stimuli overall (left) and optimal stimuli (right) were not significantly different between any of the seven neuronal categories. Results of the one-way ANOVA on neuronal category are as follows: stimuli overall: F=1.8, df=6, P=0.11, optimal stimuli: F=0.3, df=6, P=0.93.

The Preburst ISI by Stimuli Overall showed significance for neuronal response but by neither stimulus response nor interaction. The Preburst ISI by optimal stimuli showed no significance in this analysis. Statistics for stimuli overall were stimulus response; F=1.1, df=2, P=0.58, neuronal response: F=5.2, df=1, P=0.02, and interaction: F=0.4, df=2, P=0.69. Statistics for optimal stimuli were stimulus response: F=0.5, df=3, P=0.58, neuronal response: F=0.2, df=1, P=0.65, and interaction: F=0.2, df=2, P=0.78.
Figure 1
Figure 2

A

B

Spontaneous activity

Large Clip

Medium Clip

42°C

45°C

24°C

18°C

1 sec

50 µV

50 msec

1 msec

Medium Clip

Large Clip

Spontaneous activity
Figure 3

(A) NEURONAL RESPONSE

(B) STIMULUS RESPONSE

(C) INTERACTION

* $p<0.05$  ** $p<0.01$  *** $p<0.001$
(A) NEURONAL RESPONSE

(B) STIMULUS RESPONSE

**p<0.01  ***p<0.001

Figure 4
(A) NEURONAL CATEGORY

Preburst interval/inverse of primary event rate

Overall

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<tr>
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<th>MRH</th>
<th>WDR</th>
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Mean±2.7SEM

Optimal

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</tbody>
</table>

Mean±2.7SEM

(B) INTERACTION

Mechanical | Cold | Heat
non graded | graded | graded

Preburst interval/inverse of primary event rate

Mean±SEM

p<0.001

p<0.05

Figure 5
Figure 6

(A) NEURONAL CATEGORY

(B) NEURONAL RESPONSE