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Simultaneous Reorganization in Thalamocortical Ensembles Evolves Over Several Hours After Perioral Capsaicin Injections

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Katz, Donald B., S. A. Simon, Aaron Moody, and Miguel A. L. Nicolelis. Simultaneous reorganization in thalamocortical ensembles evolves over several hours after perioral capsaicin injections. J. Neurophysiol. 82: 963–977, 1999. Reorganization of the somatosensory system was quantified by simultaneously recording from single-unit neural ensembles in the whisker regions of the ventral posterior medial (VPM) nucleus of the thalamus and the primary somatosensory (SI) cortex in anesthetized rats before, during, and after injecting capsaicin under the skin of the lip. Capsaicin, a compound that excites and then inactivates a subset of peripheral C and Aδ fibers, triggered increases in spontaneous firing of thalamocortical neurons (10–15 min after injection), as well as rapid reorganization of the whisker representations in both the VPM and SI. During the first hour after capsaicin injection, 57% of the 139 recorded neurons either gained or lost at least one whisker response in their receptive fields (RFs). Capsaicin-related changes continued to emerge for ≥6 h after the injection: Fifty percent of the single-neuron RFs changed between 1–2 and 5–6 h after capsaicin injection. Most (79%) of these late changes represented neural responses that had remained unchanged in the first postcapsaicin mapping; just under 20% of these late changes appeared in neurons that had previously shown no plasticity of response. The majority of the changes (55% immediately after injection, 66% 6 h later) involved “unmasking” of new tactile responses. RF change rates were comparable in SI and VPM (57–49%). Population analysis indicated that the reorganization was associated with a lessening of the “spatial coupling” between cortical neurons—a significant reduction in firing covariance that could be related to distances between neurons. This general loss of spatial coupling, in conjunction with increases in spontaneous firing, may create a situation that is favorable for the induction of synaptic plasticity. Our results indicate that the selective inactivation of a peripheral nociceptor subpopulation can induce rapid and long-evolving (≥6 h) shifts in the balance of inhibition and excitation in the somatosensory system. The time course of these processes suggest that thalamic and cortical plasticity is not a linear reflection of spinal and brainstem changes that occur following the application of capsaicin.

INTRODUCTION

The last 15 years of research on the somatosensory system has revealed that low-threshold mechanoreceptor receptive fields (RFs), once thought to be static, reorganize after a variety of peripheral manipulations, including amputation (Elbert et al. 1997), nerve cut (Garraghty and Kaas 1991; Merzenich et al. 1983; Rasmussen 1996), injection of local anesthetics such as lidocaine (Nicolelis et al. 1993b) or injection of irritants (mustard oil, formalin, bradykinin, or most relevantly for our purses capsaicin; see Baumann et al. 1991; Cook et al. 1987; Fitzgerald and Woolf 1982; Hoheisel et al. 1993; Kwan et al. 1996; Lin et al. 1998; McMahon et al. 1993; Nusismauner and Wall 1985; Raboisson et al. 1995; Simone et al. 1989, 1991; Wall 1987; Yu et al. 1993).

Recent analyses of electrophysiological data obtained from rats has suggested that this reorganization may arise from changes in the feedback and feedforward interactions throughout the trigeminal somatosensory system that tend to maintain a dynamic equilibrium between excitatory and inhibitory affrents (Krupa et al. 1997; Nicolelis 1996, 1997; Nicolelis et al. 1998). It has been suggested that this excitatory/inhibitory balance sets up a situation whereby a fraction of the neural connections that could transmit information from a peripheral (skin) region to a CNS neuron are “latent,” suppressed by patterns of inhibition (Jacobs and Donoghue 1991; Schroeder et al. 1995). In many cases peripheral manipulations induce a different fraction of the RF to become active (Byrne and Calford 1991). Subcutaneous lidocaine injections well outside the recorded RFs of single neurons, for instance, have been shown to cause both unmasking and masking of sensory responses throughout multiple relays of the somatosensory system almost immediately (Faggin et al. 1997; Nicolelis et al. 1993a).

There is substantial evidence that the C- and Aδ-fiber nociceptors may play an important role in modulating RFs of somatosensory system neurons (Baron and Maier 1995; McMahon et al. 1993; Simone et al. 1991). For example, methods used to produce RF changes frequently alter nociceptor activity. Notable among these methods is the administration of capsaicin, the pungent ingredient in hot pepper, which specifically targets C-fiber nociceptors and Aδ-thermoreceptors, first exciting and then silencing them (Baumann et al. 1991; Caterina et al. 1997; Green 1991; Serra et al. 1998; Szolcsanyi et al. 1988). Capsaicin induces RF changes when it is applied directly to the peripheral nerve (Fitzgerald and Woolf 1982; Mannion et al. 1996; Nusismauner and Wall 1985; Wall 1987), injected subcutaneously (Calford and Tweedale 1991; Pettit and Schwark 1996; Rasmussen et al. 1993; Simone et al. 1989), or applied systemically in neonates (Cervero and Plenderleith 1985; Chiang et al. 1997; Kwan et al. 1996; Wu and Gonzalez 1995). RF changes induced by these means have been detected at the cortical (Calford and Tweedale 1991; Nusismauner and Wall 1985; Toldi et al. 1995), thalamic (Rasmussen et al. 1993), brain stem (Chiang et al. 1997; Kwan et al. 1996), and spinal cord (Cook et al. 1987; Ma and Woolf 1996; Pettit and Schwark 1996; Simone et al. 1989) levels of the somatosensory system.
In this study, we further investigated the involvement of the nociceptive pathway in the process of somatosensory reorganization in anesthetized rats by simultaneously recording the extracellular activity of populations of single neurons located in the SI “barrel” cortex and the ventral posterior medial (VPM) nucleus of the thalamus, before and after injecting small amounts of capsaicin under the skin of the lip. Chronic multisite, multielectrode techniques and computer control of stimulus delivery enabled us to quantify how, after capsaicin injections, the spatial and temporal relationships among these simultaneously recorded neural ensembles change. We found that capsaicin injections caused spontaneous levels of activity to simultaneously increase in VPM and SI. More importantly, RFs in VPM and SI rapidly changed and continued to reorganize over the course of 6 h. The extended time course of the VPM/SI changes, compared with the much shorter time course previously reported for capsaicin-related reorganization in second-order nociceptive relays (Chiang et al. 1997; Cook et al. 1987; Kwan et al. 1996; Ma and Woolf 1996; Pettit and Schwark 1996; Simone et al. 1991), suggests that the processes responsible for the observed reorganization are at least partially intrinsic to the thalamocortical loop of the somatosensory system. In addition, our ensemble analyses revealed that manipulation of the capsaicin-sensitive nociceptive pathway decreases the local interaction between neighboring cortical neurons without changing their temporal relationships.

