Nonlinear Processing of Tactile Information in the Thalamocortical Loop

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Ghazanfar, Asif A. and Miguel A. L. Nicolelis. Nonlinear processing of tactile information in the thalamocortical loop. *J. Neurophysiol.* 78: 506–510, 1997. Rats explore tangible objects in a manner such that, at any given moment in time, multiple facial whiskers simultaneously contact the surface of the object. Although both thalamic and cortical neurons responsible for processing such tactile information have large, multiwhisker receptive fields, it remains unclear what kinds of computations can be carried out by these neuronal populations when behaviorally relevant multiwhisker stimuli are used. By simultaneously recording the activity of up to 78 cortical and thalamic neurons per animal, we observed that the magnitude of sensory responses and the spatial spread of ensemble activity increased in a nonlinear fashion according to the extent and spatial orientation of the multiwhisker stimuli. Supralinear responses were seen more frequently with vertically than with horizontally oriented stimuli. These data suggest that thalamocortical interactions in the rat somatosensory system can generate complex spatial transformations of multiwhisker stimuli that go beyond the classic inhibitory interactions previously observed.

INTRODUCTION

Rats use their facial whiskers to explore their environment by making simultaneous and sequential multiwhisker contacts with object surfaces (Carvell and Simons 1990). It is of significant interest, therefore, to understand how the rat somatosensory system processes tactile information derived from multiwhisker displacements. Previous single-unit and optical imaging studies have indicated that sequentially activated, dual-whisker stimuli produce primarily inhibitory interactions whereby excitatory responses elicited by displacement of a whisker are suppressed if an adjacent whisker is previously displaced (Kleinfeld and Delaney 1996; Simons and Carvell 1989). These results have been obtained largely by stimulating in the center and immediate surround of the receptive field (RF) of layer IV ''barrel'' neurons or supragranular layer II/III neurons of the rat primary somatosensory cortex (SI). Furthermore, ventral posterior medial thalamic (VPM) neurons have been reported to show little, if any, multiwhisker integration following identical stimulation paradigms (Simons and Carvell 1989). These findings and the modular anatomy of the rodent trigeminal system have been used to support the notion that barrel cortical columns should be considered as single-whisker processing units (Kleinfeld and Delaney 1996; Simons and Carvell 1989). Here we investigate this issue further by comparing the responses of large populations of simultaneously recorded cortical and thalamic neurons following stimulation of single-whisker versus coincidentally displaced multiwhisker stimuli.

METHODS

Processing of tactile information was quantified by simultaneously recording the sensory responses of populations of layer V neurons, located in SI, and neurons located in VPM. Recordings were obtained 1 wk after chronic implantation of two electrode arrays, each of which contained 16 Teflon-coated stainless steel microwires (50 μm diam, NBlabs, Dennison TX). Electrode arrays consisted of two rows, separated by 1 mm, of eight microwires spaced 200 μm apart. This allowed $\sim$2 mm^2 of tissue to be sampled in each structure. During surgery, neural activity was recorded and RFs were mapped to ensure proper placement of electrodes in layer V of SI and in VPM. Histological analysis of Nissl-stained sections was used to confirm electrode placement for all animals. Real-time characterization of single neurons and recording of neuronal ensemble activity were carried out with the use of a multineuronal acquisition processor (MNAP, Spectrum Scientific, Dallas TX). Ensemble recordings were carried out in lightly anesthetized animals (pentobarbital sodium, 50 mg/kg ip) during discrete mechanical deflection of individual whiskers or whisker rows and columns. Overall, 209 cortical and 98 thalamic neurons were recorded in adult Long-Evans rats ($n = 9$). Discrete whisker deflections were produced by step pulses (100 ms in duration) at a frequency of 1 Hz. Statistical significance of sensory responses was assessed with the use of a one-way Kolmogorov-Smirnov test. The spread of cortical activity across the tissue sampled by our electrode arrays was quantified by counting the number of statistically significant responses in 2-ms epochs from 0 to 38 ms poststimulus time. The mean number of significantly responding neurons per epoch was plotted in a cumulative plot. Pairwise comparison (*t*-test; confidence interval set at 2%) was used to test the significance of differences observed at each epoch. All these methods have been described in detail elsewhere (Nicolelis and Chapin 1994; Nicolelis et al. 1997).

