Short-Lasting Classical Conditioning Induces Reversible Changes of Representational Maps of Vibrissae in Mouse SI Cortex—A 2DG Study

It has been known for several years that receptive field properties of sensory cortical neurons can be altered by learning experiences. We attempted to visualize a global change of the cortical body map induced by learning. In order to do this a short-duration classical conditioning involving stimulation of a row of mystacial vibrissae in mice was followed with 2-deoxyglucose (2DG) mapping of functional activity. Three conditioning sessions that paired stimulation of a row of whiskers with a tail shock produced an increase of the functional representation in somatosensory cortex (SI) of a row of the whiskers stimulated during the training. This plastic change of vibrissal representation in SI was visualized with 2DG autoradiography a day after completion of training. The expansion of representation was the most pronounced in cortical layer IV, and to a lesser extent, in layer IIIb. The expansion was observed in conditioned but not in pseudoconditioned mice or in animals that received only the conditioned stimulus. If training was discontinued, the enlargement of vibrissal representation progressively faded. The reversal could be accelerated by a behavioral extinction procedure. This study gives the pictorial demonstration of rapid, transient, and extinguishable learning-dependent changes in SI cortical maps.

The primary somatosensory cortex (SI) of adult mammals can undergo plastic reorganization as a result of eliminating peripheral inputs (Wall and Egger, 1971; Merzenich et al., 1983; for a recent review, see Kaas 1991) or changing sensory experiences (Delacour et al., 1987; Jenkins et al., 1990; Allard et al., 1991; Diamond et al., 1993). The evidence is accumulating that neuronal properties in primary sensory cortices also alter during a special form of plasticity, for instance, learning (Brons and Woody, 1980; Diamond and Weinberger, 1986; Gonzalez-Lima and Scheich, 1986; Bakin and Weinberger, 1990; Recanzone et al., 1992a; Edeline et al., 1993; Recanzone et al., 1993; Weinberger et al., 1993). The process of learning may influence the properties of neurons at all levels of the brain (also see Dudai, 1989). A permanent and stimulus-specific change in neuronal properties can be regarded as a part of the permanent storage of information induced by behavior. Such change may also be an adaptation of the sensory processing brain structures for better perception of behaviorally relevant stimuli. The work of several laboratories has demonstrated that in adult primary sensory cortical areas excitability of neurons and receptive field properties can be changed as a result of sensory experience (Brons and Woody, 1980; Diamond and Weinberger, 1986; Gonzalez-Lima and Scheich, 1986; Bakin and Weinberger, 1990; Recanzone et al., 1992a; Edeline et al., 1993; Scheich et al., 1993). Particularly in somatosensory and auditory cortex, topographical maps reorganize as a result of sensory training (Gonzalez-Lima and Agudo, 1990; Recanzone et al., 1992a, 1993). The organization of neuronal properties can be strongly correlated with improved perceptual skills (Recanzone et al., 1992b). An increase of the cortical representation of a sensory surface magnifies the power of stimuli activating this surface in the global pattern of cortical activity. In this way, engagement of specific sensory inputs presumably results in their more salient brain representation. Merzenich et al. (1990) have claimed that such changes of cortical maps are normal, dynamic cortical processes by which selective, distributed responses of cortical neurons are shaped by experience throughout life.