**METHODS**

**Subjects**

Eight female Long Evans rats (230–280 g at the time of surgery) served as the subjects in this study. Animals were maintained on a 12 h/12 h light/dark schedule, with experiments carried out in the light portion of the cycle. Animals in home cages had ad libitum access to normal rat chow and water.

**Implantation of microwires**

A complete description of these techniques can be found elsewhere (Nicolelis et al. 1997). Briefly, animals were anesthetized using a 5% halothane/air mix, quickly followed by an intraperitoneal injection of pentobarbital (50 mg/kg). Stable levels of anesthesia (i.e., no tail pinch or eye blink reflexes) were maintained with small (0.05 ml) additional pentobarbital injections. The anesthetized animal was placed on a standard stereotaxic frame, after which the scalp was excised. Small craniotomies were made for four to six ground screws and two microelectrode assemblies (NBLabs, Denison, TX). Each microelectrode assembly included 16 50-μm Teflon-coated stainless steel microwires (see Fig. 1 in Nicolelis et al. 1997). After resection of the dura over cortex and thalamus, microwire arrays (SI cortex) and bundles (VPM thalamus) were lowered slowly into layer V of SI cortex and the barreloid region of the VPM, guided by stereotaxic measurements, constant examination of the electrophysiological signals, and monitoring for responses to whisker stimulation. Thalamic bundles were lowered in the vertical stereotaxic plane, and cortical arrays were lowered normal to the exposed cortical surface. Once in position, the electrodes were cemented to the skull with dental acrylic, the scalp was sutured or stapled around the implant, and antibiotic ointment was applied liberally to the wound. Postoperative analgesic (buprenex) was administered as needed.

**Simultaneous multisite single-unit recording**

Details of this technique have been described elsewhere (Nicolelis and Chapin 1994; Nicolelis et al. 1997). Briefly, spontaneous and evoked neural activity from all implanted microwires was simultaneously digitized at 40 kHz (Plexon, Dallas, TX). Single and multiplet

**Subcutaneous injection of capsaicin**

After this initial RF mapping, 25–30 μl of a 10% capsaicin dispersion in 30% ethyl alcohol with 0.5% Tween-80 (added to improve solubility) or, alternatively, 25–30 μl of the vehicle alone, was injected subcutaneously to the perioral region through a 30-gauge needle. Injections were made into the upper lip, ~6 mm away from the whisker pad, nearest to whiskers E2 and E3 (see Fig. 3D). RF mapping was then repeated one to three times, using parameters identical to those used in the original protocol.

Data sets also included 30-min periods of spontaneous activity, recorded before each full mapping protocol. Capsaicin or vehicle injection took place halfway through the recording made between the first and second RF mappings. Small (0.05 ml) intraperitoneal pento-
barbitol boosters were given if breathing became light and fast or if the rat responded to tail pinch.

Data analyses

POSTSTIMULUS TIME HISTOGRAMS (PSTHs). The spiking responses of each unit were first summed into PSTHs for all trials in which a given single whisker was stimulated. This was repeated for all the stimulated whiskers. A one-way Kolmogorov-Smirnov test was used to determine, for each PSTH, whether the stimulus-locked firing rate rose to a level significantly above spontaneous activity (difference between baseline and response, \(P < 0.05\)). If a significant change occurred, the neuron was categorized as responsive to that whisker, and that whisker was considered a part of that neuron’s RF. For purposes of visualization, population poststimulus time histograms (PPSTHs) were used to depict the simultaneous sensory responses of ensembles of simultaneously recorded neurons in a single graph (Nicolelis et al. 1999). The gain and loss of significant responses across time was tabulated, allowing us to characterize the reorganization that followed capsaicin and vehicle injections for several hours.

MULTIDIMENSIONAL SCALING. To gain insight into population level processes that might be driving activity in the system, linear multivariate techniques were used to examine the trial-by-trial activity across the recorded ensembles of neurons during individual whisker stimulation. One such technique, multidimensional scaling (MDS), offers a straightforward, quantitative way to measure how neurons may cluster into “functional groupings” (Borg and Groenen 1997; Davison 1985; Erickson et al. 1993; Johnson and Wichern 1992) by setting the neurons into a space of predetermined dimensionality in which the proximity of any two neurons estimates their similarity in temporal response property. The use of concatenated single trials as the input matrices (see following text) caused MDS to calculate these spaces in terms of temporal firing relationships between the neurons on a trial-by-trial basis. Correlations of the pairwise distances between the neurons in separate MDS solutions offered estimates of how functional groups changed with different experimental manipulations, and according to stimulus identity.

