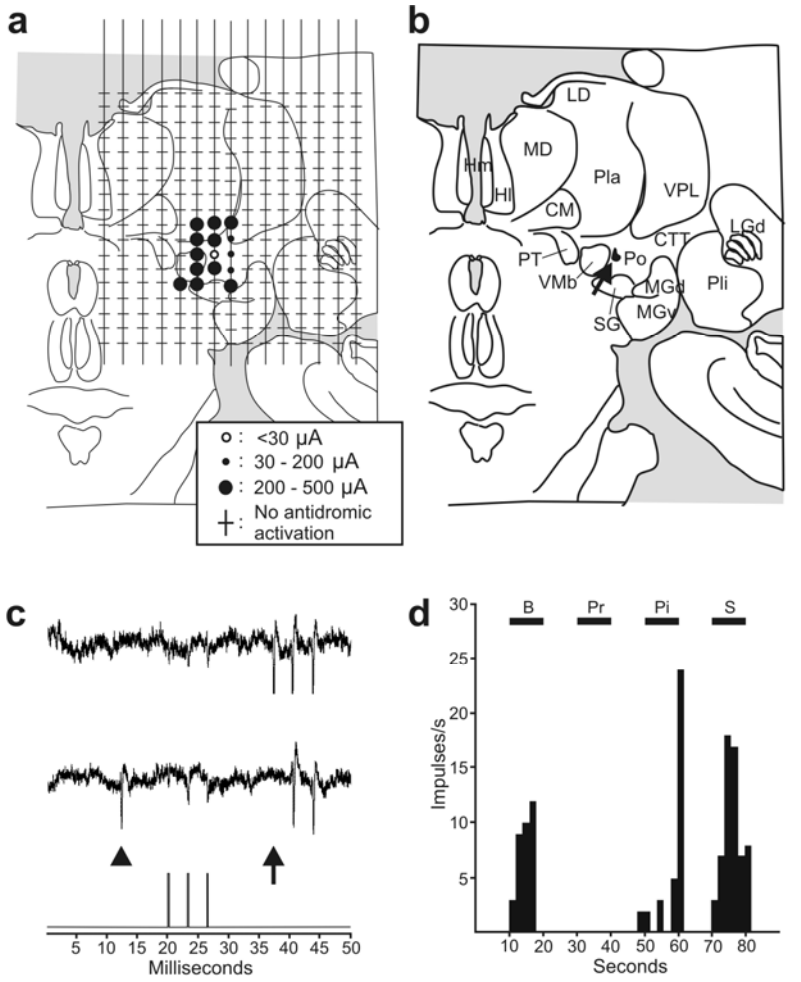
Relief of itch by scratching: state-dependent inhibition of primate spinothalamic tract neurons

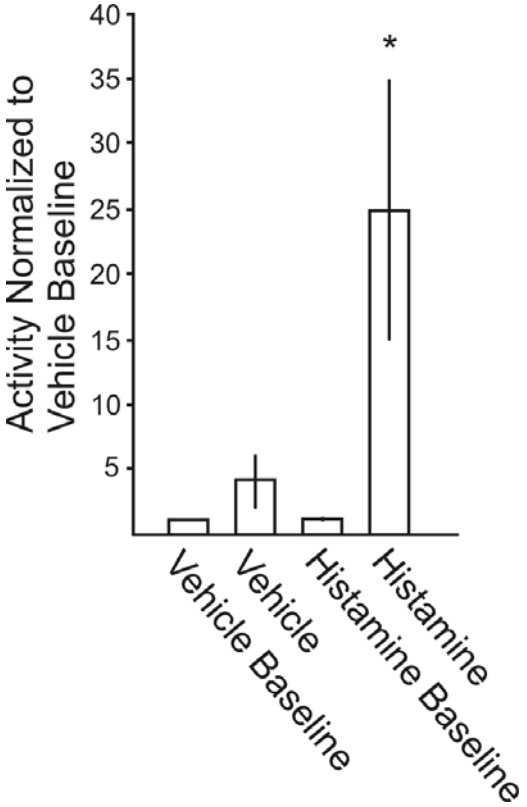
Steve Davidson, Xijing Zhang, Sergey G. Khasabov, Donald A. Simone, Glenn J. Giesler, Jr.

