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ISAS - INTERNATIONAL SCHOOL FOR ADVANCED STUDIES

LEARNING DEPENDENT MODIFICATIONS IN THE CORTICAL REPRESENTATION OF THE VIBRISSAE IN ADULT RATS

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ACKNOWLEDGMENT

GOOD HELP IS HARD TO FIND...

...Thanks to those who gave it, when I needed it*.

Mathew Diamond

Rasmus Petersen
Justin Harris

(In alphabetical order)
Kevin Ainger
Tuncay Altug
Andreas
Blasie Aguera y Arcas
Resit Canbeyli
Onur Celikel
Guido Comar
Irina Erchova
Adrienne Fairhall
Claudio Fedele
Micaela Galante
Sabina Gionotta
Tatjana Gorski
Murat Gulsoy
Irina Harris
Heike
Galma Jahic
Ida Kozma
Adnan Kurt
Irene Martin
Natasa Savic
Daniela Schulz
Mohinish Shukla
Brenda Townes
Ziyneti Ulku

*Not necessarily during or for my Ph.D. work

INTRODUCTION

BASIC OBSERVATIONS CONCERNING CORTICAL PLASTICITY

One of the cardinal questions in neurosciences is how the nervous system adapts to the ever-changing environment. A number of methods, from classical and instrumental conditioning to sensory deprivation and sensory training have been developed and applied to a variety of species ranging from *Aplysia californica* and *Caenorhabditis elegans* to Guinea pig (Robertson and Irvine, 1989), raccoon (Rasmusson, 1982), mouse (Van der Loos and Woolsey, 1973), rat (Waite and Taylor, 1978), cat (Kalaska and Pomeranz, 1979), New and Old World monkeys (Merzenich *et al.*, 1983, 1984), and human (Brion *et al.*, 1989). From the ubiquitousness of cortical modifiability, it seems safe to include that it is a property present throughout the course of mammalian evolution.

Aforementioned studies have shown that the functional circuits of the brain are not static, but are actively shaped by ongoing sensory and motor experience, not only in the developing brain but also in the mature brain. Adult cortical plasticity was first described in the somatosensory cortex and studied in detail in both somatosensory (Kaas 1983, 1991) and auditory primary sensory cortex (Irvine, 2000) as well as the visual cortex of primates and lower mammals (Gilbert, 1998). These studies showed that sensory cortex possesses the capacity to adapt to changes in an animal's sensory experiences. Functional and anatomical modifications after alterations in sensory information have been studied in mammals using a wide variety of manipulations from partial denervation (reviewed in Kaas *et al.*, 1983), sensory organ damage (Knecht *et al.*, 1998), and training (Jenkins et al, 1990), to intracortical microstimulation (Recanzone *et al*, 1992b) and sensory learning (Recanzone *et al.*, 1992a, 1993).

The sensory cortex as a model

Experience-dependent plasticity can be most easily studied in detail in the primary sensory areas of mammalian cerebral cortex and this will be the focus of the thesis. Sensory cortex possesses several advantages that lend it to the study of plastic changes. These include:

1. Easy access in the intact animal: Primary sensory cortices, by virtue of their position of the brain surface, are readily accessible by physiological probes for neural responses in the whole-animal preparation.

2. Map-like organization of the primary somatosensory cortices: Primary sensory cortices as well as subcortical components of the sensory pathways consist of topographic maps of the receptors at the body surface. This map-like anatomical structure makes it possible to specify the locus to be studied after modifications in peripheral organ use, and to document within-animal and across-animal variations.
3. Well studied neuroanatomical connectivity between loci of the sensory pathways: Sensory pathways starting from the peripheral sensory receptors to higher order cortical sensory areas have been studied in-depth for about a century and the neuroanatomical connectivity of the sensory pathways are described in detail in a wide range of experimental animals.

The first demonstration of plasticity in the adult cortex was the study of Kalaska and Pomeranz (1979), which investigated the functional reorganization of the kitten and cat primary somatosensory cortices (SI) after chronic paw denervation. In normal cats and kittens, neurons in the paw region of the SI exclusively respond to the inputs from the contralateral front paw, but not from the wrist, or forearm. Deafferentation of the paw results in a functional reorganization such that the neurons that previously responded to paw (now deafferented) start to respond to wrist stimulation.

Topographical reorganization of the sensory maps following chronic nerve denervation has been further studied in adult owl and squirrel monkeys to describe the time course of reorganization of sensory representations in higher mammals (Merzenich *et al.*, 1983, 1984). Monkey SI contains two complete and highly topographic sensory maps of the body surface within cytoarchitectonic Areas 3b and 1 (Merzenich *et al.*, 1978). Each area receives topographically organized inputs from the sensory surface receptors of the body. These maps of the body surface have been shown to undergo, as do the kitten and cat primary somatosensory cortices, reorganization of the sensory representation after manipulation of the peripheral somatosensory receptors. Cortical map plasticity is reviewed in more detail below.

Following peripheral denervation (nerve section, digit amputation, or local anesthesia application) somatotopic reorganization of the monkey primary somatosensory cortex seems to include at least three types of changes in the deafferented neurons' responsiveness. The first one is seen immediately after the denervation of the peripheral receptors and is characterized by unresponsiveness of the cortical neurons to stimulation of any site. Functional reorganization of the deprived cortex starts when deafferented neurons show responsiveness to sensory receptors

neighboring their original receptive field. Although in some cases this reorganization can start immediately after the denervation of their principal sensory receptors (Calford and Tweedale, 1988), complete functional reorganization of the previously 'silent' cortex takes up to 22 days (Merzenich *et al.*, 1983a). An additional form of reorganization can occur whereby topographically distant and separated sensory receptors (rather than neighboring ones) can come to be represented in the previously 'silenced' cortex. These 'new' representations have been found after median nerve section (Merzenich *et al.*, 1983a). In SI of the monkey, *glabrous* surfaces of digits 1,2, and 3 of the hand normally activate neurons in the areas 3b and 1 of SI, via the median nerve. After median nerve section, new representations of the *dorsal* surfaces of the digits 1,2, and in part 3 have been found in the locations previously responsive to stimulation of the glabrous surfaces of these digits.

As suggested earlier, changes in the functional organization of the brain occur not solely after pathological alterations of the sensory organ, but also after differential use of sensory organs during learning. Several such forms of plasticity have been demonstrated in a variety of models including monkey (e.g. Jenkins *et al.*, 1990) and humans (e.g. Braun *et al.*, 2000). In their classical studies, Merzenich and colleagues showed that the response properties of the neurons in primary somatosensory cortical area 3b were altered during preferential use of one fingertip to attend a tactile stimulus to receive food reward (Jenkins *et al.*, 1990) or during learning of a frequency-discrimination task (Recanzone *et al.*, 1992).

Expanded cortical representation of the utilized receptors – A general rule?

All these different kinds of experimental manipulations, pathological or innocuous, seem to include as a common component of the cortical modification an increased response in SI (both in magnitude and in spatial extent) to stimulation of the spared/trained part of the sensory apparatus. A main focus of this thesis is to test the idea that increased use of one part of the sensory apparatus inevitably leads to an expanded cortical representation of the utilized receptors. Although much of our understanding of cortical plasticity comes from studies of primate somatosensory system, a fact evident in the preceding sections, we have elected to examine the "expansion hypothesis" using the vibrissae system of rats. This sensory system has received growing attention because of a number of advantages:

1) Capacity to carry out non-invasive, reversible modifications of the sensory receptors. Facial hairs (vibrissae, or whiskers) are readily accessible on the snout of the animals

and 'sparing' and 'deprivation' protocols can be easily applied without invasive surgery. Manipulations can be "reversed" simply by allowing the vibrissa to regrow.

2) Capacity to deliver reproducible stimulation of a single vibrissa. The discrete nature of the receptor distribution in this sensory system makes it possible to deliver a desired stimulus repeatedly in a highly controlled and reproducible manner, equivalent for the different whiskers in the same or different subjects. Similarly, the topographic organization of the barrel field make between-animal comparisons more reliable than in primates. This is especially important in electrophysiological studies where the responsiveness of the neurons is defined by averaging neural responses over a number of stimulus presentations.

3) Observable animal behavior. The animals' vibrissae use can be observed and quantified in a number of different paradigms.

4) Demonstrated role of sensory cortex. It is known that learning and performance of several tactile behaviors (Hutson and Masterton, 1986; Guic-Robles *et al.,* 1992) depend on primary somatosensory cortex. This makes sensory cortex a likely site of learning-related modifications.

5) Plasticity in adults as well as young animals. Any model system developed to study brain plasticity should preferably show plastic changes not only during development but also in the adulthood. Alterations in the neural responsiveness of the barrel field neurons to the changes in the sensory organ use are seen both in the adult and developing primary somatosensory cortices.

The aforementioned features of the whisker-to-barrel field pathway in rodents make it an important model for the study of brain plasticity.

FUNCTIONAL CONNECTIVITY IN THE VIBRISSAE-TO-BARREL PATHWAY

Vibrissae taxonomy

Vibrissae are not unique to the rats and rodents; they are found in all mammals, except humans, studied (see Figure 1).

