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"Induction of immediate spatiotemporal changes in thalamic networks by peripheral block of ascending cutaneous information"

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Induction of immediate spatiotemporal changes in thalamic networks by peripheral block of ascending cutaneous information

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PERIPHERAL sensory deprivation induces reorganization within the somatosensory cortex of adult animals[1–6]. Although most studies have focused on the somatosensory cortex[1–6], changes at subcortical levels (for example the thalamus) could also play a fundamental role in sensory plasticity[7–11]. To investigate this, we made chronic simultaneous recordings of large numbers of single neurons across the ventral posterior medial thalamus (VPM) in adult rats. This allowed a continuous and quantitative evaluation of the receptive fields of the same sample of single VPM neurons per animal, before and after sensory deprivation. Local anaesthesia in the face induced an immediate and reversible reorganization of a large portion of the VPM map. This differentially affected the short latency (4–6 ms) responses (SLRs) and long latency

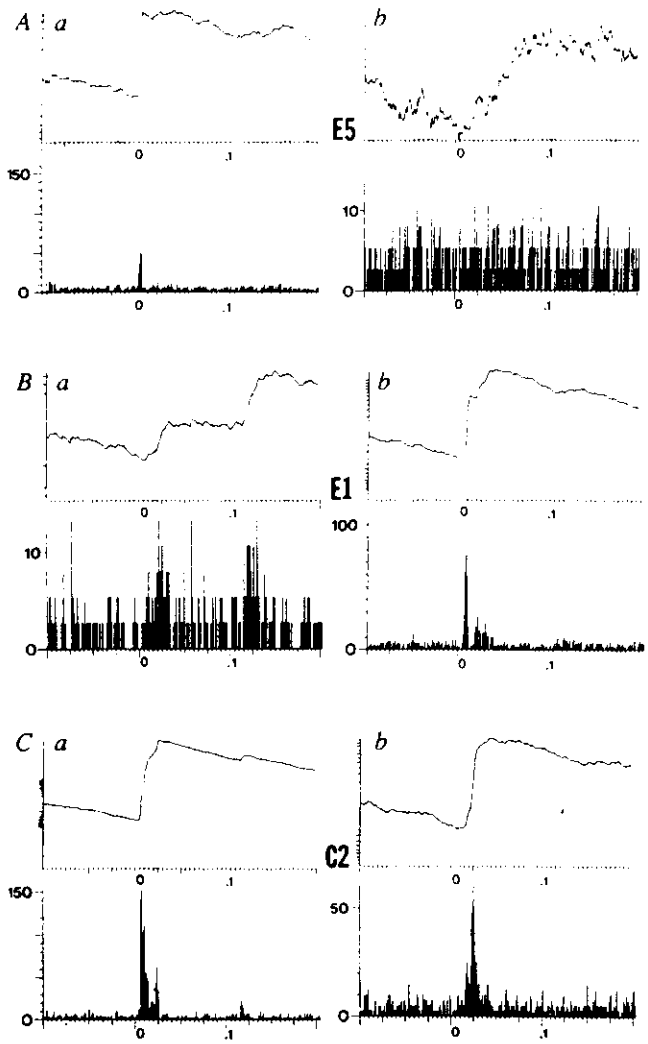

FIG. 1 Local anaesthesia of the rostral face alters the sensory responses of three simultaneously recorded neurons in the VPM of a pentobarbital anaesthetized rat. For each cell, cumulative frequency histograms (CFHs; above) and post-stimulus time histograms (PSTHs; below) show the magnitude and timing of different latency responses of these neurons to 360 single stimulations of different whiskers (E5, E1 and C2) before (*Aa–Ca*) and after (*Ab–Cb*) subcutaneous lidocaine injection in the maxillary gum. Response magnitudes, in average instantaneous firing rate per bin (in spikes per s) are shown on the vertical axes of PSTHS. The scales are different for each PSTH to highlight the effects. The vertical dimension in CFHs is used to plot the deviation of the cumulative firing above or below the average firing rate. Scales on the vertical axes of CFHs show negative log *P*-values (for example at the first scale mark $P=0.1$, at the second, $P=0.01$ and so on). These show the probability that the overall distribution of cumulative frequencies differs from a random distribution, as computed using a one-way Kolmogorov-Smirnov test. Thus, the highly significant response in *A* ($P<10^{-5}$) became insignificant ($P>0.1$) after the lidocaine injection. Horizontal axis: pre- and post-stimulus time in s; whisker deflection up at 0, down at 0.1 s; bins = 1 ms, scale marks = 5, 25 and 100 ms.