RESULTS

We found that both cortical and thalamic neurons could be robustly driven by simultaneous deflection of three whiskers, despite responding weakly or not at all to stimulation of each individual whisker (Fig. 1*a*). Because it has been shown that both thalamic and cortical neurons have large RFs (Chapin 1986; Nicolelis and Chapin 1994), this suggests that the supralinear summation did not occur in the center RFs of these neurons, but in the far surround. When the sensory responses were plotted as a function of the spatial orientation of the stimulus, we observed that 60% of all SI neurons exhibited nonlinear summation (sum $>$ 100%) to the stimu-

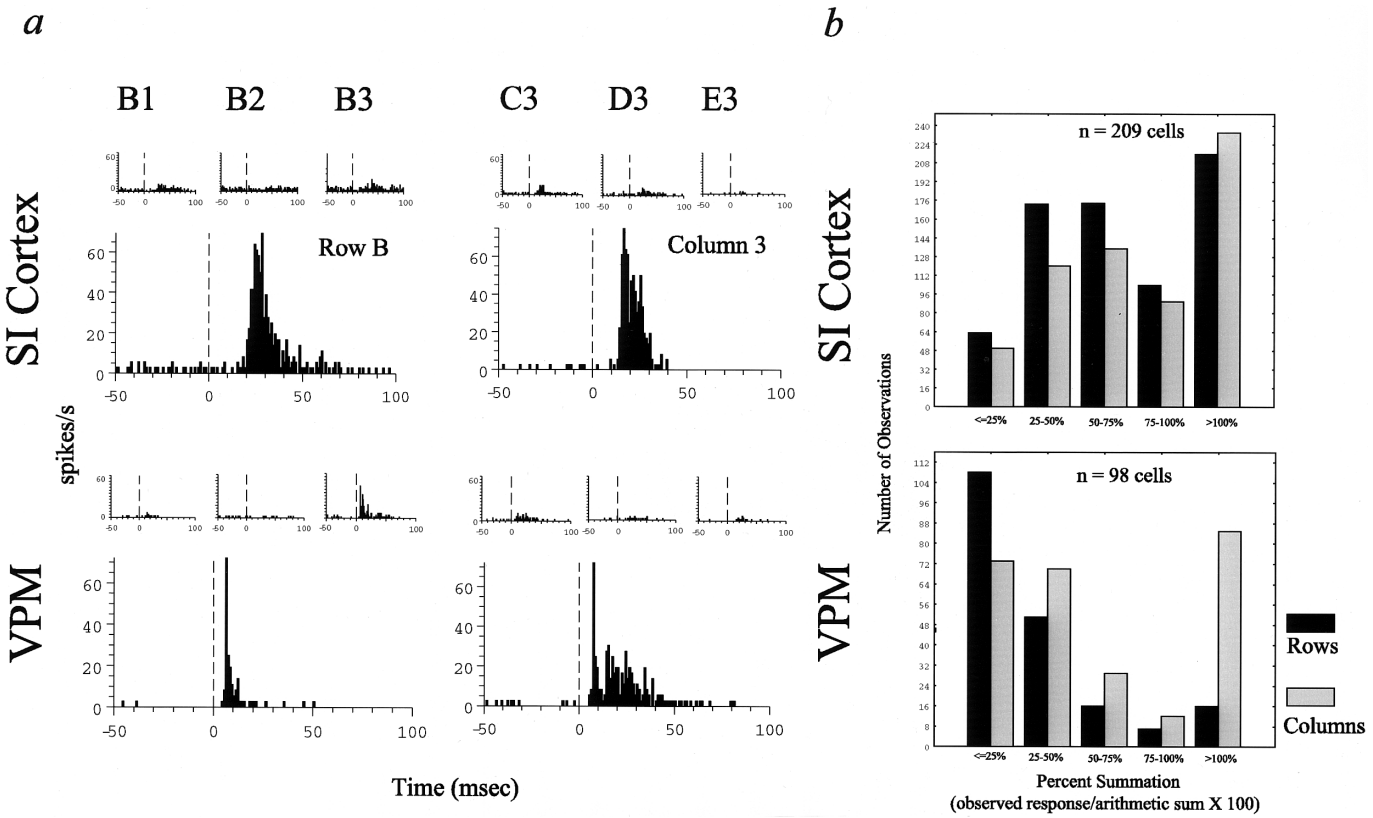

FIG. 1. *a*: poststimulus time histograms of 4 different neurons demonstrating that stimulation of individual whiskers (B1–B3, representing a row of whiskers, or C3, D3, and E3, representing a column of whiskers), elicits little or no firing. Nevertheless, simultaneous stimulation of 3 whiskers, in either rows or columns, generated a robust neuronal response that was greater than the arithmetic sum of the individual whisker responses. *b*: comparison of frequency of occurrence of nonlinear summation following row or column stimulation in primary somatosensory cortex (SI) and ventral posterior medial thalamus (VPM). Each neuron was presented with 5 row stimuli and 4 column stimuli. Histogram depicts number of observations for a range of interactions: supralinear responses are >100%, inhibitory interactions are <100%.