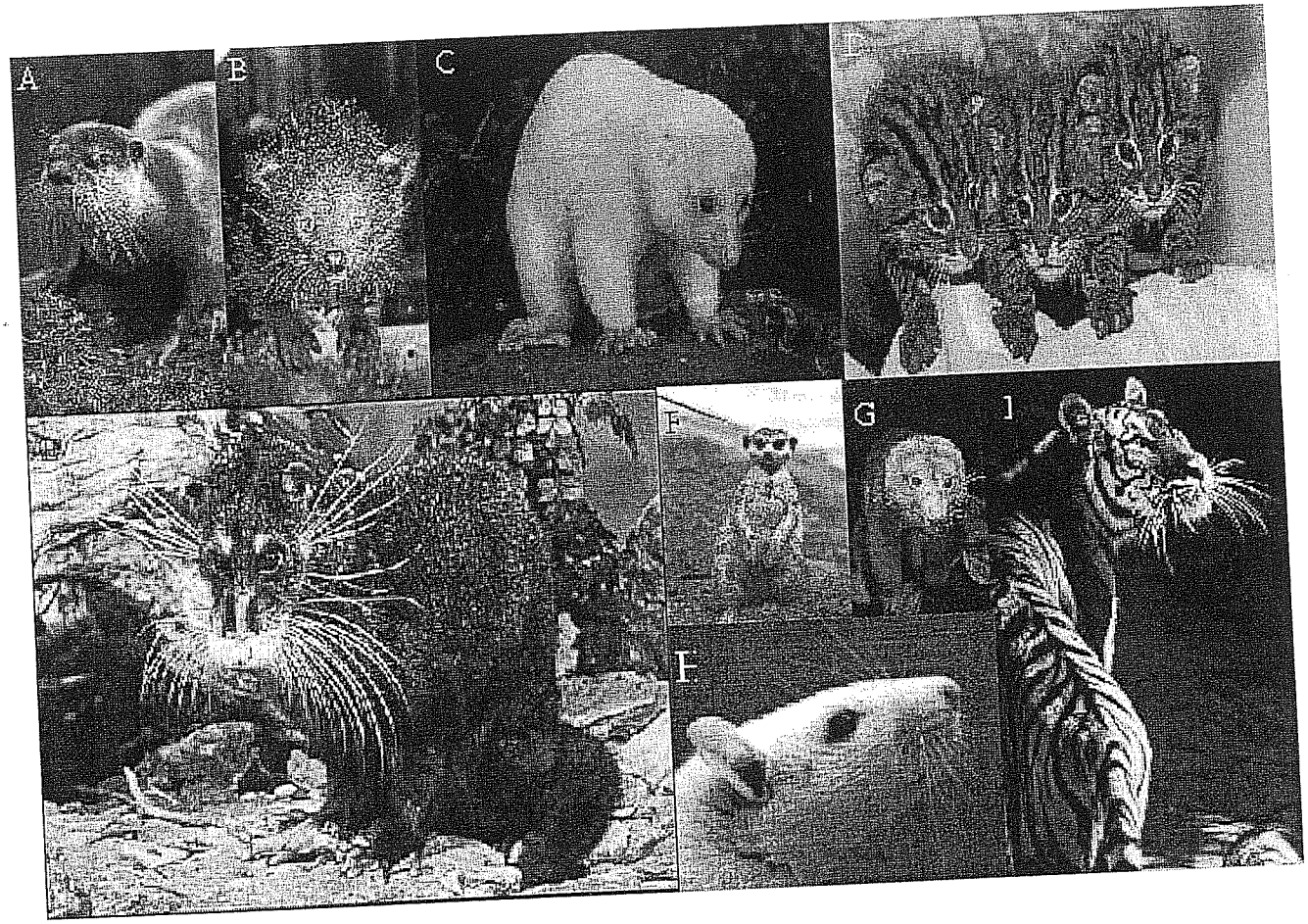


Figure 1. Vibrissae in all mammals. The length and the number of the vibrissae vary across species, and correlate with the type of habitat in which the animals live. (A) Asian small clawed otter, (B) Bornean binturong, (C) Cuscus, (D) Kittens, (E) Otter-civit, (F) Southwest African meerkat, (G) Spotted cuscus, (H) Rat, and (I) Tiger. (All except (H) from the collection of San Diego zoo at **www.sandiegozoo.org/postcards/send.php3**. (H) from **www.neurobio.pitt.edu/barrels/**

Mammals that lack vibrissae as adults (i.e. Rhinocerotide) possess them *in utero* but lose them before birth (Cave, 1969). The vibrissae of the mammals can be classified as *facial* and *body*. Even though the location and number of vibrissae differs significantly between species, the most common plan comprises vibrissa groupings on (i) the palmar surface of the forearm near the wrist (so called ulnar-carpal vibrissae), (ii) the forearm (medial antebranchial vibrissae), (iii) skin near the elbow (anconeal vibrissae), (iv) the medial side of the ankle (calcaneal vibrissae), (v) the ventral surface of the body (venter vibrissae), and (vi) the face. The facial vibrissae pattern is largely conserved across species (Ahl, 1986). Vibrissae are usually located (i) on the muzzle

(mystacial vibrissae), (ii) dorsal to the muzzle (rhinal vibrissae), (iii) over the eye (supraorbital), (iv) beneath the eye (suborbital) and (v) caudal to jaw and eye (genal vibrissae).

Mystacial vibrissae: The major tactile organ of rodents

In rodents, mystacial vibrissae (called simply *vibrissae* or *whiskers* hereafter) are organized in a grid-like matrix on the muzzle, consisting of 5 rows (A through E) and 4 to 7 columns (Figure 2).

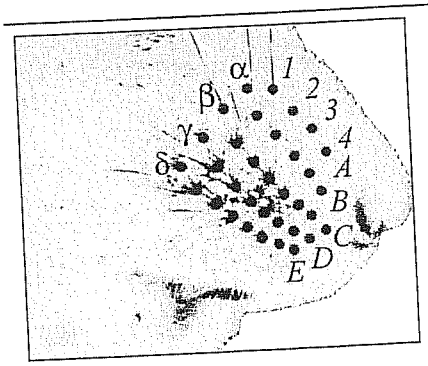


Figure 2. Vibrissae of the rodents organized in a Manhattan-like of grid. The vibrissa rows are designated with capital letters ('A' through 'E'). Each row consists of 4 to 7 caudo-rostrally organized. Arcs of vibrissae span the rows; e.g. Arc 2 = A2-B2-C2-D2-E2. An additional 4 vibrissae, named α, β, γ, δ, are located caudal to arc 1. (Adapted from Woolsey and Van der Loos, 1970)

Early observations concerning the importance of the whisker system as a tactile organ came from the studies of Broughton (1823) and Odenius (1866) who studied the connectivity between vibrissae and their massive nerve supply (briefly reviewed in Vincent, 1912). From these studies, it was suggested that the nocturnal natural history of rodents implicates the vibrissae as one of the essential sensory organs.

Initial behavioral observations confirming the importance of vibrissae came from studies of Richardson (1910) and Vincent (1912), who described how the vibrissae are used in a task that requires exploration of a complex environment. Running along a raised labyrinth towards a goal, rats moved much more slowly and made many navigational errors after clipping of the vibrissae (Vincent, 1912). Vibrissae-clipped rats sometimes even fell from the platform.

Only in the 1970s did researchers begin to quantify role of the vibrissae using controlled tasks. Schiffman and others studied rats (1970), and hamsters (1971) to show that rodents use vibrissae to perceive depth. They also demonstrated that use of vibrissae to perceive depth changes as a function of age and photic conditions. Depth perception is a part of active exploration of the environment. Vibrissae are used to collect information about the immediate environment of the animal. Whether the vibrissae have a "survival value" (which would be expected if the vibrissae are the dominant sensory organs) or, alternatively, their loss of function

can be substituted by other sensory organs, is largely unanswered. Pearson (1962) questioned the survival value of the vibrissae in an ecological setting. He first caught wild mice and then divided them into three experimental groups, two receiving either unilateral or bilateral vibrissae trimming, and the last receiving no vibrissae trimming. After the mice were tagged, they were released to the wild and retrapped after 3-6 weeks. Pearson found that clipping vibrissae did not affect the probability of a mouse's survival. The results suggested that loss of vibrissae might be substituted by some other tactile or non-tactile sensory organ(s). Nonetheless, it is also possible that because vibrissae grow about a millimetre a day (unpublished observations; also see Lillesaar and Hildebrand, 1999), they had already regrown and regained their functionality before the animals were retrapped. Hofer and others overcame this complication by cutting the infraorbital nerve, which connects the vibrissal follicles to the trigeminal ganglion (Hofer *et al.*, 1981). They operated on rat pups at different postnatal ages (day 1-17), and found that if sensation through the vibrissae was blocked, pups stopped sucking for food, lost weight, and some died after 3-6 days of weight loss. Thus, vibrissae are used to actively explore the environment, and loss of vibrissal sensation after infraorbital nerve cut cannot be substituted (at least in pups) by other sensory modalities.

During the last two decades, researchers have investigated (i) the role of vibrissal information in higher order cognitive processes (i.e. sensory discrimination, learning and memory) and (ii) the brain loci where vibrissal information is integrated to subserve learning and memory functions. One important step in the inquiries came from Hutson and Masterton's influential study, where they showed that rats could learn a perceptual task depending solely upon vibrissal sensation and, equally important, that one of the loci of vibrissal sensory learning was the barrel field of primary somatosensory cortex. Their behavioral paradigm – which we have also adopted in the present study – was the gap-crossing paradigm. There is also evidence, however, that the vibrissa-barrel cortex system is fundamental to roughness discrimination (Guic-Robles *et al.*, 1989; Cybulska-Klosowicz and Kossut, 2001), numerical discrimination (Davis *et al.*, 1989), size discrimination (Tomie and Whishaw, 1990), width discrimination (Krupa *et al.*, 2001) and distance discrimination (Shuler *et al.*, 2001) (also see Table 1).

Vibrissal role in	Species studied	Citation
Aggressive behavior	**Mouse**	Katz, 1976
Depth perception	**Rat**	Schiffman *et al.*, 1970
Distance assignment and discrimination	**Rat**	Richardson, 1910
	Rat	Shuler *et al.*, 2001
Edge and distance detection	**Rat**	Hutson and Masterton, 1986
	Rat	Harris *et al.*, 1999
Locating source of food	**Pig**	Morrow-Tesch and McGlone, 1990
	Rabbit	Hudson, 1985
	Rat	Hofer, 1981
Intraspecies communications	**Rat**	Ahl, 1986
Maze exploration	**Cat**	Vincent, 1912
	Rat	Mirabella, 2000
Numerical discrimination	**Rat**	Davis *et al.*, 1989
Orientation	**Kitten**	Larson and Stein, 1984
	Rat	Hofer *et al.*, 1981
Predatory behaviors	**Rat**	Ahl, 1986
Size discrimination	**Rat**	Tomie and Whishaw, 1990
		Brecht *et al.*, 1997
Somatosensory conditioning	**Rat**	Landers and Sullivan, 1999
Swimming	**Rat**	Ahl, 1982
Texture discrimination	**Mouse**	Cybulska-Klosowicz and Kossut, 2001
	Rat	Guic-Robles *et al.*, 1989
		Mirabella, 2000
Vibration detection	**Seal**	Dehnhardt *et al.*, 1995, 2001

Table 1. Vibrissae as multi-functional tactile organs. Facial vibrissae take a role in many kinds of sensory processing

Vibrissae-to-barrel field pathway: Anatomy of the functional connectivity between peripheral sensory organs and primary somatosensory cortex

The vibrissa is a thickened hair structure whose base is situated in a follicle. Each follicle is innervated by a separate branch of the vibrissal nerve, called the infraorbital nerve, containing about 100-250 myelinated fibers, which carry sensory information about the movement of the vibrissae (Vincent, 1913; Rice *et al.*, 1986). Cell bodies of the infraorbital nerve fibers are located in the ipsilateral trigeminal ganglion, and the first axonal termination is in the trigeminal nuclei of the brain stem. Trigeminal nuclei consist of the principal sensory nucleus (PrV) and the spinal trigeminal nuclei (SpV). The PrV, as well as the magnocellular portion of the the spinal trigeminal nuclei (spinal interpolaris, SpVi) represent the vibrissae in a topographic manner. The somatotopic map at this level is represented by clusters of neurons called **barrelettes** – see Figure 3a (Ma and Woolsey, 1984). The trigeminal nuclei project to cerebral cortex after a relay in the thalamus; they also project to cerebellum via superior colliculus. Additionally, they project to the lateral subnucleus of the facial nucleus contra- and ipsilaterally. Still another projection from these nuclei includes feedback connections with the papillary muscles surrounding each vibrissa.