For purposes of this analysis, neural activity was collapsed into 10-ms bins, and a square matrix of dissimilarities was computed from the neuron \(\times\) time matrix, using Euclidean metrics. This analysis was first carried out using the entire ~150-s file corresponding to the stimulation protocol of each single whisker and again using only the 100 ms of each file that included the period of strongest response. MDS solutions in several different numbers of dimensions were created from the dissimilarity matrices. Both metric and nonmetric MDS routines were used. The latter algorithm, based on the work of Kruskal and Shepard (see Venables and Ripley 1997) and encoded for a commercially available statistical package (S-plus, MathSoft) by Brian Ripley, relaxes assumptions about the nature of the distances, instead making use only of the rank order of pairwise dissimilarities.

SEMIVARIANCE. Spatial statistics imported from physical geography (Burrough and McDonnell 1998; Cressie 1993; Kitanidis 1997) were employed to provide estimates of how the measured cortical population activity could have resulted from orderly transmission of information across the “cortical field.” For the purposes of this analysis, it was first necessary to approximate the locations of the discriminated cortical neurons on a \(2 \times 8\) grid representing the tips of the rigid arrays implanted normal to the cortical surface.

The spatial analysis is conceptually similar to ANOVA, whereby the variability in a data set is divided into one part that can be attributed to differences between groups and another part that is variability within groups (error). Spatial statistics use the distances between pairs of neurons to pull out the portion that can be related directly to spatial structure (by definition, the semivariance). Semivariance is characterized in terms of the unshared variance between pairs of neurons at a range of “spatial lags” (relative distance, analogous to temporal lag on the \(x\) axis of a cross-correlogram). The relationship between spatial lag and semivariogram is called the variogram.

As unshared variance is the inverse of spatial covariance, a variogram made from data with spatial structure should be in the form of an increasing function of variance across lag. Examination of these functions provides information regarding the spatial processes at work in the data set. A comparison of two such functions can reveal differences in both overall spatial covariance and in the “functional spread” of spatially correlated activity.

This analysis allowed us to test whether and how the cortical RFs (summed firing rates, as opposed to trial by trial variability of spiking time) changed through time in terms of “spatial coupling.” PSTHs of the first 100 ms of cortical responses were divided into 10-ms bins, each of which was analyzed separately. The resultant 10 semivariograms for a particular stimulus response were laid side by side to produce graphic output showing spatial structure through time. These individual whisker analyses then were aggregated to produce overall mean “time-semivariograms” for each subject and for the group as a whole.

Histology

After the experimental sessions, subjects were deeply anesthetized with pentobarbitol and perfused through the heart with saline followed by 5% formalin in saline. Seven seconds of DC current (7 \(\mu\)A) were passed through selected microwires in preparation for staining. The brain was then removed and immersed in a 30%/10% sucrose formalin solution, and was refrigerated until saturated. Sections (80 \(\mu\)m) cut through the implanted areas were stained with Prussian blue for ferrous deposits blasted off of the electrode tips and counterstained with cresyl violet for cell bodies.

RESULTS

Neural data

Eight animals received chronic VPM and SI implants. Of these, four provided single neuron records in both thalamus and cortex, two provided mainly cortical single units, and two provided mainly thalamic single units. A total of 139 single neurons were recorded—60 in VPM and 79 in SI. The average number of neurons recorded from these implants was thus 13.2 for SI and 10.0 for VPM.

Effect of capsaicin injections on single-unit SI and VPM whisker responses

CHANGES IN SPONTANEOUS FIRING. Increases in the spontaneous firing rates of cortical and thalamic neurons were observed after subcutaneous capsaicin injections (Fig. 1). These increases appeared within 15 min of injection in both VPM and SI and continued for up to 15 min (whereupon the RF mappings began). The increases partially consisted of bursts of high activity (Fig. 1)—similar capsaicin-induced bursting has been reported previously in recordings from neurons in the spinothalamic tract (see Fig. 4 in Simone et al. 1991).

To directly assess the differences between capsaicin and vehicle injections, overall population firing rates were calculated for each 5-min period after capsaicin or vehicle (ethanol/Tween-80) injection. Similar averages were calculated for purely spontaneous firing, recorded for 30 min at the very beginning of the session. Figure 2 shows data from thalamus and cortex merged, as spontaneous changes in the two areas were not statistically different (see following text). Analysis of
Fig. 2 reveals that neural firing rates after vehicle (ethanol/Tween-80) injection did not differ from spontaneous rates. Firing rates did increase, however, after capsaicin injections. Most of this increase appeared during the 11- to 15-min period after injection.

A mixed ANOVA for injection condition, brain region, and time (5-min periods after injection) revealed the expected time \times injection interaction (\(F(10,1455) = 10.25, P < 0.0001\)), confirming the observation that firing rates increased only after capsaicin injection. Subsequent t-tests demonstrated that differences between capsaicin and vehicle injections were significant at each time point from 11–15 min on. The only other effect to reach statistical significance was the overall effect of time (\(F(5,1455) = 11.40, P < 0.0001\)). Neither the main effect for brain region nor the interaction between brain region and time was significant. It appears that SI and VPM reacted to the capsaicin injections with similar increases in spontaneous firing at similar times.