Supplemental Figure 1



Supplementary Figure 1: Antidromic activation and mechanical classification of the STT neuron in Figure 2a. a) Illustration of section through the thalamus where antidromic stimulation occurred. Within each track the current amplitude required to activate the neuron is indicated. b) The lesion site (arrow) was located in the posterior nucleus of the thalamus. c) This cell was activated by antidromic stimulation of 28 µA from this location (bottom trace) at a constant latency, followed a >300Hz train and an orthodromic action potential (arrow head) collided with an antidromic action potential (arrow). d) This cell was classified as wide dynamic range and responded to brushing of the receptive field (B), pinching (Pi) and squeeze of the skin with forceps (S). Pr = pressure. Bin width=2s. Abbreviations: CM, centre median n.; CTT, corticotectal tract; Hm, medial habenula; Hl, lateral habenula; LD, lateral dorsal n.; LGd, dorsal lateral geniculate; MD, medial dorsal n.; MGd, dorsal medial geniculate; MGv, ventral medial geniculate; Pla, anterior pulvinar; Pli, inferior pulvinar; Po, posterior n.; PT, pretectal n.; VMb, basal ventral medial n.; VPL, ventral posterior lateral n.

Supplemental Figure 2



Supplementary Figure 2: Magnitude of the response to histamine. In eight STT (7 wide dynamic range and one high threshold) neurons that met the criteria for a response to histamine (see Methods) the number of action potentials was summed from a 30 second period before (baseline) and a 30 seconds period immediately after an injection of saline (vehicle) or histamine. The data were normalized to the vehicle baseline level for each cell and the mean ± s.e.m. for each group is shown. Histamine injection produced a significant increase in mean activity compared with each of the other groups. One way repeated measures ANOVA with Tukey post-test; p=0.002.

Supplemental Methods

Animal Preparation

Macaca fascicularis were used in accordance with guidelines from the University of Minnesota. Monkeys were sedated initially with ketamine (10 mg/kg intramuscularly) and a catheter was placed in the median vein of the forelimb. Sodium pentobarbital (20 mg/mL) was given intravenously (IV) to produce a depth of anesthesia sufficient for intubation. Alpha chloralose (65 mg/kg IV) was administered and monkeys were placed on a feedback controlled heating pad and fixed in a stereotaxic frame. A mixture of sodium pentobarbital (3 – 10 mg/kg/hr), gallamine triethiodide (Flaxedil; 5 – 14 mg/kg/hr), and saline was delivered IV continuously with a pump for the duration of the experiment. A laminectomy was performed over the lumbar enlargement and a bilateral craniotomy (interaural 0 to 20 mm rostral and from the midline to 15 mm lateral, bilaterally) exposed the cortex over both thalami. The dura was opened over the exposed areas of the brain and spinal cord. The spinal cord was covered with warm mineral oil and the brain was covered with a mixture of mineral oil and petroleum jelly. Pneumothoraces were placed to reduce movement of the spinal cord. The monkey was artificially ventilated and CO_2 was monitored continuously along with arterial blood pressure, heart rate and body temperature.

Antidromic Technique

A low impedance recording electrode was placed into the thalamus (initial coordinates: interaural: +8.0 mm, midline: +8.0 mm, depth from cortical surface: -18.0 mm) in search of neurons within the ventral posterior lateral (VPL) nucleus that were activated by tactile stimulation of the contralateral hindlimb. Once the borders of VPL were determined, the electrode remained in place and the headstage was removed and replaced by the cathode from a stimulus isolator. The anode was placed in contact with the animal, which was grounded. A repeating square-wave pulse (search stimulus: 5 Hz, 200 μs, 500 μA) was delivered through the electrode for the stimulation of somatosensory thalamus.