lation of whisker rows (i.e., horizontally oriented stimuli) and 63% exhibited nonlinear summation to the stimulation of whisker columns (i.e., vertically oriented stimuli; Fig. 1*b, top*). Interestingly, only 15% of thalamic neurons showed nonlinear summation for whisker row stimuli, whereas 49% displayed nonlinear summation to column stimuli (Fig. 1*b, bottom*); thus thalamic neurons showed a bias toward column stimuli that was not seen in SI. Maximal inhibitory interactions in VPM (that is, multiwhisker responses that are less than the arithmetic sum of individual whisker responses) were observed primarily with whisker row stimuli ($\leq$25%, Fig. 1*b*). These results demonstrated that the nonlinear summation effect observed here depends both on the spatial extent (single vs. multiple whiskers) and the spatial orientation (rows vs. columns) of a given tactile stimulus. Overall, up to 81% of the layer V cortical neurons and 49% of the thalamic cells showed sensory responses to multiwhisker stimuli that exceeded the arithmetic sum of the responses to individual whisker stimulation.

Next we assessed the integration of multiwhisker deflections at the thalamocortical ensemble level by reconstructing the spatiotemporal spread of activation across the same simultaneously recorded neurons following single-and multiwhisker stimuli. Figure 2 depicts a typical example of the results obtained with this analysis in which color-coded maps were used to represent the magnitude and spatiotemporal spread of neuronal activation across the tissue sampled by each electrode array. In this example, stimulation of a single whisker (Fig. 2*a*) induced neuronal responses that were first observed in the thalamus 6–8 ms after the stimulus onset and in SI at 14–16 ms. Once the neuronal activation reached the cortex, it spread horizontally, in many directions, across the infragranular layers. Multiwhisker stimuli, however, produced a very different pattern of thalamocortical activation (Fig. 2*b*). When a column of whiskers (which included the single whisker deflected in Fig. 2*a*) was stimulated, sensory responses spread faster and recruited more of the thalamocortical ensemble than when any single whisker belonging to that particular column was stimulated (compare Fig. 2, *a* and *b;* see Fig. 3*a*). Indeed, for whisker column stimulation, the resulting spatiotemporal wave in SI could not be predicted by the simple arithmetic summation of the responses obtained by stimulating each of the individual whiskers (Fig. 3*a*). Statistical analysis (*t*-test) revealed significant differences, starting at 12 ms, between the amount of cortical activation following whisker column stimuli versus the arithmetic sum of individual whiskers ($P < 0.02$). Moreover, multiwhisker responses displayed shorter latencies than sin-