RF REORGANIZATION. Extremely small (25–30 \(\mu\)l) injections of a 10% capsaicin solution caused rapid, spatially distributed, and lasting RF changes in VPM and SI neurons. That is, neurons developed or lost responses to at least one whisker at the very first measurement and maintained these changes for \(\leq 6\) h. An example showing mainly excitatory changes can be seen in Fig. 3, which contains a series of PPSTHs (and some of the constituent PSTHs) taken from animal 97–103. This figure shows the responses of all simultaneously recorded single-units to deflection of whisker D5 before and 0.5 and 6.5 h after capsaicin treatment. New responses appeared in both cortex and thalamus (see the extracted PSTHs as well) soon after the injection, and most of these new RFs were still apparent 6 h later. Analysis of all eight animals revealed that subcutaneous capsaicin injections consistently caused long-lasting changes in thalamocortical responses.

Many RF changes emerged between the first postcapsaicin mapping and later mappings. Figure 4 shows the responses of four simultaneously recorded neurons neurons (2 SI, 2 VPM) before and 1 and 6 h after capsaicin injection. All four of these developed new RFs at the later mapping. The first three changed only late in the recording session (the 1st and 3rd
show unmasking, whereas the 2nd shows a lost response), and the fourth gained in response strength immediately after injection and then lost even the small precapsaicin response during the next few hours. Changes subsequent to capsaicin were thus both spatially distributed and, in many cases, slowly evolving (during the course of several hours).

Overall, 57% of the recorded neurons underwent RF changes to at least one whisker immediately after capsaicin injection. Within this subpopulation, 55% of the changes involved unmasking of new responses. Tested ≤6 h later, the percentage of neurons with at least one RF that had changed since the previous postcapsaicin RF mapping was 50% (66% of which were unmasked responses), a percentage mainly (79%) made up of responses that had not changed immediately after capsaicin injection. A subset of these new responses (19%) involved neurons that previously had shown no capsaicin-related responses to any whisker.

Control injections caused some response changes themselves, but the alterations wrought by vehicle injections were far less extensive than those caused by capsaicin injections. Overall, 24% of recorded neurons developed new RFs soon after vehicle injections (69% of these were unmasked responses). An additional control was performed on one subject, involving a dry needle stick without injection. A negligible rate of RF change (12%) was observed after this procedure, suggesting that the changes after vehicle injections were not merely the result of damage from puncture. Still, for every neuron that developed or lost a whisker response after vehicle injection, more than two did so after capsaicin injection.

For one subject, vehicle-related changes were followed ≤6 h postinjection. Only 22% of the recorded RFs changed in between the earlier and later postvehicle mappings. It is unclear whether this percentage reflects long-term effects of the vehicle injection or “spontaneous” changes occurring across recordings of several hours. It is clear, however, that a much higher percentage of neurons changed after capsaicin injection, both soon after and long after the injections.

The percentages of neurons for which RFs changed in response to capsaicin and vehicle injection were compared via a simple one-way ANOVA for mapping time (1 h postcapsaicin, 6 h postcapsaicin, and 1 h postvehicle). This analysis revealed that significantly more neurons gained or lost responses after capsaicin injection than vehicle injection (F(2,28) = 3.46, P < 0.05). Overall, these results demonstrate that capsaicin reorganizes the somatosensory system, that this reorganization is much larger than that caused by control injections, and that this reorganization continues for ≥6 h.

Similar percentages of neurons changed in thalamus or cortex, both 1 and 6 h after capsaicin injection (Fig. 5). In cortex, 59% of the neurons changed immediately after capsaicin injection, 54% 6 h after; in thalamus, the 54% of the neurons developed new RFs rapidly, and 44% developed new RFs in later mappings. A 2 × 2 mixed ANOVA for recording site and time postcapsaicin revealed no differences in the percentage of SI and VPM neurons showing reorganization (all P values > 0.25). Similarly, the number of whiskers in the unmasked responses did not differ between cortex and thalamus. On average, SI neurons began responding to 0.68 ± 0.10 (mean ± SE) new whiskers soon after injection, while VPM neurons began responding to 0.52 ± 0.14 new whiskers. At the later mapping, similar RF unmasking was found (0.61 ± 0.11 in SI; 0.45 ± 0.17 in VPM); none of these differences between SI and VPM was significant.

Another way of visualizing the large and evolving changes caused by capsaicin is presented in Fig. 6, which depicts a spatiotemporal RF for a single SI neuron. For this analysis, the responses of a single neuron to all of the stimulated whiskers were grouped into 10-ms epochs after stimulus onset, and plotted sequentially. Note that the differences in firing intensity, represented by the color change between dark red/black (low) and bright red/white (high), have been normalized individually for each panel, to highlight which whiskers caused the strongest response in each individual epoch (Nicolelis and Chapin 1994). As can be seen in this example, which is representative of much of the neuronal sample, the spatial pattern of peak response changed as a function of poststimulus time, and the overall spatiotemporal pattern changed after capsaicin injection. For this cortical neuron, shifts in the spatial location of the short-latency RF center predominated, but longer latency
Changes could be observed as well. Although the RF changes were noticed within 1 h after the injection, RFs also changed in the hours after the initial change. Once again, this demonstrated that the subcutaneous capsaicin injection caused immediate reorganization, and that this reorganization continued to evolve in both SI and VPM for ≤6 h.

**FIG. 4.** Responses of 4 simultaneously recorded neurons from animal 97–59 to whisker stimulation before (A), immediately after subcutaneous capsaicin injection (∼15–30 min; B), and long after injection (∼6–7 h; C). x axes represent poststimulus time in milliseconds (dashed vertical line represents stimulus onset), and y axes represent response magnitude in spikes/s (horizontal line represents 95% confidence interval for prestimulus firing rate). Top 2 rows depict the responses of SI cortical units, and the bottom 2 VPM thalamic units.