Body maps in the somatosensory cortex can be altered by training involving sensory stimulation. For example, Jenkins et al. (1990) and Recanzone et al. (1992a) described topographical reorganizations of hand representations in the SI cortices of monkeys trained in regulated finger contact or frequency discrimination behavioral tasks. Hand and Hand (1995) reported that the area of 2DG labeling of cortical functional representation of a mystacial vibrissa (a vibrissal column) in a rat was enlarged following a prolonged period of pairing sensory stimulation with alimentary reward. A long-lasting sensory stimulation of whiskers, unrelated to behavioral experience, which can be considered habituation, leads to a decrease in activation (2DG uptake) of cortical representation of vibrissae (Welker et al., 1992). The vibrissal pathway is well suited for such cortical response "mapping" experiments because rodent SI cortex contains barrel-shaped neuronal aggregates ("barrels") in layer IV that form a pattern replicating the whisker distribution on the snout; the special anatomical and cytoarchitectonic structure allows for an easy localization of the central representations of particular vibrissae (Woolsey and Van der Loos, 1970). Considerable plasticity of the vibrissal system in adult rodents has been reported by several investigators working with different experimental approaches and experimental techniques (Hand 1982; Kossut et al., 1988; Levin and Dunn-Meynell, 1991; Fox 1992; Kossut, 1992; Diamond et al., 1993; Armstrong-James et al., 1994; Glazewski and Fox, 1994; Siucinska and Kossut, 1994a,b). We have used this pathway in mice to determine whether or not changes in cortical body maps can be generated by a brief training that does not involve an extensive use of special sensory stimulation, and to assess the stability of any induced plastic changes. In particular, we wanted to know if a body map change could be induced in SI by a few minutes of classical conditioning and whether such changes could be reversed by an extinction procedure, designed to obliterate the previous signaling significance of the conditioned stimulus.

Materials and Methods

Behavioral Training

The experiments were performed on 34 young adult (7–8 weeks old during conditioning) Swiss mice. The mice were accustomed to a neck restraint by being placed in a restraining apparatus for 10 min a day for 3 weeks prior to behavioral conditioning. The training consisted of stroking the whiskers of row B on one side of the muzzle (conditioned stimulus, CS) with a hand-held fine camel hair brush in a posterior to anterior direction. The brush was held at an angle so that it was parallel to the vibrissae. Great care was taken not to touch adjacent rows of whiskers. The CS lasted 9 sec and consisted of three strokes. One stroke took 3 sec, at the last second of the last stroke a single tail shock (UCS) was applied (0.5 sec, 0.5 mA). The electrical stimulation was discontinued simultaneously with the end of stroke.
ing. The stimulator was controlled manually. After a 6 sec interval the trial was repeated. Pairings were repeated four times/min for 10 min/ d for 3 d. Altogether, these animals (n = 7) received 120 pairings of CS + UCS. One day after the end of training the cortical representation of row B was mapped with 2DG. A second group of mice (n = 4) had the 2DG mapping experiment done with a delay of 3 d after the end of training, and a third one (n = 4) with a 5 d delay.

The control group (n = 4) received only the CS delivered at the same schedule. In a pseudoconditioned group (n = 4) the stimuli were presented as random; these mice received the same number of CS and UCS per session as applied for conditioning. In the extinction procedure, after 3 d of associative CS + UCS conditioning, the mice (n = 4) received whisker stroking only, delivered at the same frequency as during the training, but with no UCS.

In a separate control group (n = 4) of mice the heart rate was monitored during the CS + UCS pairing. Pin electrodes were inserted in to the skin under local anesthesia and an ECG trace was registered with a polygraph.

2-Deoxyglucose Mapping of Cortical Representation of Row B of Vibrissae

Images of the cortical representation of row B of vibrissae were obtained with [14C]2-deoxy-D-glucose functional activity mapping (Sompolinski et al., 1977). 2DG experiments were performed on animals that were trained to respond using the previously established criterion (Kossut et al., 1988), which considered as activated the regions with a level of 2DG radioactivity standards (American Radiolabeled Chemicals). The brains were dissected and cut into 200-μm sections. The sections were rapidly dried on a hot plate and the autoradiograms of sections and [14C] radioactivity standards (American Radiolabeled Chemicals) were obtained on Kodak mammography film. After obtaining the autoradiograms, the brain sections were counterstained with cresyl violet for identification of cortical layers and the barrels in layer IV.