Thalamus plays a relay-center role for sensory information, before transmission to cortical processing centers (Sherman and Guillery, 2001). There are two nuclei, ventral posteromedial (VPM) and posterior (POm), in the dorsal thalamus which receive projections from the trigeminal nuclei. Projections at this level of sensory pathway are somatotopic (Van der Loos, 1976; Nothias *et al.*, 1988) and the representations of the vibrissae at this level take the form of **barreloids** – see Figure 3b. VPM projects densely to the posteromedial barrel subfield of the primary somatosensory cortex, **barrel cortex**, and diffusely to the secondary somatosensory cortex (SII) (Diamond, 1995). Although the main sensory input to the barrel cortex is through VPM, there are two additional projections carrying sensory information to the cortex. The first of these is from POm, whose axons terminate exclusively in extrabarrel space (i.e. septa) in the barrel cortex. A second portion of the posterior complex, termed caudal PO (POc; see Diamond, 1995) projects to SII and motor cortex; POc may also be the source of a small projection to the septa of barrel cortex. POm and POc project to the dysgranular portions of SI, for example between the whisker representation and the forelimb representation.

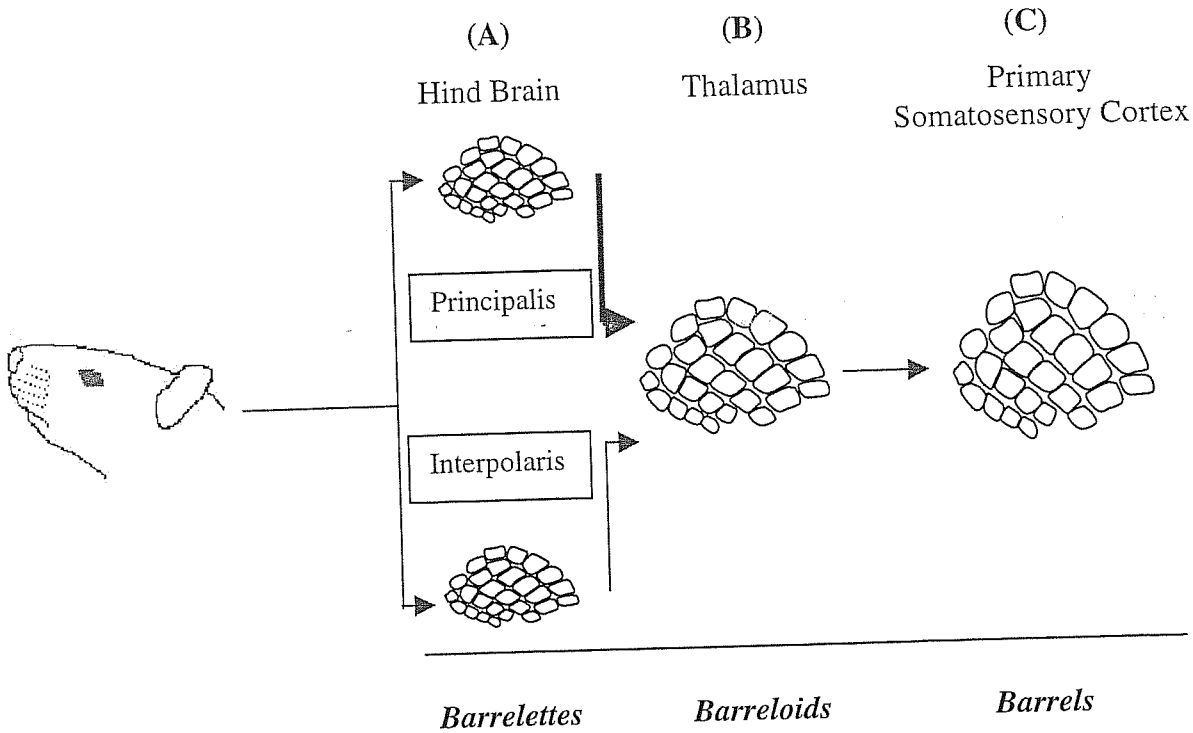


Figure 3. Somatotopic maps of the vibrissae in several stations of the sensory pathway. **A)** Orderly projections from the vibrissae are preserved in some of the trigeminal nuclei of the hind brain. **B)** Vibrissae representations in the VPM thalamus are termed barreloids. The size of vibrissae representations in this relay center is bigger than the barrelettes in the hind brain, **C)** Cortical representations of the vibrissae (i.e. barrels) are the largest representations of the vibrissae.

VPM projections to the barrel cortex terminate in layer IV and, to a lesser extent, in layer III (Lu and Lin, 1993). The granular layer of the SI cortex has two cytoarchitectonically distinct and interposed regions (see Figure 3b) termed barrels and septa (Woolsey, and Van der Loos, 1970). Barrels are cell-dense structures in layer IV with flame-like extensions into layer III. Septa contain fewer cells and are interposed between barrels (Woolsey, and Van der Loos, 1970). Barrels and septa have different inputs from the thalamus. Barrels receive projections from the VPM; in contrast septa receive projections mainly from POm and to a lesser extent from POc (Koralek *et al.*, 1988; Lu and Lin, 1993). Neurons in the barrels, then, project vertically mainly to the supragranular layers, and horizontally to the other neurons in the same barrel and to surrounding septa (Kim and Ebner, 1999). Projections to the septa, in turn, are not distributed through the thickness of the cortex, but are restricted to the close-by septa.

Neurons in the infragranular layers of barrel cortex receive connections from neurons in granular and supragranular layers, and project efferents to adjacent cortical areas. Although the

major output of infragranular neurons is to secondary somatosensory cortex (SII), there are also projections to surrounding dysgranular cortex, motor cortex, posterior medial parietal cortex (Staiger *et al.*, 1999), and to many subcortical centers.

FUNCTIONAL PLASTICITY AFTER ALTERATIONS IN VIBRISSA USE

Expanded cortical representation of spared vibrissae

As Kossut (1985) originally noted, functional representations of the vibrissae are modified by alterations in vibrissa use. Although the history of the study of functional plasticity in the vibrissae system does not reach back as far as that of other sensory modalities. A large variety of techniques ranging from 2-deoxyglucose (2-DG) and immediate early gene mapping to intrinsic optical imaging and electrophysiology have been utilized to examine functional plasticity in the vibrissa-to-barrel neuroaxis.

Several early studies made use of the 2-DG autoradiography method to describe experience-dependent changes in developing and mature barrel cortex. In her original study, Kossut (1985) spared one vibrissa (all others clipped) for varying periods prior to a 2-DG experiment. In the intervening period (days or weeks), rats were left in their home cage. The spatial extent of the metabolic activity associated with a vibrissa increased when that vibrissa was spared. Subsequently, Simons and Land (1987) studied the change in the neural responsiveness upon vibrissa-sparing using electrophysiological recordings. After trimming a row of vibrissa in the neonatal rats for 45-60 days and allowing them to regrow for 3-15 weeks, they found that representation of the intact vibrissae neighbouring the cut row increased. Independently from the experimental method used, both studies have concluded that changes in vibrissa use alters barrel cortical functional organization.

Factors influencing somatosensory system plasticity

When plastic changes in the central representations of the somatosensory system were first described, it was assumed that such reorganization occurred at the cortical level (Merzenich *et al.*, 1983). It was believed that cortex, but not thalamus or the other subcortical structures, modifies its organization by increasing or decreasing the connectivity between columns of neurons (Merzenich *et al.*, 1983). However, later studies showed that under some circumstances plasticity

may also occur in the subcortical stations of the somatosensory pathway (reviewed by Jones,2000). They also suggested that the nature, extent and magnitude of the plasticity depend upon a number of experimental factors. Below, we will summarize the factors that influence plasticity, and subsequently we will discuss the locus and mechanisms of plasticity.

Age: The differences in plasticity between the developing and adult brain have been studied mainly using anatomical methods, and these studies showed that the brain responds to the alterations in the peripheral organ use in distinct ways at different ages. One remarkable finding in support of this conclusion came from the measurement of barrel size after cauterization of a row of vibrissae at different ages: representations of the cauterized vibrissae diminished in size only if the cauterization was performed before the age of 7 days (PND-7) (Woolsey and Wann, 1976). Cauterization after PND-6 did not induce changes in the size of the anatomical representations of vibrissae (the barrels). However, intracortical connectivity of barrel field neurons is influenced by sensory input even at later ages. When the sensory input from a subset of vibrissae was blocked for several weeks by sensory nerve sectioning at PND-7, intracortical projections in the deprived barrels decreased relative to the normal controls, although the 'map' organization of layer IV – the arrangement of barrels -- did not change (McCasland *et al.*, 1992). When the sensory input of the vibrissae was blocked after the animals reach to adulthood, on the other hand, intracortical projections were not reduced (Kossut and Juliano, 1999). *Critical periods* of the plastic changes in anatomical representations of vibrissae and intracortical projections of barrel cortex neurons suggest that there might be multiple mechanisms of plasticity which control the organization of barrels and horizontal connectivity between neurons differently in developing and adult brains.

Method of deactivating sensory inputs: The majority of studies in the field of sensory cortical plasticity described changes in the anatomical and functional representations of the sensory organs after amputation, nerve block, or sensory training. These manipulations produce different kinds of reorganization both in cortex and at subcortical levels. For example, a relatively short period of vibrissa sparing in juvenile animals leads to reorganization of the representation of the spared vibrissa in SI, but not in the subcortical loci of the pathway (Wallace and Fox, 1999). However, if the modifications in vibrissa use are performed through injecting local anaesthetics in the vibrissal pad, changes occur concomitantly in SI and subcortical centers (Faggin *et al.*, 1997).

Changes in sensory receptor use (not associated with any peripheral nerve damage) tend to produce exclusively cortical plasticity, whereas lesions tend to produce both cortical and subcortical changes. Besides the locus of plasticity, the type of manipulation also affects the time course of the plasticity. Use-dependent plasticity induced by stimulation of a sensory organ in a training task, or sparing a vibrissa without damaging the peripheral nerve, is typically induced more gradually (days to weeks) than lesion-induced plasticity, which can occur immediately following denervation of the sensory organ in both cortical and subcortical centres of the somatosensory neuroaxis (reviewed by Jones, 2000).

Quantitative extent of peripheral manipulation: Local and mid-ranged interactions between neurons and columns in the cortex are believed to have a computational role in processing the information gained through peripheral sensory organs (Philips and Singer, 1997). In the case of vibrissa representations, the functional units of the brain perform this computation through inhibitory and excitatory interactions between neurons and columns. Because functional units of the cortex and thalamus interact with each other during normal functioning, modifications in the number of whiskers activating neighboring barrels and barreloids might be expected to alter the extent, and perhaps nature of this interaction. This notion has been supported by experimental studies in which animals were subjected to restricted sensory experience with some combination of whiskers left intact, and the others trimmed (Wallace and Fox, 1999). They showed that plastic changes (i.e. potentiation) in responses to spared vibrissae occurred faster in animals with multiple vibrissae spared. Similarly, the amount of the depression in the responses to the deprived vibrissa stimulation was greater when more vibrissae were spared (Wallace and Fox, 1999). Clipped whisker representations seem to lose a "competition" for cortical space with the intact whiskers, and the loss is greater when the number of spared whiskers is larger.