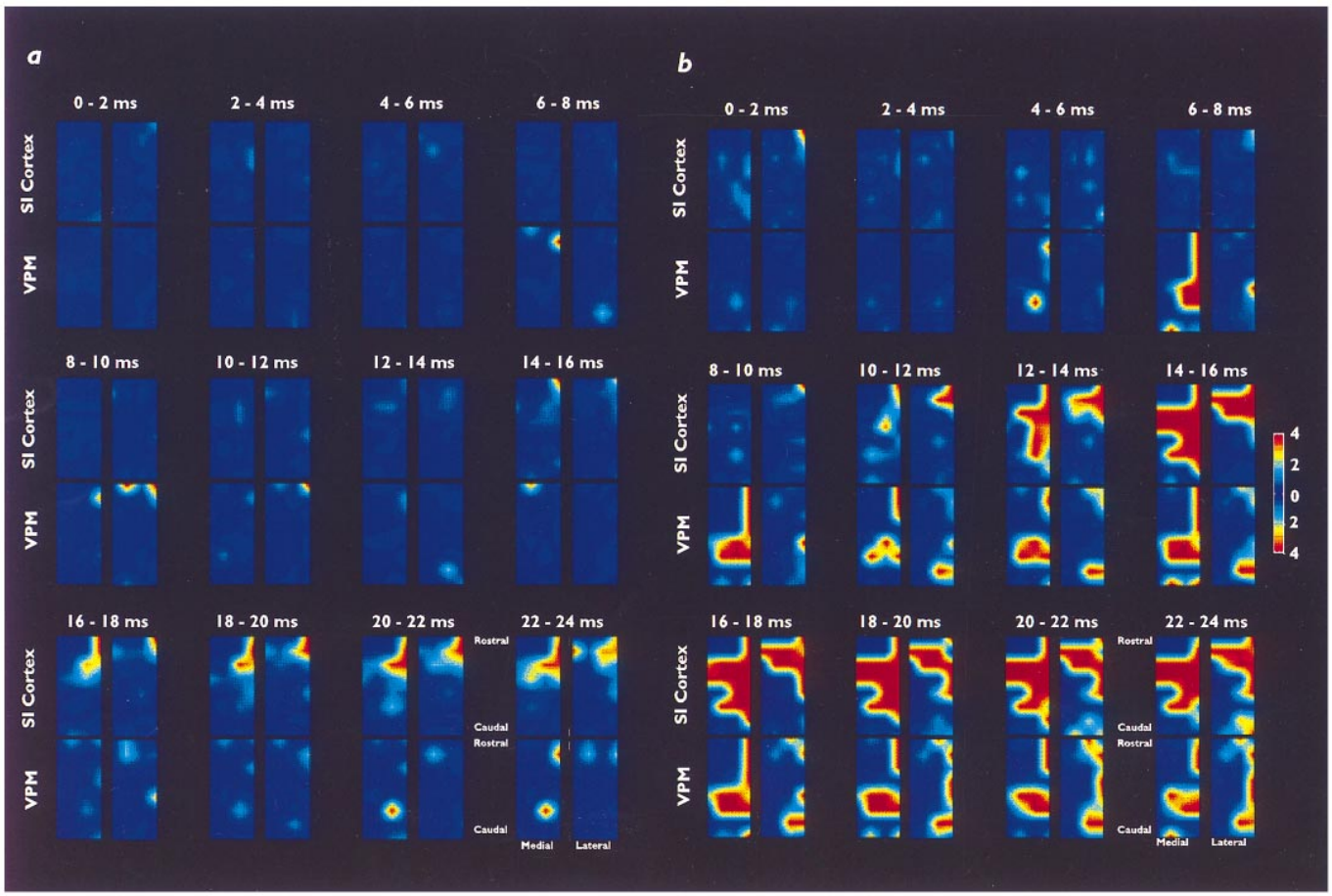

FIG. 2. Color-coded 3-dimensional matrices were used to represent poststimulus firing of neurons in VPM and SI neurons according to their location on the 2 × 8-electrode arrays implanted in each of these structures. Data were plotted on consecutive 2-ms time epochs depicted atop each matrix. In each electrode array (represented by 2 panels plotted side by side and separated by an empty space), *X*-axis represents mediolateral position (left: medial) of neurons in the recording probe; *Y*-axis represents rostrocaudal position (top: rostralmost wires 1 and 9; down: caudalmost wires 8 and 16); and *Z*-axis, plotted in a color gradient, represents variation in neuronal response magnitude (dark red: >4 SD of spontaneous firing rate; dark blue: baseline firing rate). All sensory responses were extracted from poststimulus time histograms obtained after 360 stimulation trials. *a*: spatiotemporal activation of thalamocortical ensembles following stimulation of whisker D3. *b*: spatiotemporal activation of same set of neurons following simultaneous stimulation of whiskers C3, D3, and E3.

gle-whisker responses. The spread of cortical activity, however, was not significantly different between whisker row stimuli versus the arithmetic sum of individual whisker stimulation (Fig. 3*b*).

DISCUSSION

The above results demonstrate that individual VPM and layer V SI neurons are capable of nonlinear summation of responses following multiwhisker stimuli. The spatial extent and sometimes the orientation of the tactile stimulus was fundamental in shaping the magnitude and pattern of activity across the thalamocortical pathway. In addition, we also observed that the spatial spread of cortical activation also increased nonlinearly with whisker column stimuli and it was not limited to the barrel columns isomorphic to the displaced whiskers.