Quantification of the Autoradiograms

The autoradiograms were analyzed with a computer-controlled image analysis system (Visionetics). Images were corrected for film background and optical distortions by subtraction of a clearfield image of the film. A calibration curve was created on the basis of the absolute gray levels of the [14C] standards on the film. The width of the labeled region and its labeling intensity were measured on serial sections through the barrel cortex. Usually about 40 sections from each hemisphere were measured. The software allowed us to display on a computer screen the image of a stained section from which the autoradiogram was obtained, and to mark the barrel outlines, which were superimposed on the autoradiogram so that the relations of the labeled regions to the morphological barrels could be accurately determined. The barrels of row B were measured on all sections (6-9) on which they were visible. The measurements were performed for each barrel, with its long axis as the main orientation. For each barrel the measurement was taken close to its wall with B3. The labeled representation of row B whiskers in layer IV was measured in exactly the same locations (Fig. 1A,B). The labeled representation was also identified and measured on all sections through layers II/III and V, where the barrels were not present. We determined the width of labeling of row B representation using the previously established criterion (Kossut et al., 1988), which considered as activated the regions with a level of 2DG labeling ([14C] concentration) 15% higher than in the surrounding cortex (Fig. 1C). We have found that the 15% difference corresponds to two standard deviations above the mean value of labeling in the surrounding cortex (Chmielowska et al., 1986). Values obtained in histological and autoradiographic measurements were averaged to obtain the mean value of the barrel dimensions and the labeling dimensions for the entire row B. In order to correct for the possible slight differences of the plane of sectioning, which could produce differences of dimensions of row B barrels, the results were initially expressed as a ratio of the width of the labeled area to the width of row B barrels.

In the measurements of labeling intensity the outline of the labeled area was marked on the screen and the average gray level reading was taken. The ratio of labeling on the experimental and control side was calculated for each animal.

Results

Row B Representation 1 Day after CS + UCS Pairing

The effects of training upon row B representation were assessed by comparing the labeling evoked by stimulation of the “trained” row B of whiskers and the control row B, unstimulated during the behavioral training, on the other side of the snout. We previously ascertained (Chmielowska et al., 1986; Kossut et al., 1988) that there were no ipsilateral changes of 2DG uptake produced by unilateral whisker stimulation in the way applied during the 2DG mapping, and therefore we could directly compare the labeling of “trained” and untrained row B in the same animal. We also found previously that in intact untrained mice, the 2DG uptake evoked by stimulation identical to that used in the present experiment applied to a row of vibrissae on both side of the snout is very similar, the width of labeling varying by about 10% (Siucińska and Kossut, 1994b; Jablonska et al., 1995).

Comparison of the extent of 2DG uptake evoked in the barrel cortex by stimulation of the “trained” row B and control row B of whiskers on the other side of the muzzle (not stroked during the classical conditioning) revealed that following training, the “trained” rows had a larger representation in layer IV of all examined animals than the control row (Figs. 2-4). The 2DG labeled representation of row B in layer IV was wider, on the average, by 45% (490 ± 41 μm in control rows and 710 ± 105 μm in trained rows; p < 0.01, Mann-Whitney U test). We measured the ratio of the width of 2DG labeled zone to the width (long axis) of cortical barrel in order to correct for variations in the plane of sections; however, in most cases the long axes of the corresponding barrels of row B in both hemispheres were the same and no corrections were made. We measured the width of labeling rather than the area because in some cases the sections from the level of layer IV did not contain the entire row B, and parts of either B1 or B4 barrel were missing; the width gave us a more reliable measure. All quantitative data come from measurements of autoradiograms of tangentially cut sections; one brain was sectioned parasagittally to visualize the effect in a different plane (Fig. 5). No statistically significant changes in labeling were found in cortical layers other than IV when layers II/III values were averaged together (Fig. 6). If the data were taken only from the deepest four sections of layer III, a statistically significant enlargement of the width of labeling was found (25%, Mann-Whitney U test, p < 0.05) (Figs. 2, 4, 6). The remaining sections of layers II/III, although they did not differ in the width of labeling, as determined by the applied criterion, showed an altered pattern of labeling. Around the central dense core there was a radiating fringe of lower intensity label (Fig. 4). On the control side the labeling terminated more abruptly. The radiating projections were also observed in the labeling of layer IV sections, but they were usually of higher labeling density.

The labeling intensity of row B representation remained unchanged after CS + UCS pairing, as compared to labeling of control row B representation in the opposite hemisphere (mean difference between intensity of labeling between rows B in the two hemisphere amounted to 3 ± 17, Mann-Whitney U test, p > 0.05).
Figure 1. Method of autoradiogram analysis. A, Nissl-stained section at the level of layer IV and pseudocolored autoradiogram of the same section showing row B labeling in a control mouse. Row B labeling marked with an arrow. B, Enlargement of fragments of A. Lines across the labeled zone on the autoradiogram and across the barrels on the Nissl-
In the groups of mice that received only CS or were trained in a pseudoconditioning schedule, in which CS and UCS were randomly applied, no changes in the dimensions or labeling intensity of cortical representations of row B of vibrissae were found (Figs. 3, 6).