Type of stimulation: The type of stimulation during the "mapping" session also determines the nature of cortical representational plasticity. Braun and colleagues (2000) first trained subjects to discriminate the orientation of stimuli applied on the thumb and 'pinky', simultaneously. In the testing session, they used fMRI to quantify the distance between the representations of the two fingers during the discrimination task, and during passive stimulation of the two fingers. Although the stimuli used for both conditions was the same, representations of the fingers become spatially distant when subjects were required to carry out the discrimination task,

while finger representations overlapped when the subjects were not required to make any discrimination.

Availability of loci of circuitry: The layers of neocortex have distinct roles in processing information coming from the peripheral sensory organs. The granular layers, of course, receive the main sensory input from the thalamus. Supragranular layers receive input from the granular layers and project intracortically to other cortical columns. Infragranular layers collect information from other layers and other columns of cortex and project to close-by parietal cortical regions (for example secondary somatosensory cortex, and surrounding dysgranular cortex). How is sensory processing influenced by selective removal of one part of this circuitry? Huang *et al.* (1998) examined the vibrissa-pairing-inducedreceptive field plasticity in the granular and infragranular layers after lesioning the supragranular layers, and found that supragranular layers play a role in the generation of *normal* receptive fields and are required for the full expression of vibrissae-pairing plasticity in the other layers.

In this thesis, we wanted to further describe the variables effecting cortical plasticity. We first studied how vibrissa use in the home cage for 13 days altered the functional organization of the supragranular layers of barrel cortex. We then asked whether use of the vibrissa In a perceptual learning task for 13 days would cause a different sort of alteration in the functional organization of the supragranular layers of barrel cortex. In order to compare the types of modifications seen in different laminae of the cortex, we also studied infragranular layer plasticity in another group of animals after 13 day long alteration in vibrissa use in home cage or in a perceptual learning task (see Methods).

Another objective of the current serie of experiments was to study the possible mechanism(s) of the cortical plasticity after home cage as well as learning experience. To address this objective we studied plasticity in the supragranular layers after differential time courses (i.e. 13 vs 26 days of alterations in vibrissa use) with a variety of vibrissa use (see Chapters 3-4).

The results of the studies are discussed in terms of the role of type of sensory organ use, time span of altered experience and laminar differences in cortical plasticity (Chapter 5).

Chapter 2. METHODS AND OVERVIEW OF EXPERIMENTAL DESIGN

INTRODUCTION

As one of the purposes of this thesis is to investigate how the precise use of a vibrissa shapes its cortical representation, the study required a paradigm to force rats to utilize their vibrissae in specific, documentable ways.

Hutson and Masterton developed an ecologically valid behavioral task, the so called gap-crossing task, which took advantage of the active use of vibrissae by rodents during exploration (Hutson and Masterton, 1986). The apparatus consists of two elevated platforms, placed end-to-end with a gap between them (see below for details). Hutson and Masterton showed that, when a blinded rat is put on one of the platforms, and left to explore the apparatus, the animal used its vibrissae to collect information about the availability of the opposite platform. They further showed that when vibrissae of the animals were cut subjects failed to perform the task successfully suggesting that the success in the task is vibrissa-dependent. Ablating barrel cortex had the same effect as cutting the vibrissae (absolute failure to perform the task), pointing to barrel cortex as an essential component of the neuronal system involved in gap-crossing.

More recently Guic-Robles *et al.*(1992) studied roughness discrimination in the gap-crossing apparatus and showed that when the barrel cortex of the rat is lesioned, subjects which already learned how to gap-cross using their vibrissa before lesioning failed to gap-cross even if the vibrissae of the animals were intact. Most recently, Harris *et al.* (1999) adopted the task to study the functional topography of the barrel field in rat. They showed that functional representations of vibrissae are topographically organized in the barrel cortex: the capacity to "transfer" learning between any two vibrissae corresponded directly to the spatial relationship between the representations of the two vibrissae in barrel cortex.

The studies cited above suggested that (i) rats use their vibrissa to perform the gap-crossing task, and (ii) barrel cortex is one important locus of the neural processing that leads to vibrissal sensation. Therefore we adopted the gap-crossing task to investigate plastic changes following alterations in vibrissa usage. In this chapter we describe the methods we utilized to induce barrel cortex plasticity and to quantify it.

MATERIALS AND METHODS

Subjects: 50 adult male Wistar rats (weighing 238-450 g) were used in this study (see Table 1). Upon delivery of the rats from the local supplier (University of Trieste), littermates were housed in cages (n=4 per cage) with a 12 h-12 h light-dark cycle and randomly assigned to one of the groups (n=5 each, see below). The subjects were acclimatized for a 2-week period, during which each animal received 10-min of handling per day. Three days before the start of behavioral experiments, a food restriction protocol was initiated to reduce the subjects' weights to 85% of their free-feeding value. Because the behavioral training lasted two (see Chapter 3) to four weeks (see Chapter 4), the subjects were allowed to increase their weights throughout the training period but maintained at 85% of their projected free-feeding mates. Water was available *ad libitum*.

Experimental groups: There were ten groups of rats studied in the present series of experiments (see Table 1).

				Gap Cross Training	
Name of group	**Number of Rats**	**Recording Site**	**Vibrissae Trimming***	**At vibrissa distances**	**At nose distances**
Naïve	10	SG / IG	None	None	None
13 day groups					
Homecage	10	SG / IG	Day 1-13	None	None
Vibrissa-crossers	10	SG / IG	Day 1-13	Day 2-13	None
Nose-crossers	10	SG / IG	Day 1-13	None	Day 2-13
26 day groups					
Homecage	5	SG	Day 1-26	None	None
Vibrissa-crossers	5	SG	Day 1-26	Day 14-26	None

Table 1. Experimental groups. (*Day on which vibrissae were first trimmed designated as day1; SG= supragranular layers, IG= infragranular layers.) SG/IG means that the recordings in 5 of the rats of the group were made in SG, in the other 5 in IG.

Three kinds of manipulation of the vibrissa were made. All groups except naïve animals received vibrissa-trimming for 13 or 26 days. Vibrissa-trimming was performed under

ether anaesthesia (SIGMA, Milano, Italy) and consisted of clipping the vibrissae to their base. The entire vibrissa-clipping procedure took about 3 min per animal.

Naïve animals had all their vibrissae intact at the time of the electrophysiological recordings and were not subjected to any behavioral training protocol. Animals in the 'homecage' group received vibrissa-trimming but no training. All animals in the training groups received vibrissa-trimming throughout the 13 or 26 days of gap-crosstraining. All animals that underwent vibrissa-trimming had a single vibrissa spared – in every case D2 on the right side of the face. The trimming was repeated every other day to assure that cut vibrissae were not used in the gap-cross apparatus of in the home cage.

Among the rats trained to gap-cross, two training protocols were utilized, and these differed by the gap distances that the animals had to cross. For the "vibrissa-crossers", the gap distances presented after several days of training (see below) were large enough to ensure that animals had to use the spared vibrissa to perform the task (vibrissa-distances, typically 13-15 cm). By contrast for the "nose-crossers", the maximum gap-distance presented was 6 cm (so called nose-distances). This distance allowed animals to use not only the spared vibrissa but also the other tactile receptors around the nose (see below).

After 12 sessions of gap-cross training for the vibrissa-crosser and nose-crosser groups, and after 13 days of vibrissa-sparing for the home cage group, electrophysiological recordings were made.

Physiology experiments

The main features of the physiology protocol are given in Rousche *et al.* (1999). Multi-unit activity was recorded under urethane (i.p., 1.5 g/kg body weight) anaesthesia. During the recording session, anaesthetic depth was held constant by monitoring respiration rate, hindpaw withdrawal, and corneal reflexes. When needed, supplemental doses of urethane (0.15 g/kg) were administered. Body temperature was maintained at 37.2 ± 1 °C. After injection of the anaesthetic, rats were placed in a Narashige stereotaxic apparatus for small rodents. An anterio-posterior incision was made over the midline from 5 mm anterior to bregma, to 5 mm posterior to lambda. The bone covering barrel cortex and the surrounding region (approximate coordinates, in mm, AP: 1.5 – 5.0; ML: 2.5 – 7.5) were removed using a micro drill. After the craniotomy, a 10 x 10 microelectrode array (Bionic Technologies, UT) with 1.5 mm long electrodes was implanted through the dura into barrel cortex to a depth of 200-400 μm for the SG groups or 1100-1400 μm for the IG groups (Figure 1). Insertion was done using a pneumatic impulse inserter (Bionic Technologies, UT).

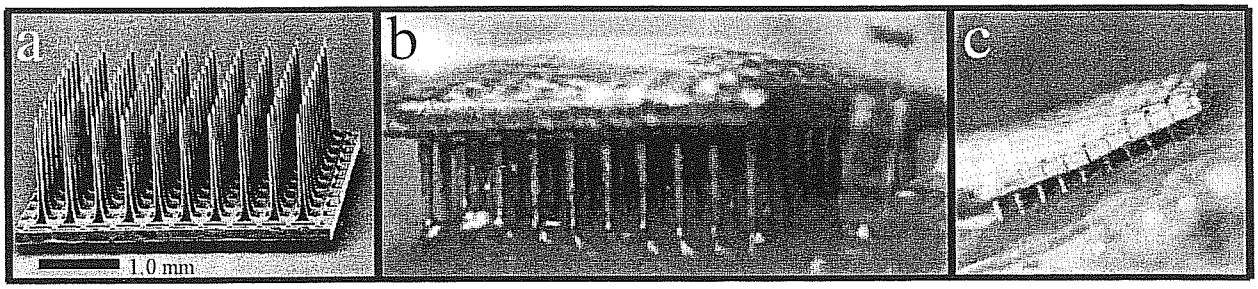


Figure 1. Microelectrode arrays (a) consisted of 100 electrodes arranged in a 10x10 grid. Distance between electrode tips was 400 μm. (Image courtesy Bionic Technologies, UT). (b) An array implanted in supragranular layers. Ruler placed by the array was used to estimate recording depth. (The distance between bars in the ruler is 500 μm). (c) An array implanted in infragranular layers. Please note that electrode pad is 250 μm thick and electrode length in (b) and (c) is 1500 μm.

A reference wire was placed posterio-medial to the array in the layers corresponding the recording depth.

Neural signals were filtered at bandpass 250-7,500 Hz, amplified (gain=5,000) and digitized at 30 kHz using data acquisition system (Bionic Technologies, UT). A threshold for event detection on every channel was automatically set using the control software (Bionic Technologies). Events crossing the threshold (typically 3 times greater that the RMS background noise) on any given channel were stored on a Pentium workstation.

Vibrissa deflection parameters: Vibrissae (which had regrown 4-5 mms after the last trimming) were deflected individually 3 mm from their base using a piezoelectric wafer (Morgan Matroc, OH). The wafer was controlled by a voltage generator (A.M.P.I., Jerusalem). The stimulus was an upward deflection of 80 μm amplitude lasting for 100 ms, delivered once per second.