During the search stimulation, a stainless steel recording electrode (epoxylite insulated, ~10 MΩ) was inserted through perforations in the pia-arachnoid and into the dorsal horn of the spinal cord. The recording electrode was repositioned until an action potential was found meeting the criteria for antidromic activation: constant latency from the stimulation, ability to follow a > 300 Hz train, and collision between an orthodromic and an antidromic action potential (Lipski 1981). The stimulus amplitude was then lowered to such a level that the action potentials were generated at a 50% success rate. This amplitude of current was recorded and the electrode then repositioned to determine the current thresholds around the axon. The most rostral position at which antidromic action potentials could be generated by ≤ 30 μA was determined. This amplitude has been demonstrated only to activate axons that are within 400 μm of the tip of the stimulating electrode (Burstein et al. 1991; Dado et al. 1994; Ranck 1975; Zhang et al., 2000a).

Functional Assessment

For each STT neuron, the receptive field location, size and shape were determined using innocuous mechanical stimuli. Mechanical sensitivity was determined during brushing of the receptive field with a soft bristled brush, pressure applied using an arterial clip, and pinch with a smaller, frankly painful arterial clip. Cells that did not respond to these mechanical stimuli were tested further with a more intense stimulus: squeezing the skin with forceps. Cells were classified as either high threshold (those that did not respond to innocuous stimuli), or wide

dynamic range (those that responded to both innocuous stimuli and, at higher frequencies, to more intense mechanical stimuli).

Scratching was performed with a metal edge dragged across the receptive field from proximal to distal. Scratches only contacted the skin in one direction and occurred at a frequency of ~2Hz. Two hand-held devices were used. Initially, a serrated coping blade (0.6mm thickness) was used with an applied force of 0.7-1.0 N. A second device was fashioned to approximate three, side-by-side monkey fingernails. Each "fingernail" had a thickness of 0.6mm and a width of 12mm and could be used independently or together at an applied force of 0.6N each. Both scratching instruments left superficial abrasions on the experimenters' skin that disappeared within a few minutes.

Histamine dihydrochloride (20 µg in 10 µL 0.9% saline; pH 5.0) or a pH matched vehicle was delivered intradermally through a 28 gauge needle (Simone et al., 2004). Every cell received two injections and the vehicle always preceded histamine. STT neurons were considered responsive to histamine when: 1) the rate of action potentials for >60 s after the injection increased ≥ 1.5 times the mean baseline frequency calculated during 30 s or 60 s immediately prior to the application and 2) outlasted any response to the vehicle by ≥ 60 s. Sensitivity to an intradermal injection of capsaicin (10 µg in 10 µL of 7% Tween-80 in normal saline) was determined using a 28 gauge tuberculin syringe. Action potentials were collected for one minute prior to the injection, and then until 10 minutes had elapsed after the injection of capsaicin.

Histology

At the end of the experiment, an electrolytic lesion was made at the stimulation site in the thalamus (15 µA, 45 s) and at the recording point in the spinal cord (25 µA, 20 s). Monkeys were perfused with 1 L of room temperature normal saline and then with 3 L of cold 10% formalin with 1% ferrocyanide to produce a Prussian blue reaction at the locations of the lesions. The brain and spinal cord were removed and placed in 10% formalin/1% ferrocyanide overnight and then blocked the next day. Sections were cut on a freezing microtome in 75 um or 50 um sections for the brain and spinal cord, respectively. The tissue was stained in neutral red and the sites of the lesions were identified.

Data Analyses

Analog voltage recordings were amplified, filtered (10 - 30K Hz), digitized at 33 kHz, and then wave-form discriminated and saved on a PC using DAPSYS software (www.dapsys.net). Statistical analyses were performed with SigmaPlot v10.0 (Systat Software, Inc). The pre-scratch period was defined as the 10 seconds before scratching and the post-scratch period was the 10 seconds immediately following scratching. The comparison between pre- and post scratching periods in the same cells was done with the Wilcoxon signed rank test and a p value of <0.05 was considered significant. To compare changes in activity between different groups of cells a one-way Kruskal-Wallis ANOVA was used followed by Dunn's post-test.

References

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