**Row B Representation after Longer Delays Post-CS + UCS Pairing and after Extinction Procedure**

The changes in row B cortical representation were tested after a waiting period of 3 and 5 d following training. During the waiting period the animals were left in their cages. Three days after training the row B representation in layer IV was smaller than 1 d posttraining, but still significantly larger than in controls (p < 0.05); 5 d after training it returned to control values (Fig. 6).

In a group where extinction procedure applied for 2 or 5 d after training, no increases of row B representation were observed. Already 2 d of extinction completely obliterated the results of training—the width of the trained row B representation was not significantly different from the control values (Fig. 6). The intensity of 2DG uptake was the same in both hemispheres of control mice and in all experimental groups; it was not affected by any of these experimental procedures.

**Behavioral Results**

During the CS + UCS pairing we observed that the behavior of mice changed visibly. By the end of the first session stroking of row B of vibrissae evoked a rigid posture, signifying an expectation of an unpleasant stimulus. This behavior was not observed in pseudononconditioned mice. In a group of four mice where the heart rate was measured during the CS + UCS pairing, deceleration of the heart rate was observed during the pairing sessions. Heart rate was measured for 30 sec before the first trial in each session when the mouse was in the experimental setup and during the CS applications. The heart rate values were averaged for blocks of 10 trials. The heart rate declined significantly during the experimental session (p < 0.02, ANOVA, mixed design followed by Duncan test). The mean decline during the last block of trials in each session, as compared to the presession rate, was 7% (p < 0.01). Heart rate decelerations developed in all four animals, in two during the first training session and in two during the second day of training.

**Discussion**

**CS + UCS Pairing**

The main finding of this study is that under the influence of a relatively short lasting (30 min), behaviorally salient stimulus, the responses of neurons of mouse barrel cortex can be modified so that the cortical representation of the stimulated mechanoreceptors is enlarged in thalamorecipient layers. This change is transient, no longer observable 5 d after the end of training.

In naive mice, 2DG labeling in the barrel cortex obtained with this type of stimulation overlays the appropriate barrels in layer IV of SI cortex and extends beyond the barrel bound-aries, over the septa and parts of adjacent barrels (Chmielewksa et al., 1986; Siucinska and Kossut, 1994b). This pattern of labeling reflects an activation of barrel neurons by inputs not only from their principal whiskers, but also from adjacent vibrissae, presumably at least partly due to activity of intra-cortical excitatory connections (Armstrong-James et al., 1991).

In untrained animals and on the control sides of mice from all the experimental groups, the ratio of the width of the labeled representation of row B to the long axis of the cor-responding row B barrel was about 1.4. In the trained group, it was 2.03. The expansion of the row representation over the adjacent rows of barrels increased on the average by 245 μm on each side and overlapped about a half of adjacent row A and C of barrels.

Modifications of responses of cortical neurons during the process of learning have been investigated using several experimental models (for review, see Weinberger, 1995). The results of these studies revealed changes in excitability and in receptive field properties. Specific CS-linked alterations in receptive fields were found, such as increases of cortical representation of skin receptors used during conditioning, increases response to frequency of conditioned stimulus and of cortical representation of the tone frequency used as CS. The cause for increasing the cortical representation of sensory input paired with reinforcement may lay with the action of neuromodulatory systems regulating arousal, attention, and the stimulus-to-background ratio in cortical activity (Singer, 1987). It was shown that elimination of cholinergic input prevents plasticity in the adult somatosensory cortex (Juliano et al., 1991) and impairs tactile learning (Jacobs and Juliano, 1995), while in the auditory cortex, microapplications of chol-linergic agonists can modify receptive field characteristics of neurons in a manner similar to those obtained during classical conditioning (Cox et al., 1992). Coactivation of basal forebrain and cutaneous receptors leads to a long-term enhancement of somatosensory evoked potentials (Rasmussen and Dykes, 1988).