Vibrissae A1-4, B1-4, C1-5, D1-6, E1-6, together with α,β,γ,δ, were deflected in this way for 60 seconds each.

Influence of the number of stimulus trials: Earlier work from this laboratory has used data collected across more than 279 trials per whisker (Petersen and Diamond, 2000). This paper showed that some results depend upon the number of stimulus trials. In the present study, we were less interested in quantifying the absolute parameters of cortical response, and more interested in comparing across groups with different sensory histories. We wished to exclude the possibility that the results of such comparisons were somehow related to the number of trials. Therefore, in some experiments, a subset of vibrissae (C1-3, D1-3, E1-3)

were further stimulated for 279 trials (same parameters as above) to find out how the basic results, to be described in the following chapters, depended on the number of vibrissa stimulations. An example of how a larger number of stimulus trials affected the spatial extent and magnitude of the vibrissa-evoked activity is depicted in Figure 2. This example comes from the 13-day home group, but here the reader's attention should not be focused simply on the role of number of trials, rather than on any findings related to cortical plasticity.

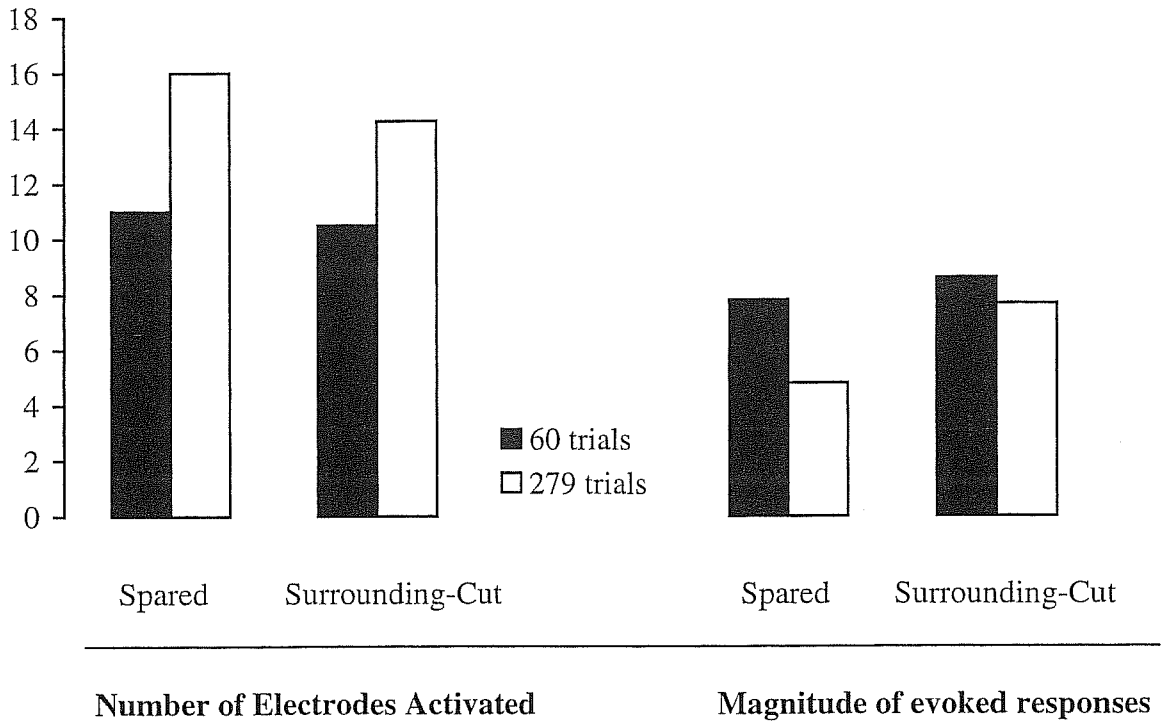


Figure 2. An example (subject GC129976) of how increasing number of vibrissae deflections affects the spatial extent and magnitude of the evoked responses (see the text for details).

In general, a larger number of vibrissa deflections increased the spatial extent of measured neural activity. After 13 days vibrissa sparing in the home cage group, for example, 60 vibrissa deflections of spared and surrounding-cut vibrissae evoked significant activity at, on average 8.6, and 7.5 electrodes, respectively. 279 vibrissae deflections, on the other hand, increased the spatial extent of the evoked activity to 16.0 electrodes for the spared vibrissae deflections, and 14.25 electrodes for surrounding-cut vibrissae deflections. In order to determine whether the increased number of electrodes activated after 279 vibrissae deflections affected the relationship between the representations of spared and cut vibrissae, we calculated the difference scores in every animal of the two groups after 60 and 279 trials of vibrissa deflections and compared the two sets of data using the chi-square test. This comparison

showed that there was no significant difference between the two sets of data ($p > 0.91$), suggesting that within each animal the relationship between spared whisker representation and clipped whisker representations is maintained independently of the number of vibrissa deflections.

Similar insignificant modifications in the magnitude of the evoked responses were seen after 279 vibrissae deflections. The number of spikes collected after 60 vibrissa deflections of spared and surrounding-cut vibrissae were, on average 7.8, and 8.6 spikes, respectively. After 279 vibrissae deflections, on the other hand, the magnitude of the evoked responses were 4.8 for the spared vibrissae deflections, and 7.7 spikes for surrounding-cut vibrissae deflections.

We repeated the same analysis after 26 days of alterations in vibrissa use. An example of how an increased number of stimulus trials affected the measured spatial extent and magnitude of the vibrissa-evoked activity is depicted in Figure 3.

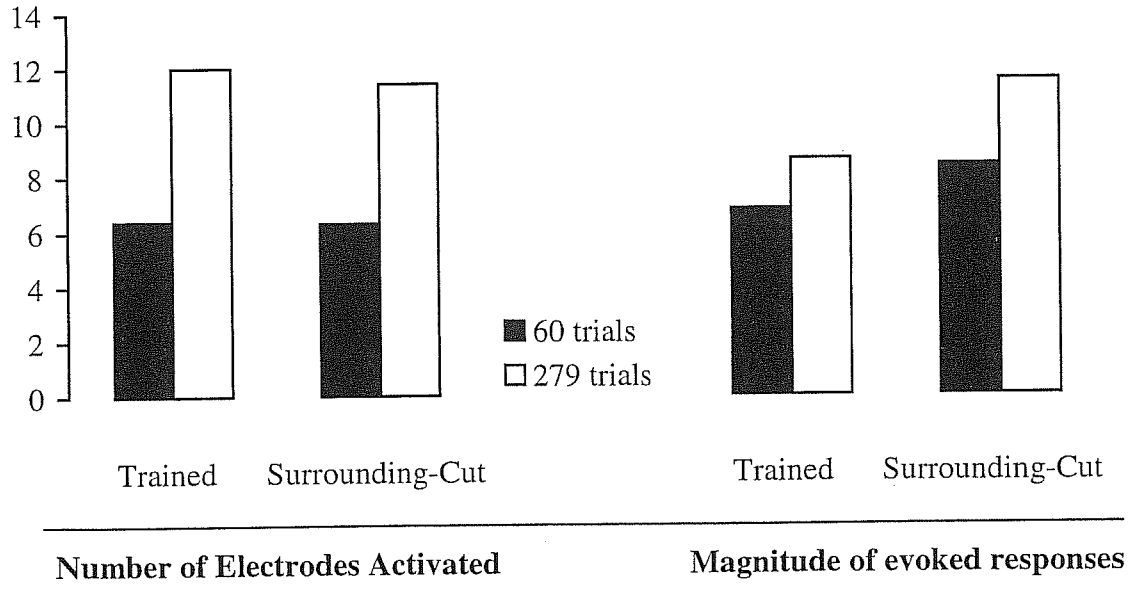


Figure 3. Number of electrodes activated increased noticeably as a function of the number of vibrissa deflections. The number of the evoked spikes, however, was less significantly affected (see the text for details).

Increasing the number of vibrissa deflections mainly increased the spatial extent of the neural activity evoked after vibrissa deflections. In the vibrissa-crossers, for example, upon 60 vibrissa deflections trained and surrounding-cut vibrissae evoked significant activity on average at 6.4, and 6.3 electrodes, respectively. After 279 vibrissae deflections, on the other hand, the extent of the evoked activity increased to 12.0 electrodes for trained vibrissa

deflections, and 11.4 electrodes for surrounding-cut vibrissae deflections. A similar pattern was seen in the homecage group, where spared vibrissa-evoked activity was increased to 13.2 and 8.3 electrodes from 7.4 and 4.5 electrodes for spared, and surrounding-cut vibrissae, respectively. Nonetheless, elevated measures of response due to increased numbers of vibrissa deflections did not affect the relationship between cortical representations of different "classes"of vibrissa (i.e. spared, trained, and cut vibrissae) within experimental groups (Chi-square, $p > 0.96$ for vibrissa-trained, and $p > 0.67$ for vibrissa-spared groups). Moreover, the increased number of vibrissa deflections (see Table 2) did not significantly affect the magnitude of the evoked response (i.e. number of spikes). Therefore, in rest of the analysis, the data collected with 60 deflections per vibrissae will be used.

	After 60 trials of vibrissa deflections		**After 279 trials of vibrissa deflections**	
After...	D2	Cut vibrissae	D2	Cut vibrissae
Vibrissa-Sparing	4.2 ± 1.4	2.3 ± 0.9	5.1 ± 3.4	2.9 ± 1.5
Vibrissa-Training	6.1 ± 3.4	6.1 ± 4.6	7.6 ± 3.4	6.7 ± 3.1

Table 2. Magnitude of vibrissa-evoked activity did not change with increased number of vibrissa deflections. Comparisons of number of spikes evoked after 60 and 279 trials of vibrissa deflections as a function of the experimental group and vibrissa-type.

Analysis of neural activity: In off-line analysis, a spike-sorting program (Bionic Technologies, UT) was used to separate neural signals from potential non-neural artifacts.

A representative "screen-shot" showing typical data from the spike sorting program is given in Figure 4. This illustrates the waveforms that have been discriminated and thus designated as the multi-unit recording, or cluster recording, from channel 35 for experiment GC129982.