The finding that multiple-whisker stimulation can elicit nonlinear excitatory summation responses in both VPM and SI neurons may seem to be at odds with previous studies demonstrating little or no multiwhisker excitatory integration in these structures (Kleinfeld and Delaney 1996; Simons and Carvell 1989). However, there are several reasons why these results are not mutually exclusive. First, for both thalamic and cortical neurons, previous studies focused on integration around the center of the neurons' RFs, whereas in the present study nonlinear summation was largely observed in the far surround of the RF. Second, for cortical neurons, past studies have focused either on layer IV or layer II/III neurons, whereas results presented here were from layer V neurons, which are known to have much larger RFs with ill-defined centers (Chapin 1986). Last, our multiple whisker stimulation included three coincidentally displaced whiskers as opposed to sequentially displaced, dual-whisker stimulation. In this context, our results do not support the hypothesis that barrel cortical columns are ''single-whisker processing units.''

Recent anatomic studies of the rodent somatosensory system may provide a clue for the differential nonlinear effects

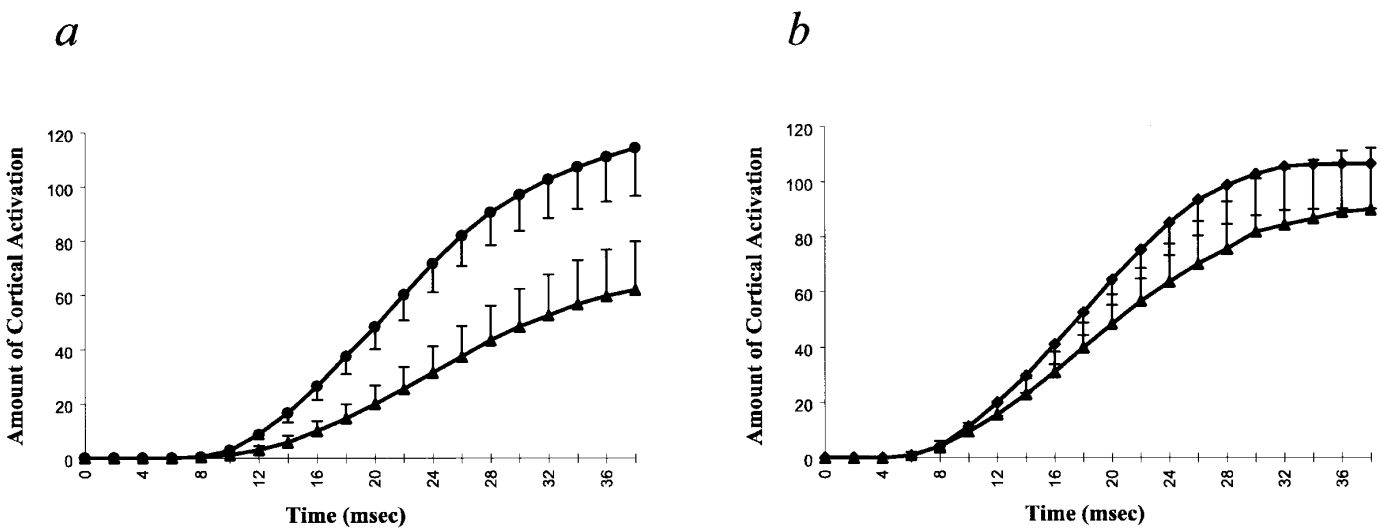

FIG. 3. Cumulative plots depicting amount of cortical activation predicted by linear summation of sensory responses obtained by stimulating each of 3 individual whiskers (▲), and amount of cortical activation obtained experimentally by stimulating all 3 whiskers simultaneously. Amount of activation was quantified by counting number of neurons with statistically significant responses at each time point (every 2 ms), and each point in this plot represents mean ± SE obtained with the use of 7 animals. *a*: amount of cortical activation for whisker column deflections (●) vs. summated, individual whisker deflections (▲). Statistically significant differences (*t*-test, $P < 0.02$) were observed from 12 ms on. *b*: amount of cortical activation for whisker row deflections (♦) versus summated, individual whisker deflections (▲).

observed by us in the thalamus. Corticothalamic projections from the rat SI have been shown to terminate in a distinct topographic manner in VPM and in the reticular nucleus (RT) of the thalamus. Glutamatergic axons (Salt and Eaton 1996) originating in the underlying infragranular layers (V and VI) of one cortical barrel column terminate precisely in regions of VPM representing the entire whisker column, including the vibrissa represented by the cortical barrel (Bourassa et al. 1995; Hoogland et al. 1987). Conversely, collaterals of the same axons terminate along the entire whisker row representation in the RT (Hoogland et al. 1987). Individual GABAergic RT neurons project to a single VPM barreloid (Pinault et al. 1995). This overall pattern of connectivity is consistent with our physiological observations. First, the observed strong bias toward nonlinear summation of responses for whisker column stimuli in VPM is consistent with the anatomic bias of the excitatory corticothalamic projections. Second, maximum inhibition was observed in VPM following row stimuli, a finding that is supported by the rowlike pattern of termination of corticothalamic fibers in the RT neurons and the inhibitory projections between RT neurons and VPM barreloids. Our interpretation of the current findings in the rat somatosensory thalamocortical pathway is in agreement with data obtained in other sensory systems (visual: Murphy and Sillito 1987; auditory: Yan and Suga 1996) and argues in favor of the hypothesis that the corticothalamic projection performs similar computational tasks, i.e., enhancement of spatial contrast or modulation in neuronal tuning, in different sensory systems.