**FIG. 3.** A: series of population peristimulus time histograms (PPSTHs) showing the response of an ensemble of SI cortical and VPM thalamic units from animal 97–103. Time since stimulus onset is on the x axes, and the neurons are arrayed along the y axes. Response intensity (in spikes/s) is represented on the z axes, and also by the color of the response peak. New responses in both SI and VPM can be seen in the first responses after capsaicin injection and are mostly maintained for the next 6 h. Note that units within SI and VPM are arrayed nontopographically—no specific spatial patterning of the response is revealed by this plot. B: individual PSTHs for a VPM single neuron (DSP31b) pulled out of the PPSTH. The axes are time in msec poststimulus and response magnitude in spikes/s, as in a. The dashed vertical line represents stimulus onset. C: individual PSTHs for a SI single neuron (DSP5b), pulled out of the PPSTH. D: schematic of the rat’s snout, with the row (A–E) and column (1–5) whiskers identified. Specific whisker stimulated to create A–C (D5) is shown, as is the injection site, ∼6 mm from whiskers E2 and E3.
Ensemble analyses of changes after capsaicin injections

CHARACTERIZATION OF TEMPORAL RESPONSE PROPERTIES OF ENTIRE ENSEMBLES VIA LINEAR MULTIVARIATE ANALYSIS: METRIC MDS. The recent advent of multiple electrode simultaneous recording technology has allowed researchers to obtain information concerning the temporal relationships between the firing patterns of two or more neurons (see, for instance, Deadwyler et al. 1996; Nicolelis et al. 1995; Seidemann et al. 1996; Vaadia et al. 1995). An initial characterization of these relationships, at zero time lag, can be made with linear multivariate techniques such as MDS. MDS arranges a space of predetermined dimensionality, in which the relationships between a set of variables can be estimated as Euclidean distances. We used this technique to measure the temporal structure of the ensembles’ responses to particular whiskers at each RF mapping time and then compared the changes between maps (MDS solutions) that could be related to the effect of capsaicin.

A variety of parameters were manipulated to test the robustness of the analysis, including number of time bins from each response used (all vs. the first 100 ms), algorithm used (metric versus nonmetric), and dimensionality (2 vs. 3). As each of these variants gave qualitatively similar results, we report here only data pertaining to two-dimensional metric solutions for the initial 100 ms of the responses.

Changes between MDS solutions calculated before and after capsaicin or vehicle injections were quantified in terms of the correlations between pairwise distances. If the relationships among the neurons in one scaling solution (i.e., the grouping based on synchrony of firing) is precisely the same as those among the neurons in the second scaling solution, then the correlation should be 1.0. Changes, therefore, are quantified as reductions in the correlation between solutions.

According to this analysis, the application of capsaicin had little or no effect on the stability of the calculated MDS solutions and thus had little or no nonrandom impact on the temporal structure of the thalamocortical neural ensemble responses to whisker stimulation. Correlations between protocols separated by a capsaicin injection were similar to the correlations between protocols separated by a vehicle injection and to correlations between halves of the same protocols. None of the differences were significant. Thus it appears that capsaicin injections did not cause changes in the patterns of neuronal synchrony that distinguished particular individual whisker stimuli in anesthetized rats.

Next, we considered the possibility that capsaicin injections changed the temporal structure of ensemble responding only for whiskers that were close to the injection site. Given that SI and VPM contain topographic representations, and given that the effects of the subcutaneous injections were relatively localized, reorganization of temporal patterning could have been obscured in the previous analysis by the aggregation of all whisker responses into a single analysis. To test this possibility, we repeated the analysis, dealing separately with whiskers at four distances from the injection site. This manipulation of the data had no impact on the outcome: even for whiskers D2–3 and E1–4 (group 1, closest to the injection; see Fig. 3D), capsaicin had no specific impact on the MDS solutions. Thus this method failed to provide evidence that nociceptor-based reorganization of somatosensory cortex and thalamus involves changes in temporal organization of neural ensemble firing.

CHARACTERIZATION OF SPATIAL RESPONSE PROPERTIES OF CORTICAL ENSEMBLES: TIME-SEMIVARIOGRAMS. The regular, grid-like arrangement of our multielectrode arrays in the SI, implanted normal to the surface of the brain, made it possible to specify, at least approximately, distances between simultaneously recorded cortical neurons. These approximate distances were used, in conjunction with the analysis of RFs from SI neurons, as input to a geostatistical analysis (Burrough and McDonnell 1998; Cressie 1993; Kitandir 1997). This analysis specifically allowed us to isolate one mechanism responsible for any particular population firing pattern—spatial spread of activity. We used this method to examine whether or not capsaicin injections affect the spatial spread and coupling of SI neuronal sensory responses. Specifically we used geostatistical analysis to test whether variance attributable to the relative proximity between pairs of neurons in layer V contributed to the patterns of ensemble activity observed before and after capsaicin injection.

The success of this analysis depends on having an adequate number of neurons in the data set and specifically on having sufficient numbers of pairs of neurons at each different spatial separation (or spatial lag). Four of our implants provided enough spatial data for estimation of multiple lags between 0 and 1,000 μm, and three of these allowed estimation of all desired lags—0, 250, 500, 750, and 1,000 μm (the 1000-μm lag is unreliable, however, as it approaches half of the distance across the entire array). Only those four subjects are included in the analysis of spatial variance.