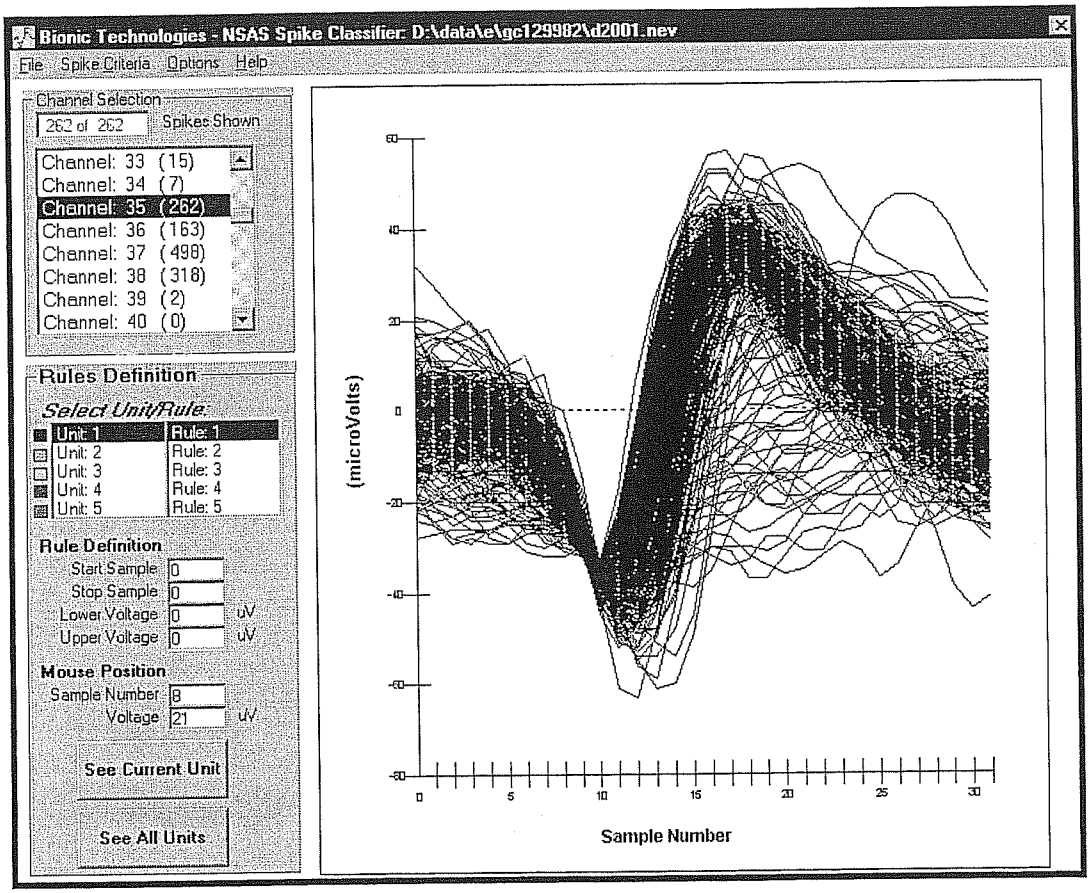


Figure 4. Spike sorting program and spike traces.

Spike-time data were analyzed using MATLAB programs (Mathworks, Natick MA) to measure (i) the spatial extent of cortical the vibrissae representations, (ii) the number of spikes elicited by vibrissa deflections, and (iii) the receptive field size of the cortical neurons. Programs (i) and (ii) were developed by Dr.Rasmus Petersen and program (iii) by Tansu Celikel.

The first step was to compute a peristimulus time histogram (PSTH) of the activity at every electrode for a given stimulus site (Figure 5). To measure the spatial extent of the activity evoked by a given vibrissa, we defined an electrode as responsive if the number of spikes occurring in a given post-stimulus time window was significantly greater than that in a corresponding pre-stimulus time window (Wilcoxon signed rank, $p < 0.01$). The magnitude of response at a given electrode was simply the average number of spikes per stimulus deflection (average spike count in the poststimulus window, minus average spike count in prestimulus window).

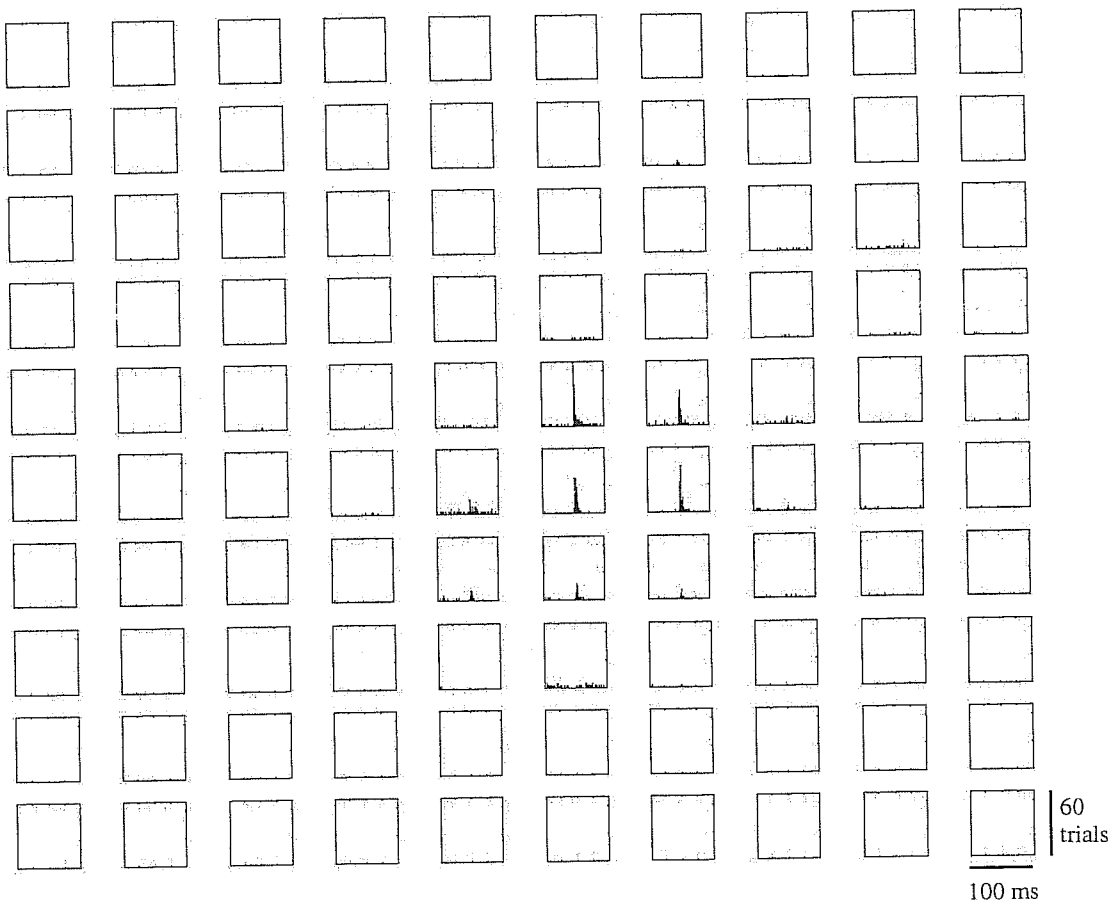


Figure 5. 100 PSTHs corresponding to 100 electrodes at which neural activity recorded was recorded (see text for details).

Receptive field size of the neurons in the barrel cortex was defined as the number of the vibrissae evoking a significant neural response at a given recording site within a 50 ms post-stimulus period in comparison to a 50 ms pre-stimulus period.

Histological evaluation: After data collection, animals were injected with an overdose of urethane, and perfused first with isotonic solution (0.09% NaCl) and then with 4% paraformaldehyde. After fixation, paraformaldehyde was replaced with 20% sucrose. 40 μm coronal sections were cut and stained with cresyl violet. Figure 6 shows examples of sections taken from experiments with supragranular and infragranular recordings, respectively.

As discussed in Rousche *et al.* (1999), histological sections prepared after 100-microelectrode recordings undoubtedly lead to a strong overestimate of the amount of damage in the cortex. This is because, unlike the situation for single micro-electrode recordings, the 10x10 recording method permits no low-trauma technique for removing the electrode array.

The electrodes are not fastened to a microdrive, but instead are freely "floating" in the brain. To remove them, the edges of the array must be grasped by a forceps and pulled upwards manually. This is not a smooth, orthogonal movement as would be achieved with a microdrive. Moreover, the 100 electrodes form a bond with the brain and do not slide up easily. Thus, we would argue that the photomicrographs illustrate the state of the tissue after electrode removal, but not during the recording session. Indeed, the health of the tissue during the recording sessions is indicated by the normal sensory responsiveness of the neurons.

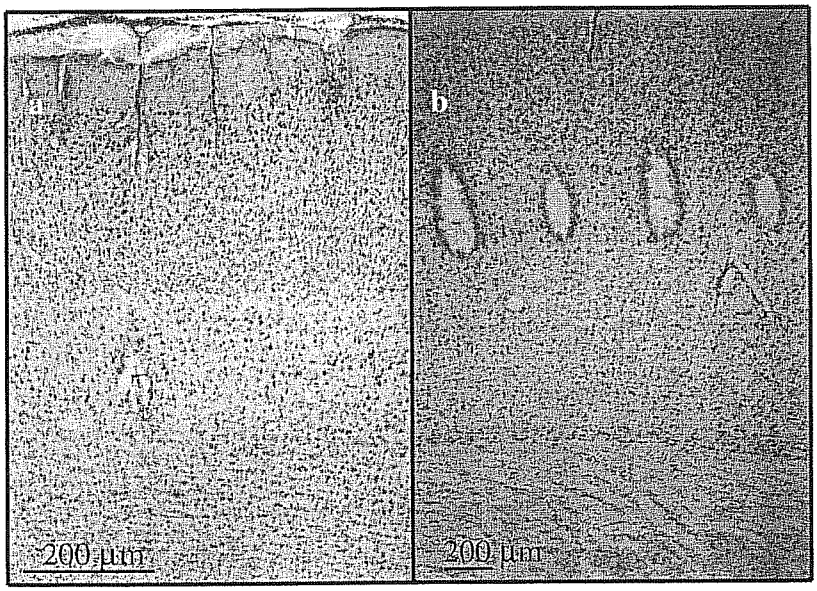


Figure 6. Examples of histological sections. **(a)** Supragranular layer implants 200-400 µm below dura. **(b)** Infragranular layer recordings from 1100-1400 µm below dura.

Behavioral Training

Apparatus: The gap-crossing apparatus consisted of two individually moveable platforms (30 X 12 X 34 cm) made of Plexiglas (see Figure 7). Upper sides of the platforms were covered with black self-adhering plastic. Three sides of each platform had 2-cm-high walls to guide the subjects to the open end. The two platforms were positioned end-to-end (see Figure 7). In order to align the two platforms, a board (11 X 70 cm) made of Plexiglas was fixed to the ground between the lateral walls of the platforms.