METHODS. Experiments were done on six adult Long-Evans (hooded) rats. One week before experimentation bundles of 8–16 teflon-coated stainless steel 25 or 50 μm tip microwires were surgically implanted in the VPM thalamic nucleus. Up to 23 single VPM neuronal signals were simultaneously amplified, filtered and discriminated using an apparatus from Spectrum Scientific (Dallas, TX). Each single extracellular spike waveform was time-voltage window discriminated, and statistically validated using principal components analysis. This ensured that the same set of single neurons was recorded throughout each experiment. Cutaneous sites were stimulated at a rate of 1 Hz, using a computer-controlled vibromechanical probe delivering 100 ms duration step pulse displacements (whiskers were moved 3°; furry skin was depressed 0.5 mm). For each experiment, 11–20 single whiskers or skin sites were independently stimulated in a random sequence.

(15–25 ms) responses (LLRs) of single VPM neurons. The SLRs and LLRs normally define spatiotemporally complex receptive fields in the VPM[12]. Here we report that 73% of single neurons whose original receptive fields included the anaesthetized zone showed immediate unmasking of SLRs in response to stimulation of adjacent cutaneous regions, and/or loss of SLRs with preservation or enhancement of LLRs in response to stimulation of regions just surrounding the anaesthetized zone. This thalamic reorganization demonstrates that peripheral sensory deprivation may induce immediate plastic changes at multiple levels of the somatosensory system. Further, its spatiotemporally complex character suggests a disruption of the normal dynamic equilibrium between multiple ascending and descending influences on the VPM.

To investigate whether peripheral deprivation produces immediate reorganization of sensory maps in subcortical structures such as the thalamus, we developed neurophysiological techniques allowing chronic simultaneous recordings of large numbers of single neurons distributed across topographic representations in sensory nuclei. This approach overcame many limitations of the classic mapping technique because it allowed: (1) the same neurons to be recorded before and after sensory deprivation, (2) a precise quantitative definition of their spatiotemporal response properties, and (3) a simultaneous sampling of short- and long-term neuronal changes occurring across the whole sensory representation embedded in the nucleus. This new approach was used to demonstrate that the map of the face in the VPM thalamus of adult rats is immediately reorganized following reversible peripheral sensory deprivation induced by small (0.01–0.04 ml) subcutaneous injections of lidocaine (1%). This reorganization was manifested by marked changes in the spatiotemporal response properties of single VPM neurons to stimulation of facial locations within the fully and partially anaesthetized zones, and also their immediate surrounds.

Figure 1 illustrates typical spatiotemporal modifications observed within an ensemble of single thalamic neurons following a small (0.04 ml) injection of lidocaine in the gum just behind the maxillary incisors in a pentobarbital anaesthetized (50 mg kg^{-1} intraperitoneally) adult rat. Before this injection, most of the total 11 neurons that were simultaneously recorded in this rat responded robustly to computer controlled vibromechanical stimulation of up to 16 whiskers across the face, including whisker E5 (as in Fig. 1*Aa*). After the injection, these responses to E5 were completely blocked (Fig. 1*Ab*), thus defining this rostral whisker as part of the zone anaesthetized by the lidocaine spread. By contrast, stimulation of more caudally placed whiskers (for example, whisker E1) yielded strong short latency (5–10 ms) responses in many neurons that had previously been unresponsive at that latency (Fig. 1*Ba, b*). Such complementary changes in response magnitudes produced a spatial shift of single neuronal receptive fields away from the anaesthetized zone towards surrounding unanaesthetized regions.