This analysis demonstrated that firing patterns from SI of anesthetized rats were spatially interpretable. Figure 7A presents the basic time-semivariogram analysis for one animal (97–103). The x axis is spatial lag (distance between pairs of neurons) from 0 to 1,000 μm; the y axis represents continuous sequences of 10-ms time bins, with time bin 1 beginning at stimulus onset. Variance progresses from low (black to dark red) to high (bright red to white) and has been interpolated into contours connecting equivariable points on the graphs.
FIG. 6. Spatiotemporal RF for cortical neuron DSP6 from animal 97–98. Each row of panels represents the activity for a particular 10-ms epoch after stimulus onset. In each row, left: preinjection responses; middle: responses recorded within ~1 h of the subcutaneous capsaicin injection; right: responses recorded ~6 h postcapsaicin injection. Response magnitude (spikes/s) is individually normalized for each panel to accentuate which whiskers caused peak firing in each epoch. Note that while each column (representing the spatial dynamics of the responses) is unique, the postcapsaicin RFs are more similar to each other than either are to the precapsaicin RF. Note in particular that the spatial location of the short-latency RF center of this neuron, marked on each series, changes after capsaicin injection.
In Fig. 7A, the brighter regions equate to higher variance and lower covariance between sites; that is, a gradient from dark to bright is generally interpretable as a progression from higher similarity to lower similarity. It thus can be seen that, at most time points, neurons separated by any distance behaved similarly to each other—at time bins 1 and 4–10, no variance appears for neurons separated by any of the five lags (these are the black regions). This is true because most of these neurons were not firing at these instances. Spatial structure emerged only 10–20 ms after stimulus onset and disappeared soon thereafter. During these epochs, neurons separated by ≤500 μm continued to behave similarly to each other (deep red region), but the variability rapidly increased between lags of 500 and ~850 μm (that is, the colors progress from deep red to pink to white as the graph is scanned from left to right). Beyond this separation, the variance appears to asymptote. Neurons found closer together tended to behave similarly to each other, whereas neurons that were further apart tended to behave less similarly. These results suggest that ensembles of single neurons recorded from the cortical sheet do behave, during stimulus response, analogously to points in a geographical data set and that the ensemble itself can be profitably thought of as a landscape. Simply put, spatial proximity seems to be an important mechanism whereby SI neurons in a population relate to each other or covary.

The general shape of this spatiotemporal pattern—fleeting emergence of an interpretable spatial structure in the population response to whisker stimulation—was reliable across subject and across repeated RF mappings. Figure 7, B and C, shows individually normalized time-semivariograms for the same neural ensemble seen in Fig. 7A recorded ~1 h after and ~6 h after capsaicin injection. It is important to note that each panel is normalized individually, to emphasize the fact that the overall pattern replicated 1 and 6 h after subcutaneous capsaicin injections. Overall after subcutaneous capsaicin injections, the similarity in response between two neurons decreased as
the distance between them increased. For this individual subject, it appears that, after subcutaneous capsaicin injections, the increases in spatial variability during epochs 2 and 3 began at a smaller spatial separation; as neuron pairs become separated by $>500 \, \mu m$, the color becomes lighter (and thus the spatial variance becomes larger).

Figure 8, which shows the across-subject average peak semivariances at each spatial lag, demonstrates that capsaicin injections reliably increased the spatial variance at all lags—that is, the capsaicin decreased the spatial coupling between neurons between the first and second RF mappings, effectively sliding the semivariance curve higher on the $y$ axis. A two-way repeated measures ANOVA for spatial lag and mapping time revealed a marginally significant main effect for time, despite the inclusion of only four subjects in the analysis ($F(2,6) = 4.28, P = 0.06$). With such a small sample, this represents a substantial effect (see Cohen 1992) and demonstrates the robustness of the increase of spatial variance after capsaicin injection. The difference between the first postcapsaicin mapping and the mapping made several hours later was not significant, but rather the spatial variance appeared to stabilize at a new, less coupled level within an hour of the injections. The interaction between mapping time and spatial lag was significant ($F(8,24) = 2.26, P = 0.05$) as well.

Although in some cases the overall variance of the whisker responses increased after capsaicin, this failed to account for the observed increase in spatial variance. The analysis was repeated with overall variance differences between protocols normalized out. The spatial structure remained unchanged as the impact of capsaicin injection on that spatial structure. Nor did the passage of experimental time account for the changes. Finally, vehicle injections had no impact on the amount of spatial variance in the data. Therefore the most parsimonious interpretation of this result is that subcutaneous capsaicin injections affected cortical ensembles by decreasing the importance of spatial relationships as a variable that influences the firing rates of individual neurons.

DISCUSSION

In the present study, we quantified the temporal evolution of changes in firing rate and RF reorganization across simultaneously recorded populations of neurons in the rat somatosensory thalamocortical loop caused by subcutaneous perioral injections of capsaicin. We found that capsaicin injections outside the whisker pad simultaneously increased the spontaneous firing of neurons in SI barrel cortex and VPM thalamus, with the primary increase occurring between 11 and 15 min after injections; rapidly changed the RFs in both VPM and SI neurons as well as the distributed patterns of neural activity caused by stimulation of individual whiskers, in both structures at rates above those after control injections; caused RFs to continue changing for $\geq 6 \, h$, in a manner that seemed largely identical in the two structures; and decreased the spatial coupling among ensembles of SI cortical neurons, causing neurons to respond more independently to whisker stimulation.

Overall, this study provides evidence of the primary nociceptive pathway’s influence on somatosensory system function and suggests that this influence can far outlast the short-term changes typically observed in the spinal and brainstem nociceptive nuclei themselves.