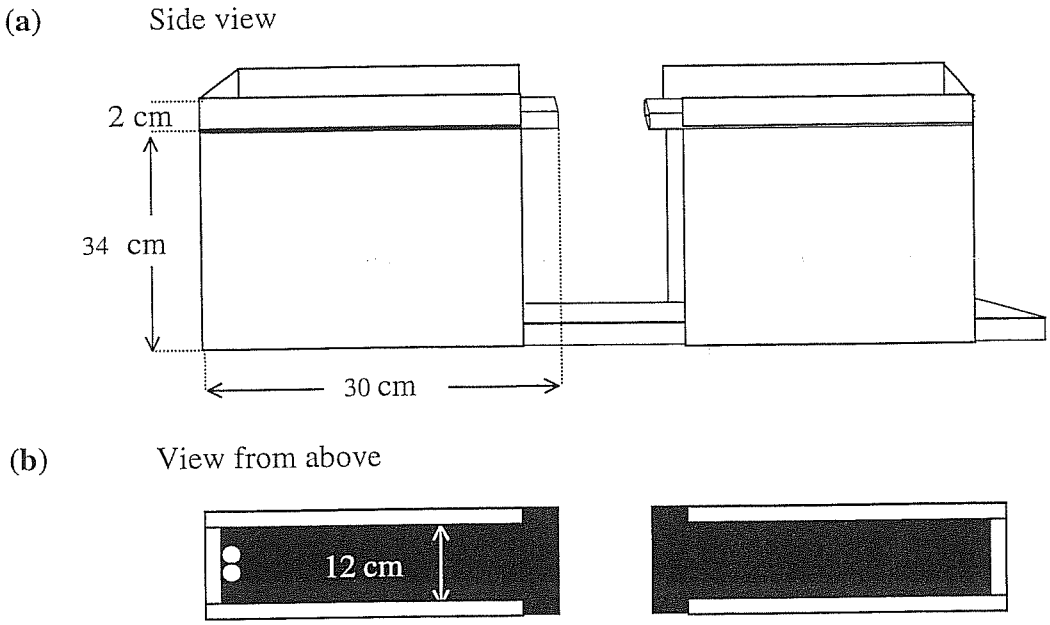


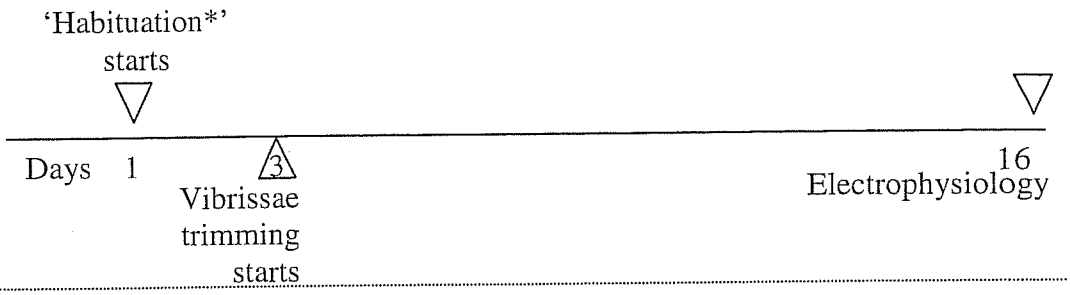
Figure 7. Gap-Crossing training apparatus consisted of two Plexiglas elevated platforms. **(a)** The two platforms were elevated 34 cm from the ground and placed end-to-end. **(b)** The width of the platforms was 12 cm. Food rewards were placed at the closed-end of each platform.

To prevent the use of visual sensory information, all training and testing sessions were performed under red light (Philips PF 712, Germany), invisible to albino rats. Possible auditory cues were masked by white noise (Cooledit, Syntrillium Software Corporation, AZ). After every session, platforms were cleaned using 0.5 % acetic acid solution (SIGMA, Milano, Italy).

Gap-cross training: The experiment started with a three-session long period of habituation to the apparatus. In the first session, subjects, in pairs, were placed onto the platforms, with no gap and allowed to explore the apparatus and the environment under white light. In the second session, subjects individually explored the apparatus, and most subjects started to receive the available food rewards (Cocopops, Kellogg's™) at the end of each platform. After this session, which was run under white light, subjects received the last session of habituation, but this time under red light. During the habituation sessions, rats had all their vibrissae intact. Following the last session, all subjects were briefly anesthetized with ether (Carlo Erba, Milano, Italy), and received vibrissae trimming. One day after the initial vibrissae trimming (see Figure 8), the subjects started to receive training sessions during which they

learned to cross from the 'home' platform to the 'target' platform to receive three Cocopops (see Figure 9). A session started when the animal was placed onto the starting platform by the experimenter. The animal was then expected to walk along the 'home' platform, lean over the gap between the two platforms, sample the target platform and jump onto it. To be certain that the rats jumped onto the target platform on the basis of tactile information collected by the spared vibrissa in the vibrissa-crossers group, 'catch trials' were presented in each training session after the rats reached distances where they could collect information about the availability of the target platform solely by the spared vibrissa. In catch trials, the target platform was placed *just beyond the vibrissa-contact distance*. If the rats were crossing without collecting tactile feedback (i.e. if they had learned simply to proceed to the edge and then jump) or if they were using visual information, they would have been expected to attempt to cross even on 'catch' trials. As reported below, this did not occur.

a. Home cage group



b. Gap-Cross training (Vibrissa- and Nose-Crosser groups)

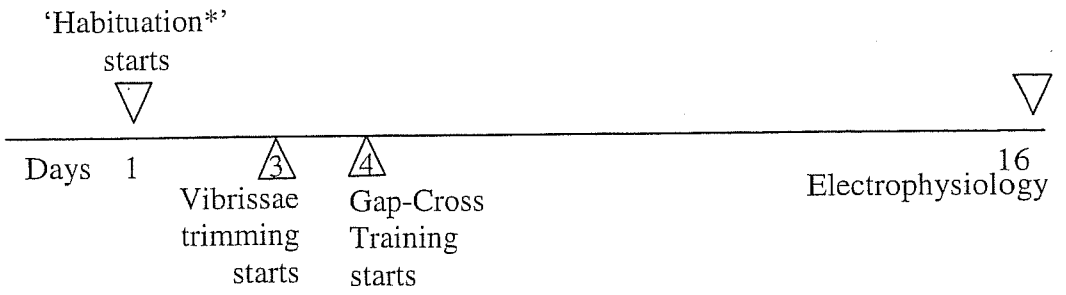


Figure 8. Experimental manipulations before the rats were taken to electrophysiology. **(a)** Home cage animals did not receive any gap-cross training throughout the vibrissa-sparing period. **(b)** Animals trained on the gap-cross apparatus received training for 12 session throughout the 13 day vibrissa sparing period (*Day on which first habitation session is given designated as day1).

In the nose-crosser group, rats received a similar training schedule, but they differed from the vibrissa-crossers in terms of the maximum gap distance presented. After these

animals reached the 6 cm gap distance, the size of the gap between the platforms was not increased any longer. For both groups, training lasted for 12 sessions.

Video recordings and behavioral data coding: To quantify vibrissa usage in the nose-crosser and vibrissa-crosser groups, the last training session ("test session," hereafter) was digitally recorded (Panasonic DVSPRO AJ-D230, Tokyo, Japan) with a 50 Hz. sampling rate onto video cassettes (Fuji DP121, Tokyo). All recordings were made under red light.

Among the 25 rats trained in the gap-crossing task, recordings from 18 animals were selected on a random basis. A total of about 180 min of data was analyzed while the animals performed the gap-crossing task at either nose-distances or vibrissa-distances (vibrissa-crossers only) on a frame-by-frame basis. A successful gap-cross trial meant that the rat reached the end of the target platform. A failure was signalled by the rat's decision to remain on the start platform. Whether a trial was logged as successful or unsuccessful, the rat's actions were coded for four dependent variables: (i) trial duration, (ii) exploration duration, (iii) number of attempts per trial, and (iv) duration of sampling.

(i) The trial duration clock started when the animal's nose passed the midway-mark of the *home platform*, and stopped when the animal's nose passed the midway-mark either on the *target platform* or, in the case of failure, on the home platform (see Figure 9).

(ii) The exploration duration clock started when the animal's nose passed a virtual line halfway across the gap, and stopped when either (a) one forelimb contacted the target platform, or (b) in the case of non-crossing, the rat's nose returned back across the (line halfway across the gap).

(iii) The number of visits to this 'exploration area' within a trial was coded as the number of attempts per trial.

(iv) The sampling duration clock started when the animal's nose passed a virtual line 2 cm before the edge of the target platform and stopped when either (a) one forelimb contacted the target platform or (b) in the case of non-crossing, the rat's nose crossed the line in the opposite direction. Because there was no target platform available for collecting data in the catch trials, we modified the description of the sampling duration in those trials and calculated the time spent sampling beyond the line described by the previous successful trial's gap distance. For example, if an animal had successfully crossed at 14 cm gap distance immediately before receiving a catch trial, in the catch trial the sampling duration clock started when the animal's nose passed 12 cm and stopped when the nose crossed the line in the opposite direction.

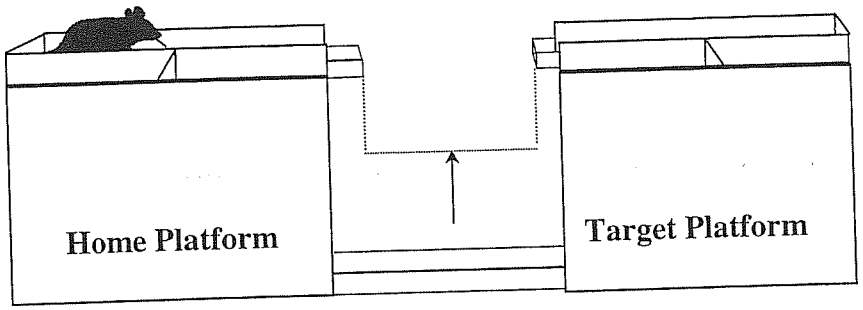


Figure 9. Animals started a trial by moving along the home platform, passing the midway-mark (the triangles) with the snout. A virtual line (the arrow) between the platforms marked the halfway of the gap.

In total, about 540,000 video frames (18 animals x 1 testing sessions/animal x 10 min x 60 sec x 50 frames/s) across 355 trials were evaluated to code aforementioned dependent variables in nose-crossers (Figure 10a) and vibrissa-crossers (Figure 10b).

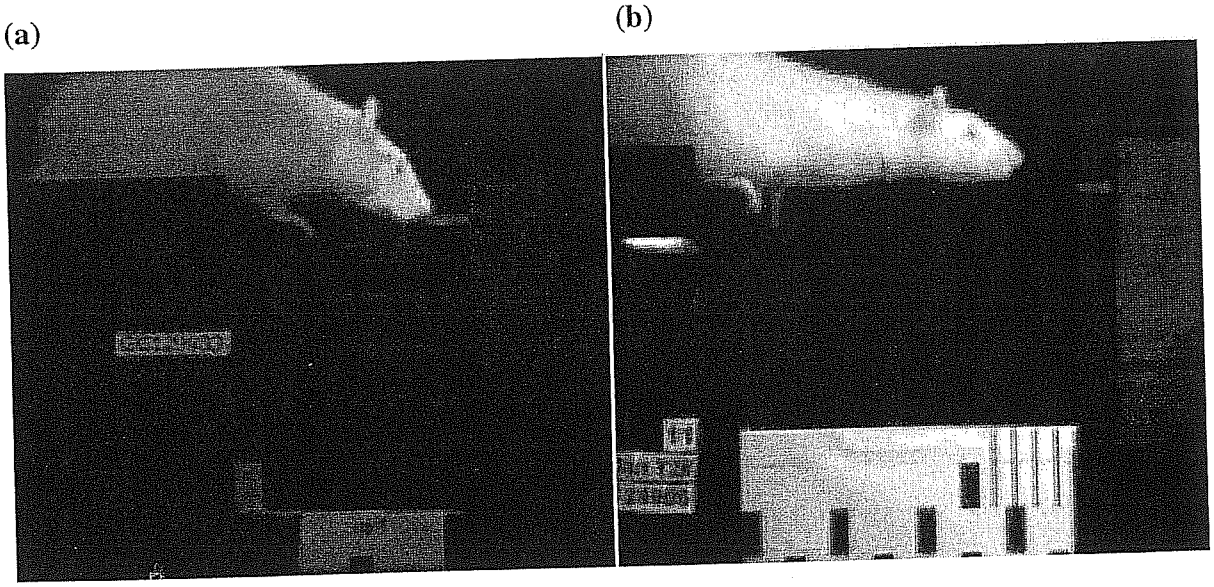


Figure 10. Gap-crossing behavior in the two experimental groups. **(a)** Animals in the 'nose-crossers' group received training at maximum 6 cm. **(b)** By the end of the gap-crossing training at vibrissa distances, animals in the vibrissa-crossers group crossed typically 13-15 cm.