Beyond these changes in the spatial domain, the focal anaesthesia also altered the normal latency distribution of sensory responses of 10 neurons within the ensemble. For example, the neuron in Fig. 1*Ca* normally responded at both short (5–10 ms) and long (15–25 ms) latencies to stimulation of whisker C2. Lidocaine selectively blocked the short latency response, thus shifting the temporal profile of the post-stimulus time histograms (see Fig. 1*Cb*).

The statistical significance of the sensory-evoked responses in these neurons was routinely assessed using: (1) the Kolmogorov–Smirnov (KS) test, which estimates the deviation from randomness of the cumulative frequency distribution, and (2) analysis of variance (ANOVA), which determines whether sensory-evoked responses in specific time epochs are significantly different from spontaneous discharge. All of the effects reported here were found to be highly significant ($P<0.01$). The deprivation-induced changes occurred immediately (within about 3 minutes) after the lidocaine injection and lasted 4–6 hours. In all cases in which the neurons were re-recorded 12–24

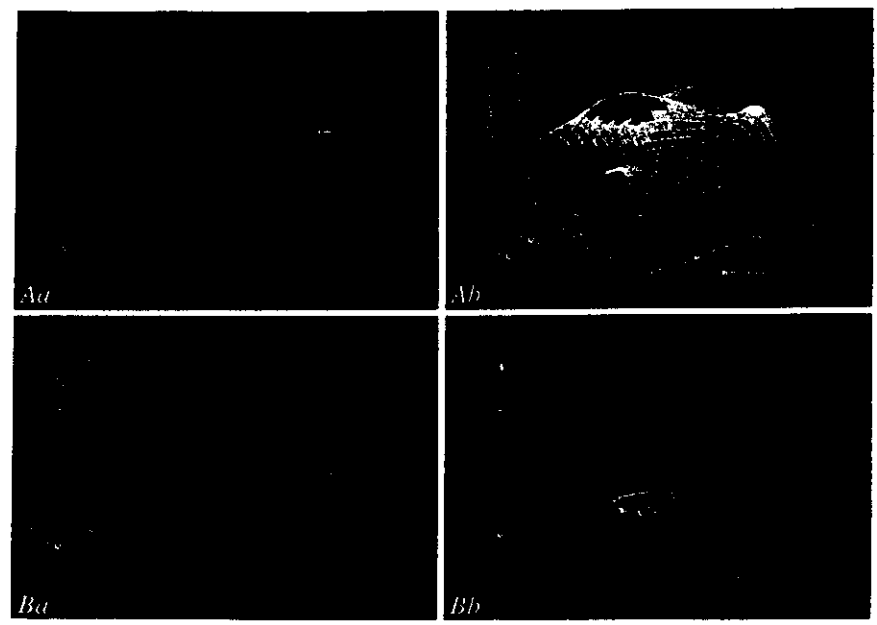

FIG. 2 Spatiotemporal receptive fields of VPM neurons are altered by lidocaine anaesthesia of the rostral face. The colour-coded three-dimensional surfaces here define the spatiotemporal receptive fields of two VPM neurons before (*Aa* and *Ba*) and after (*Ab* and *Bb*) a lidocaine injection in the maxillary gum of an adult rat. These were constructed by sequentially stimulating 11 whiskers 360 times apiece, and measuring average instantaneous evoked firing rate during each of eight different post-stimulus time epochs (shown on the left horizontal axis, ranging from 0 to 35 ms). The responses for each stimulated whisker were rank ordered according to their relative caudal-to-rostral position in the whisker pad (ordered from 1–11 as follows along the right horizontal axis: B1, E1, E2, C1, C2, D2, E3, C3, E4, E5 and E6. Response magnitudes are shown in Hz (spikes per s) on the vertical axis. The range of response magnitudes was coded by eight colours, in which red represents the greatest firing rate and dark blue the lowest. All colour codes were automatically scaled according to the vertical range, and thus vary from graph to graph. Actual data points are represented by white open circles whose distances from the spline smoothed surface are indicated by vertical dotted lines. All of the major topographical features shown here were shown to be significant ($P < 0.01$) using the ANOVA and KS tests.

hours after the lidocaine injections their receptive fields were found to be similar to pre-lidocaine controls.