Reactivity of cortical neurons is also under very strong control of inhibitory interneurons, as seen by the fact that the GABAergic transmission is influential in shaping the properties of cortical receptive fields (Dykes et al., 1984; Sillito, 1975). The GABAergic system in adult sensory cortex is downregulated by elimination of afferent input (Hendry and Jones, 1988; Warren et al., 1989; Welker et al., 1989a; Skangiel-Kramska et al., 1994). There is also evidence of increased GAD immunoreactivity after prolonged sensory stimulation of vibrissae; however, this stimulation resulted in lowering of me-tabolism of the stimulated rows of barrels, presumably due to habituation (Welker et al., 1989b, 1992). The investigation of interactions of inhibitory and excitatory systems in our conditioning paradigm revealed no changes in the GABA, recept or binding in the trained row of barrels, while the NMDA and AMPA receptor binding was transiently elevated in barrels of the trained row (Skangiel-Kramska et al., 1993).

The stimulus used during the 2DG mapping was different from that applied during the classical conditioning phase of the experiment. This experimental design allowed us to ob-

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**Figure 2.** Examples of pseudocolored autoradiograms of 2DG labeling cortical representation of "trained" (CS + UCS) and control row B in different cortical layers of one mouse. Row B labeling marked with arrows. Scale bar, 500 μm.

**Figure 3.** Examples of pseudocolored autoradiograms and Nissl-stained sections of layer IV of control and experimental hemispheres of mice belonging to A (conditioning [CS + UCS] group) and B (pseudoconditioning). Row B labeling marked with arrows: A, auditory cortex; SII, second somatosensory area. Scale bar, 1 mm. Scale bar, 500 μm.
Figure 4. Representative example of labeling pattern of row B representation in layer IIIb and IV binary thresholded image, showing in uniform black-only areas activated 15% higher than background surrounding cortex. Arrowheads point to row B representation. Scale bar, 500 µm.

Figure 5. Labeling of row B representation in a mouse from CS + UCS group, computer-reconstructed autoradiograms of paracoronal sections cut in a plane orthogonal to rows of barrels according to Chmielowska et al. (1989). A, Experimental hemisphere. B, Control hemisphere. Arrowheads point to cross section of row B representation. Scale bar, 500 µm.
serve changes of responsiveness of neurons in the involved cortical area to a standard stimulus. We therefore described changes in sensitivity to a stimulus of characteristics different than ones used during the training, but applied to the same receptive surface. This is similar to the phenomenon of reorganization of hand representation after operant conditioning described by Recanzone et al. (1992a), where changes in properties of cortical neurons were induced by training with a high-frequency flutter stimulus, but the spatial maps of the cortex were obtained with fine-pressure probes. The experiments of Recanzone et al. (1992b) demonstrated also that discrimination training brings about changes in temporal properties of neurons in the part of SI cortex where the trained skin area is represented. Moreover, some of these changes in area 3b can be correlated with perceptual abilities of the trained animals. The very simple classical conditioning paradigm used in the present study, cannot be compared with discrimination training used by Merzenich’s group or the roughness discrimination by vibrissae employed by Guic et al. (1993), which produced large changes of cortical maps. However, the restricted changes of topographical maps observed in our study also represent remodeling of the cortex by the process of learning.

Laminar Localization of the Body Map Change

In view of evidence suggesting the importance of intracortical connections, especially via supragranular layers, in plastic changes of barrel cortex of adult rats (Fox, 1992; Armstrong-James et al., 1994; Diamond et al., 1994; Glazewski and Fox, 1994), it is puzzling that this brief training regime influenced mostly the vibrissal representation in layer IV. We cannot attribute our result to peculiarities of the 2DG method, for example, preferential labeling of layer IV by 2DG, because in our previous experiments large increases in the cortical representation of whiskers spared from denervation or sensory deprivation were observed not only in layer IV but also in supragranular and especially infragranular layers (Kossut et al., 1988; Siucinska and Kossut, 1994a). Similar localization of changes in cortical representation after classical conditioning using a single vibrissa was obtained by Hand and Hand (1995), who employed a long lasting (60 d) appetitive training procedure. This effect is therefore not due to the changes being observed “in statu nascendi.” Indeed, the recent papers describing plastic changes in neuronal responsiveness after application of a whisker-pairing paradigm in rats, when all vibrissae except two adjacent ones are clipped close to the skin, report that altered receptive fields can be observed in neurons of nongranular layers already after 24 hr (Diamond et al., 1994) or that long latency responses of the barrel neurons, of intracortical origin, are the first to show receptive field changes (Armstrong-James et al., 1994). These changes later decrease and are replaced by short latency (presumably thalamocortical) alterations in receptive fields (Armstrong-James et al., 1994). It is therefore likely that in our experi-