The tangential spread of activity in layer V quantified in our study does not rule out the possibility that a similar spread of activity occurs in the supragranular layers. Nevertheless, the finding that whisker column stimulation results in greater cortical activation in layer V than whisker row stimulation is intriguing because anatomic studies have demonstrated a bias of horizontal cortical connectivity between cortical columns belonging to a whisker row in both the supra- and infragranular layers (Bernardo et al. 1990), and a whisker row bias has been confirmed physiologically for the supragranular layers (Kleinfeld and Delaney 1996). Perhaps the nonlinear excitatory summation responses in VPM for whisker columns are reverberated back up to SI, resulting in further recruitment of cortical tissue. Because whisker columns are likely to be coincidentally deflected during active tactile exploration by rats, our results indicate that response magnitude and spatial spread of activation across the thalamocortical loop may vary nonlinearly, on a millisecond scale, as rats sweep their whiskers across an object surface.

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REFERENCES

BERNARDO, K. L., MCCASLAND, J. S., WOOLSEY, T. A., AND STROMINGER, R. N. Local intra- and interlaminar connections in mouse barrel cortex. *J. Comp. Neurol.* 291: 231–255, 1990.

BOURASSA, J., PINAULT, D., AND DESCHENES, M. Corticothalamic projections from the cortical barrel field to the somatosensory thalamus in rats: a single-fibre study using biocytin as an anterograde tracer. *Eur. J. Neurosci.* 7: 19–30, 1995.

CARVELL, G. E. AND SIMONS, D. J. Biometric analyses of vibrissal tactile discrimination in the rat. *J. Neurosci.* 10: 2638–2648, 1990.

CHAPIN, J. K. Laminar differences in sizes, shapes, and response profiles of cutaneous receptive fields in the rat SI cortex. *Exp. Brain Res.* 62: 549–559, 1986.

HOOGLAND, P. V., WELKER, E., AND VAN DER LOOS, H. Organization of

the projections from the barrel cortex to thalamus in mice studied with *Phaseolus vulgaris*-leucoagglutinin and HRP. *Exp. Brain Res.* 68: 73–87, 1987.

KLEINFELD, D. AND DELANEY, K. Distributed representation of vibrissa movement in the upper layers of somatosensory cortex revealed with voltage-sensitive dyes. *J. Comp. Neurol.* 375: 89–108, 1996.

MURPHY, P. AND SILLITO, A. M. Cortical feedback influences the generation of length tuning in the visual pathway. *Nature Lond.* 329: 727–729, 1987.

NICOLELIS, M.A.L. AND CHAPIN, J. K. Spatiotemporal structure of somatosensory responses of many-neuron ensembles in the rat ventral posterior medial nucleus of the thalamus. *J. Neurosci.* 14: 3511–3532, 1994.

NICOLELIS, M.A.L., GHAZANFAR, A. A., FAGGIN, B., VOTAW, S., AND OLIVEIRA, L.M.O. Reconstructing the engram: simultaneous, multiple site, many single neuron recordings. *Neuron* 18: 529–537, 1997.

PINAULT, D., BOURASSA, J., AND DESCHENES, M. The axonal arborization of single thalamic reticular neurons in the somatosensory thalamus of the rat. *Eur. J. Neurosci.* 7: 31–40, 1995.

SALT, T. E. AND EATON, S. A. Functions of ionotropic and metabotropic glutamate receptors in sensory transmission in the mammalian thalamus. *Prog. Neurobiol.* 48: 55–72, 1996.

SIMONS, D. J. AND CARVELL, G. E. Thalamocortical response transformation in the rat vibrissal/barrel system. *J. Neurophysiol.* 61: 311–330, 1989.

YAN, J. AND SUGA, N. Corticofugal modulation of time-domain processing of biosonar information in bats. *Science Wash. DC* 273: 1100–1103, 1996.