The major conclusion to be derived from the above analyses of PSTHs is that peripheral sensory deprivation disrupts both spatial and temporal features of thalamic neuronal responses. To visualize how the responses seen in PSTHs (as in Fig. 1) contribute to the definition of single cell receptive fields, we used three-dimensional computer graphics to construct 'spatiotemporal receptive fields', which depict neuronal response magnitude (in spikes per s) as a function of stimulus position and post-stimulus time. For this, quantitative measurements from the PSTHs were used to generate a three-dimensional surface, which was smoothed using a spline algorithm based on a moving third-order polynomial function. To reduce the impact of statistical outliers, a low 'stiffness' coefficient (0.15) was used.

Figure 2 shows spatiotemporal receptive fields of two typical neurons before (Fig. 2*Aa*, *Ba*) and after (Fig. 2*Ab*, *Bb*,) the same lidocaine injection as in Fig. 1. These quantitative representations revealed that receptive fields in the VPM are much larger and spatiotemporally complex[12] than reported[13,15]. Lidocaine induced two general categories of receptive field reorganization in the VPM: (1) temporal shifts (as in Fig. 2*Aa*, *b*) and (2) spatial shifts (as in Fig. 2*Ba*, *b*). Figure 2*Aa* depicts a receptive field that covers a wide area of the whisker pad during both short and long latency post-stimulus time epochs. After the lidocaine injection, this receptive field changed dramatically (Fig. 2*Ab*). At short latencies, the centre of the receptive field disappeared, while its surround was enhanced at both edges. By contrast, the long latency peak remained almost intact, though somewhat prolonged. Thus, the main effect of the deprivation was to shift temporally the main response of this neuron from short to long latency epochs.

By contrast, Fig. 2*Ba*, *b* shows an example of a predominantly spatial shift. Lidocaine-induced sensory deprivation produced a marked spatial shift in the short latency receptive field peak of this neuron from its original location in the rostral whiskers (C3 and E3–E5) to the caudal whiskers (B1, C1 and E1–E2). In addition, two weak long latency response peaks emerged. Similar patterns of reorganization were observed in each of eight equivalent experiments, involving chronic recordings from a

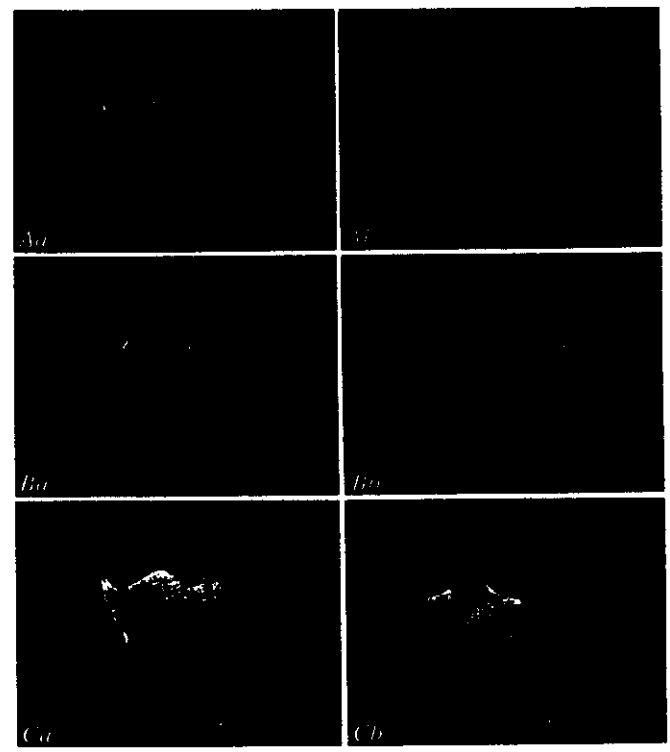

FIG. 3 Population receptive fields of VPM neuronal ensembles are altered by lidocaine anaesthesia in the rostral face. Each colour-coded three-dimensional surface here represents the sensory-evoked responses of an ensemble of 11 simultaneously recorded VPM neurons to stimulation of each of 11 whiskers (same as in Fig. 2) within each of three post-stimulus time epochs (5–10, 10–15 and 25–35 ms). *Aa–Ca* show the population receptive fields recorded during control. *Ab–Cb* show the same population receptive fields following the lidocaine injection. The vertical axis, right horizontal axis, and the colour coding of the three-dimensional surfaces are the same as in Fig. 2. The 11 neurons that comprise the left horizontal axis were rank ordered according to the relative position of their receptive field centres and their overall sensory responsiveness.