Figure 6. Width of row B representation in all experimental groups, in cortical layers II/III, IV, and V. The values represent the mean width of labeling of row B representation ± standard deviation. *p < 0.05, **p < 0.01, Mann-Whitney U test. n = 7 in CS + UCS group, n = 4 in all other groups.
mental animals the plastic changes have already proceeded far enough to be localized in primarily in layer IV. It is also possible that the classical conditioning paradigm that we used may trigger different circuits subsuming plastic changes than deafferentation or pairing-induced plasticity of representational maps, circuits where the thalamocortical component is dominant. For example, the efficiency of thalamocortical synapses was found to be altered in the motor cortex during associative conditioning (Meftaf and Rispał-Padé, 1994). Alternatively, this specific form of CS or UCS stimulation may result in different competitive or modulatory input consequences in the barrel field. Interestingly, training induced changes in cortical representation of fingers in monkeys were observed by Recanzone et al. (1992a) at cortical depth of 700–900 μm, which include layer IV.

Pseudoconditioning

The close temporal spacing of trials during conditioning sessions made it possible that sensitization and not conditioning has occurred. The controls where pseudoconditioning was applied served to clarify this situation. However, no significant change in cortical representation of row B were found after the pseudoconditioning, indicating that sensitization was not the cause of the observed body map change.

Duration of Training

We did not map the cortical representation after conditioning training shorter than three sessions. Changes in unit response have been observed by Edeline et al. (1993) after a very short conditioning training in auditory cortex (five trials, 30 sec of CS). Very fast changes were also found, with 2DG autoradiography, in gerbils auditory cortex following aversive conditioning (Scheich et al., 1995). The changes produced by nonassociative learning, habituation, in cortical maps of vibrissae seem to require more time—as previously mentioned by Hand and Hand (1995). They observed cortical maps changes after 10 sessions but not after 3 sessions of unpaired passive whisker stroking. We are presently trying to determine the minimum duration of associative pairing necessary to produce a change of a cortical vibrissal map.

Duration of Body Map Change

The enlargement of the representation of “trained” row B in this study was not long lasting. Five days after the training no changes in the labeling dimensions were observed. Three days after the training the enlargement was still present in layer IV (although it was not as pronounced as 1 d after the training) but disappeared from layer IIIb. This spontaneous disappearance was accelerated by application of extinction procedure, believed to disrupt the effects of conditioning. In this case, following 3 d of training, a daily session of CS only was given. After 2 d of this treatment the enlargement of the row B representation was no longer seen. Again, the presence of this effect strengthens the notion that the observed change is related to the learning process.

The spontaneous disappearance of the observed change is in variance with the results of Weinberger et al. (1993) who found that in the auditory cortex of guinea pigs changes in receptive field properties induced by classical conditioning persist for as long as 8 weeks. The differences in the animal species, sensory modality, and finally a much longer duration of the UCS (2 sec vs 0.5 sec) can account for these differences.

The experiments described above were not designed to map a memory trace in the brain, but to induce an experience-dependent change in a cortical body map. The training procedure was to provide an intensive stimulation of the vibrissal pathway coupled with aversive reinforcement. The observed enlargement of the row B cortical map represents a plastic change in the cortical representation induced by associative training; therefore, a learning-dependent enhancement of the power of a sensory pathway. This change may, however, constitute some part of the memory trace, since it disappears when extinction procedure is employed. In any event, this model should provide a convenient procedure for conducting pharmacologic and genetic manipulation studies of adult cortical plasticity mechanisms involved in changes induced by both conditioning and extinction.

Notes

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