Changes in spontaneous activity after capsaicin injection

The first finding of this study was that subcutaneous capsaicin injections significantly increased the basal levels of activity in VPM and SI neurons (Fig. 1). Similar changes in firing rate have been noted to occur in many preparations and thus represent a fundamental aspect of the capsaicin response and of capsaicin-induced CNS plasticity (Carstens et al. 1998; Simone et al. 1989, 1991). Although it is difficult to compare across differences in capsaicin concentration, the changes in VPM and SI appeared to occur later than those previously observed in the spinal cord and brain stem. VPM/SI increases developed most immediately after injection (Simone et al. 1989, 1991), whereas in the dorsal horn, spontaneous changes develop almost immediately after injection (Simone et al. 1989, 1991), and in the trigeminal subnucleus caudalis, they develop in 5–10 min (Carstens et al. 1998). Therefore these data suggest that increases in tonic thalamocortical firing do not temporally track brain stem activity. Rather it appears that capsaicin-related changes cause a rather sudden state change that occurs later in the brain stem than in spinal cord and later in the thalamocortical system than in the brain stem. This suggests that firing rate increases in the VPM and SI do not simply reflect alterations observed in the brain stem.
RF changes after capsaicin injections

We observed that subcutaneous capsaicin injections triggered changes in the RFs of VPM and SI neurons, above and beyond those caused by vehicle injection, that occurred either rapidly or in the following 6 h (Figs. 3–6). Similar changes have been observed previously in the dorsal horn (Cook et al. 1987; Fitzgerald and Woolf 1982; Pettit and Schwark 1996; Simoni et al. 1991), principal trigeminal nucleus (Chiang et al. 1997; Kwan et al. 1996), ventrolateral thalamus (Rasmussen et al. 1993), and SI cortex (Calford and Tweedale 1991).

Our results add to the above studies in two important regards: first, we were able to detect more subtle RF changes than had been observed previously, and also observed that these changes occurred in VPM and SI practically simultaneously. Second, we were able to chart the evolution of these changes during 6 h and to observe that unmasking and masking of sensory responses can happen in both VPM and SI throughout this period.

Complexity of Changes. Using computer-controlled stimulus delivery to individual whiskers, we observed distributed, complex RF changes (Figs. 3, 4, 6, and 8) consisting of both unmasked responses (which accounted for 55–66% of the observed RF changes) and masked responses (Fig. 4). That is, both enlargements and contractions of the RFs were observed. Although the magnitude of the unmasked portion of the RFs was smaller than that observed after subcutaneous injections of lidocaine made under similar conditions (Faggin et al. 1997; Nicolelis et al. 1993), both compounds produced similar patterns of change. This suggests that a subset of the mechanisms involved in the reorganizations activated by these two pharmacologically different compounds may be similar. Given that RF unmasking also occurs after injections of mustard oil (a C-fiber excitant) (see Hoheisel et al. 1993; Yu et al. 1993), our results support the hypothesis that any lasting change in peripheral afferents can trigger sensory reorganization throughout the CNS.

The present results further suggest that the process set in motion by capsaicin injection is likely to be more complex than simple unmasking of previously silent synapses. Capsaicin did not simply increase the number of synapses involved in production of a spatiotemporal RF (see Ghazanfar and Nicolelis 1999; Nicolelis and Chapin 1994) but appeared to change the fraction of the total population of synapses that could be activated by stimulation of particular whiskers. This finding is consistent with the suggestion that capsaicin injections altered a balance between excitatory and inhibitory afferents within the somatosensory relays contributing to the definition of spatiotemporal RFs rather than simply increasing the effectiveness of previously inhibited synapses.

RFs in the somatosensory thalamocortical loop frequently have been assumed to be purely a function of stimulation of Aβ fibers in the whisker follicles. The present data, however, represent definitive evidence of the nociceptive system’s influence on somatosensory responses.

Evolution of Capsaicin-Related Changes. Overall, our RF quantifications revealed a continuous time course (≤6 h) for changes in the somatosensory thalamus and cortex that is different from those reported in the brain stem and spinal cord. The percentage of neurons undergoing reorganization was approximately as high 6 h after injection (50%) as 1 h after injection (57%; Figs. 3–6). Previously, capsaicin-related changes in the dorsal horn and nucleus gracilis have been reported to peak or stabilize in ~1 h (Cliffer et al. 1992; Pettit and Schwark 1996; Simoni et al. 1991). The difference in time course between thalamocortical and spinal reorganization make it clear that changes in the thalamocortical system are not simply a reflection of changes at second-order nuclei.

In fact, there is good reason to expect that information should not be linearly transmitted between levels of the nociceptive and somatosensory system. Both of these pathways are characterized by the existence of massive recurrent connections to all subcortical relays. Feedback frequently causes physical systems to function nonlinearly. Moreover, it is well known that feedback connections from somatosensory cortex can affect the function of the protopathic component of the spinothalamic system (Berkley and Hubscher 1995; Dickenson and Sullivan 1987; Vaccarino and Chorney 1994; Vin-Christian et al. 1992). Cortical feedback also has been shown to affect somatosensory thalamic responses (Ergenzinger et al. 1998; Krupa et al. 1997).