total of 75 VPM neurons in six rats. Of the 75 neurons, 55 had receptive fields within the anaesthetized zone and/or its immediate surround. Of these, 30 cells (55%) exhibited the temporal shifts (category 1, as in Fig. 2*Aa*, *b*), and 27 cells (49%) exhibited the spatial shifts (category 2, as in Fig. 2*Ba*, *b*). These effects were partially overlapped, such that both were observed in 17 cells (31%). Finally, 11 cells did not change, and 4 cells were completely anaesthetized.

The next step was to determine how the spatiotemporal complexity of the lidocaine effects on the receptive fields of single neurons was translated into changes in the sensory map embedded in the neuronal population. Three-dimensional graphics were used to construct population receptive fields, which depict, within given post-stimulus time epochs, the magnitudes of sensory responses across the population of recorded neurons as a function of stimulus location. The population receptive fields in Fig. 3*Aa–Ca* show how the spatiotemporal receptive fields of 11 simultaneously recorded single neurons were integrated into a sensory map which dynamically changes over post-stimulus time. The lidocaine produced the alterations shown in Fig. 3*Ab–Cb* by creating an anaesthetized zone in which short latency responses to stimulation of whiskers C3 and E3–E5 (7–10 in Fig. 3*Ab* and *Bb*) were blocked. This induced a spatial shift of the short latency responses toward 'far surround' whiskers B1, E1, and E6 (1, 2 and 11 in Fig. 3*Bb*), distal to the anaesthetized zone. Furthermore, long latency responses tended to remain, or newly appear, in 'near surround' whiskers E2, C1 and 2, and D2 (3–6 in Fig. 3*Cb*), more proximal to the anaesthetized zone. The net effect was to increase the relative strength of the longer latency responses which partially 'filled in' the area vacated by the short latency responses, conceivably providing a 'seed' for longer term reorganization.

These results demonstrate that local peripheral sensory deprivation induces, within minutes, a marked reorganization of the somatosensory map in the VPM thalamus. The purely spatial aspects of these receptive field shifts are reminiscent of the spatial reorganizations of the sensory map in the somatosensory cortex which were defined using classic mapping studies[1–3]. Moreover, they are quite similar to the immediate unmasking of cortical neuronal responsiveness observed after similar sensory deprivation models[4–6]. The results here, therefore, show that the short-term plastic reorganization in the somatosensory system is not restricted to the cerebral cortex, and provide direct quantitative evidence for previous suggestions that the thalamic map may be reorganized after different types of sensory deprivation[7–11,14,15]. Thus, the long-term reorganizations observed in the cortex could derive at least partially from changes at lower levels such as the thalamus.

Another important issue raised by the quantitative approach used here is that thalamic receptive fields, which normally exhibit complex spatiotemporal dynamics[12], are altered by peripheral deafferentation. This spatiotemporal complexity might be attributed to the asynchronous convergence of multiple feedforward[15–16] and feedback[17] excitatory inputs to the VPM, which could be the sources of the different latency response components observed in these neurons. This is supported by our observations that short and long latency responses in the VPM have different receptive fields[12], and further that lidocaine affects them selectively. The lidocaine-induced unmasking of these various components could result from changes in the tonic activity of inhibitory neurons acting on the VPM or trigeminal nuclei. As a hypothesis, therefore, we suggest that sensory deprivation disrupts the normal dynamic state of equilibrium between excitation and inhibition within the network that comprises the entire somatosensory system, producing reorganizations at multiple levels of this pathway. As such, the population receptive fields in Fig. 3*Ab–Cb* could be considered as snapshots of the new dynamic equilibrium states of the somatosensory network as viewed at the thalamic level. This theoretical framework for a network-based model of sensory deprivation-induced plasticity might reasonably be applied to other systems of the brain. □

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