A recent paper (Ma and Woolf 1996) demonstrated that after subcutaneous capsaicin injections, repetitive tactile stimulation can cause response plasticity in the dorsal horn. It is conceivable that the protracted RF changes observed here could have resulted, not from the direct manipulation of C and Aδ fibers, but from the use of repeated stimulation of individual whiskers. Although we cannot conclusively dismiss this possibility, we consider it an unlikely explanation for our findings. Although our protocol called for individual whiskers to be stimulated 300 times during a 2.5-min period, no particular whisker was so stimulated more than once every 1.75 h (entire stimulation protocol plus 30 min of spontaneous activity recording). The infrequency of stimulation probably offered relatively little opportunity for the development of the sort of changes discussed by Ma and Woolf. We favor, instead, the interpretation that the evolving RFs observed in VPM and SI represent the intrinsic response to a long-lasting manipulation of peripheral nociceptors and that the reciprocal connections between the VPM and SI are capable of amplifying and sustaining the reorganization process long after lower subcortical structures have ceased to show their effects.

The extended time course of the observed changes further suggests the possibility that changes in C- and Aδ-fiber activity could strongly contribute both to immediate changes in the pattern of excitation and inhibition in the somatosensory system after perturbations and to longer-term changes that may be dependent on modulations of synaptic strength. Increases in activity in the thalamocortical loop, such as those that we’ve observed in this study, could potentially drive the eventual stabilization of new RF maps. Thus it is possible that the evolving changes observed here, caused by perturbation of C- and Aδ-fiber afferents, could represent a bridge between immediate reorganization (Faggin et al. 1997) and longer-term plasticity (Merzenich et al. 1983).

Reduction in spatial coupling between cortical neurons after capsaicin injection

In this study, we took advantage of classic geostatistical analysis to examine the spatial characterization of capsaicin-induced plasticity at the level of neuronal populations (see also...
Freeman and Baird 1987). These analyses revealed spatial structure in the ensemble responses, quantified in time varigrams of activity across trials (Figs. 7 and 8). The initial response to whisker stimulation had a reliable spatial component, reflecting the tendency of nearby neurons to fire in a more related fashion than distant neurons. After capsaicin injection, nearby neurons’ responses became less coupled (i.e., less covariant) to each other (Fig. 8), and thus the spatial structure of neural ensemble responses, while still present, was no longer as strong a force in shaping neural firing.

This finding supports the suggestion that the activity of C-fiber nociceptors can provide tonic inhibition of neurons located in the somatosensory system, and that the desensitization of C fibers via capsaicin injection removes this tonic inhibition (Calford and Tweedale 1991). One difficulty for this theory, already noted by Calford and Tweedale, lies in the fact that C-fiber afferents affected by subcutaneous capsaicin injection typically are reported to lack spontaneous activity (Szolcsanyi et al. 1988). Silent afferents, particularly when relatively distant from the RFs of the recorded neurons, hardly can be expected to provide tonic inhibition. It remains unclear, however, whether capsaicin inactivates only silent afferents. Nociceptors with very low spontaneous firing rates may, under certain conditions, be mistaken for silent afferents. Such low rates of spontaneous firing may be enough to provide tonic inhibition, and the loss of seldom-firing afferents may be enough to disinhibit the system. It is also possible that the disinhibition is mediated by the loss of capsaicin-inactivated Aδ fibers, known to be spontaneously active (Szolcsanyi 1990).

The precise peripheral source of the reduction of spatial coupling—and the attendant RF changes—observed in our data remains unclear. Neither vehicle injections nor electrical stimulation (Calford and Tweedale 1991) cause reorganization that approaches the spatiotemporal extent of that seen after subcutaneous capsaicin injection, suggesting that the exact excitation of nociceptors does not cause sizeable central plasticity. For several reasons, excitation of afferents in skin regions surrounding the injection site is also an unlikely candidate to explain this finding. First, similar reorganization occurs after lidocaine injections, a compound that primarily desensitizes C fibers, known to be spontaneously active (Szolcsanyi 1990).

Thus, the most likely explanation is that small subcutaneous injections of capsaicin changed the overall excitatory/inhibitory balance in the thalamocortical system and that this effect led to a reduction in the “functional coupling” between nearby neurons. Regardless of the mechanisms underlying this effect, the algorithm/method used here provided us with a useful way to quantify plastic changes at the level of neural populations.

Change in somatosensory function after capsaicin injection

The reorganization observed in VPM and SI after capsaicin injection is likely to lead to perceptual changes. When capsaicin is injected under the skin, subjects experience brief pain followed by analgesia at and around the injection site (see for instance Baumann et al. 1991; Fitzgerald and Woolf 1982; Iadarola et al. 1998; Serra et al. 1998; Simone et al. 1991; Treede et al. 1992). The subjects also experience hyperalgesia and allodynia, “tenderness” of the skin marked by lowered mechanical and thermoreceptive pain thresholds and changes in tactile perception, in a large area beyond the injection site. Simone et al. (1991) reported that the extent of hyperalgesia reported by human subjects was related to increased firing of primate spinthalamic tract neurons. Our recordings suggest that plasticity at the level of the thalamus and cortex may be part of the same system response and that a rat receiving subcutaneous capsaicin injections in its lip would experience hyperalgesia, allodynia, or alterations in somatosensory processing after the stimulation of whiskers (Giamberardino and Vecchiet 1995; Kauppila et al. 1998; Treede et al. 1992). This interpretation implies that information from low-threshold mechanoreceptor stimulation may be processed differently after capsaicin injections, perhaps due to plasticity induced in the trigeminal nuclei, thalamus, and cortex. These results further suggest that the somatosensory and nociceptive circuits are perhaps best thought of not as completely separate systems (Berkley and Hubscher 1995) but rather as a single dynamic entity, processing a continuous spectrum of stimulus modalities dependent on the condition of the peripheral afferents.

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