RESULTS

General Observations

By the end of the habituation sessions, all subjects were familiar with the environment, as evidenced by the fact that they readily shuttled between the two platforms for food rewards at the end of each platform. During the habituation, but not training and test sessions, animals were able to use extra-tactile cues because the sessions were carried out under white light. Animals in all experimental groups started to cross the gap when presented in the first session of the training, and continued to perform the task in the following sessions. Most animals in the vibrissa-distance group learned to rely on vibrissal sensation about the target platform within about 4-5 sessions, judging by their successful crossing at distances where they could sample the target platform only by using their vibrissae (Figure 11). In none of the catch trials did the subjects cross or attempt to cross the gap.

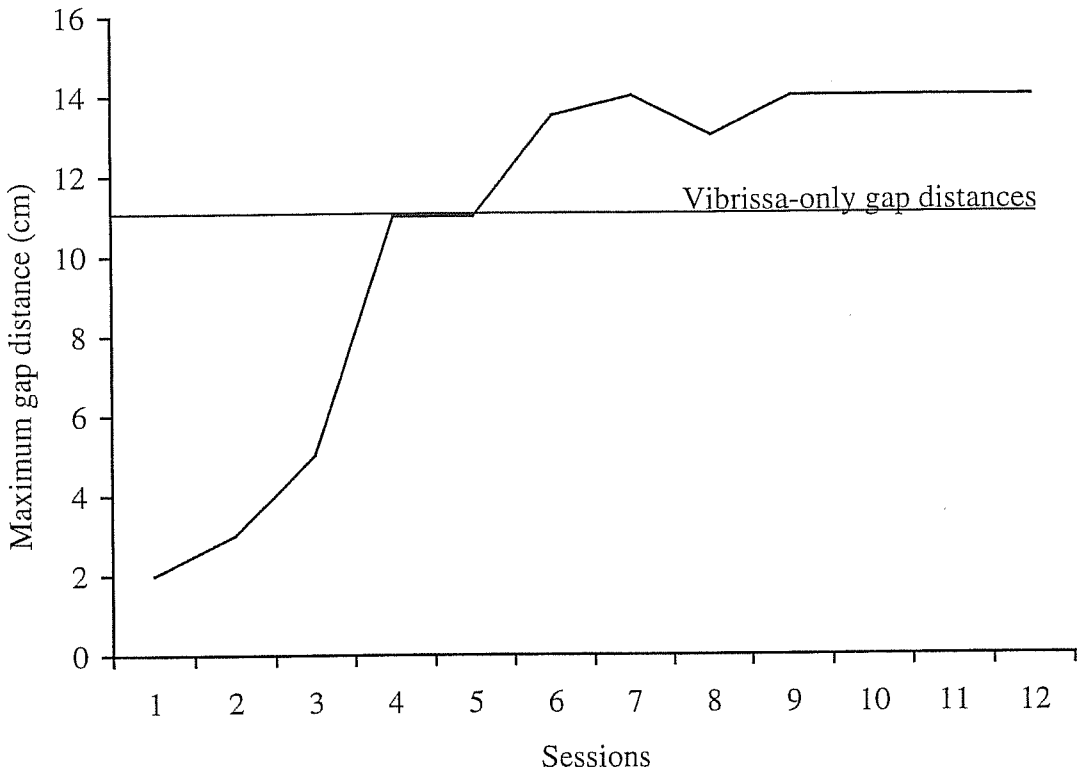


Figure 11. An example (subject no. GC129934) of gap cross training at vibrissa-distances. After reaching the vibrissa-only gap distance at the end of the 4th session, the subject continued performing at vibrissa-distances for the rest of the training period.

Temporal and behavioral components of gap-crossing for rats trained at vibrissa-distances

Subjects trained to gap-cross at vibrissa-only distances (n=9) received three types of trials, namely, nose-distance trials, vibrissa-distance trials, and catch trials. Grouping together the three kinds of trials, trial duration was, on average, 7.7 sec. Trial duration differed significantly (ANOVA, $p < 0.005$) between different types of trials. On average, subjects concluded nose-distance trials faster (1.6 sec duration) than vibrissa-distance trials (8.0 sec) and catch trials (12.2 sec) (Tukey-HSD, $p < 0.005$). Similarly, exploration durations for the three types of trials differed significantly (ANOVA, $p < 0.0001$). On nose-distance trials, subjects explored the gap for 830 ms, on average. On vibrissa-distance trials, average exploration duration increased significantly (Tukey-HSD, $p < 0.0001$) to 1750 ms and on catch trials average exploration duration increased further to 3000 ms.

Analysis of the sampling duration shows that subjects collected sensory data about the availability of the target platform for, on average, 618 ms in nose-distance and vibrissa-distance trials, grouped together. On catch trials they collected sensory data for 1.6 s before returning to the start platform. This nearly three-fold increase in the sampling duration between nose- and vibrissa-dependent versus catch trials was significant (Oneway ANOVA, $p < 0.0001$). The long period of time spent "feeling for" the target platform on catch trials suggests that the rats were collecting sensory information to falsify their expectation of the presence of the goal platform. Evidently the expectation was so strong that very clear negative evidence was required before the rat aborted the attempt.

We further classified the data of animals trained at vibrissa distances according the success or failure of the animal in a given trial (see Table 3).

Trial Type	N	Number of attempts	Trial duration (s)	Exploration duration (s)	Sampling duration (ms)
Success	**127**	1.20 ± 0.06*	6.10 ± 0.98*,ψ	1.38 ± 0.12*,ψ	623 ± 48*
Fail	**32**	1.56 ± 0.17	10.98 ± 1.56	2.50 ± 0.43	620 ± 180*
Catch	**22**	1.82 ± 0.24	12.62 ± 1.69	$3.03 \pm .30$	$1{,}648 \pm 64$

Table 3. Temporal and behavioral correlates of success and failure together with catch trial performance of animals trained at vibrissa-distances (values are mean ± standard error). (*, Statistically (ANOVA) different from the catch trial value ($p < 0.05$); ψ, statistically (ANOVA) different from the fail trials' value ($p < 0.05$)).

These results show that the average number of attempts, visits to sampling area, to gap-cross on successful trials were less than that on catch trials (Tukey's HSD, $p < 0.001$). Nonetheless, these parameters did not differ between successful and unsuccessful trials. Moreover, the average duration of successful trials was shorter than that of unsuccessful trials and of catch trials (Tukey's HSD, $p < 0.05$). On catch trials, the exploration duration was longer than for either successful or unsuccessful trials when the platform was within reach. Exploration duration was shortest in the trials when the animals successfully gap-crossed (Tukey's HSD, $p < 0.005$). Average sampling time did not differ between successful and unsuccessful trials. Thus, on unsuccessful trials the rats spent the same amount of time contacting the goal platform, yet did not cross.

We further classified use of vibrissa, and the temporal correlates of decision making in the gap-crossing task, according to whether the spared vibrissa was used alone or together with other touch receptors around the nose, and compared these two types of trials to catch trials. To carry out this analysis, on every trial we observed which receptors appeared to be in contact with the goal platform: spared vibrissa alone, or spared vibrissa plus nose. Then, we pooled all trials presented at nose- or vibrissa-distance, and re-classified them as a function of how the animal collected information about the target platform. The results showed that animals spent significantly less time in contact with the target platform when they sampled it with either their nose plus spared vibrissa or spared vibrissa alone in comparison to the trial where the target platform was out-of-reach (Tukey-HSD, $p < 0.05$).

Temporal and behavioral components of gap-crossing for rats trained at nose-distances

For subjects trained at nose-distances (n=9), we again coded the final test session to define total trial duration, time spent exploring and sampling together with number of attempts to gap-cross before crossing the gap. We also asked whether animals trained at nose distance require less time to cross when they use their single spared vibrissa. When the four dependent variables were grouped according to the subjects' performance in a given trial, the results showed that trial duration, exploration duration, and sampling duration were longer for failures than for successes (ANOVA, $p < 0.01$). On average, trial duration was 13.7 s for failures but just 3.9 s for successes. In trials where the animals failed, the reason for failure was not an insufficient exploration time or sampling time; on the contrary, in those trials, animals explored the gap and sampled the target platform longer than they did when in successful trials (ANOVA, $p < 0.001$). Although the temporal measures significantly differed between successful and failed trials, the number of attempts to gap cross did not differ (ANOVA, $p > 0.99$).

In the test session, the animals used their spared vibrissa together with other touch receptors around the nose in 107 of the 174 trials (61%). When these trials were compared with the trials when the animals used their spared vibrissa alone to perform the task (e.g. there was clearly whisker contact but no nose contact), no difference was found (see Table 4).

Trial Type	N	Number of attempts	Trial duration (s)	Exploration duration (s)	Sampling duration (ms)
Nose Touch	**107**	1.23 ± 0.08	5.09 ± 0.98	1.32 ± 0.15	832 ± 71
Vibrissa Touch	**51**	1.16 ± 0.07	2.79 ± 0.33	1.1 ± 0.2	720 ± 185
Not Discriminable	**15**	1.2 ± 0.15	2.24 ± 0.56	0.8 ± 0.2	512 ± 162

Table 4. Temporal and behavioral correlates of target platform sampling in the test session after training at nose-distances (values are mean ± standard error). (Note: One animal, in one of the trials crossed the gap without sampling the target platform first. This trial is not included above). On trials classified as Not Discriminable, the observer was not able to determine what combination of whiskers and nose contacted the goal platform.

Preference in tactile organ use in the vibrissa-dependent and nose-dependent groups

As dictated by the experimental design, nose-crossers and vibrissa-crossers did not differ from each other in terms of number of training sessions (12 each), and total number of training trials received (on average, vibrissa-crossers 181 trials, nose-crossers 174 trials). Nor did they differ in terms of duration of target platform sampling on average across different types of trials (vibrissa-crossers 747 ms, nose-crossers 766 ms; ANOVA, $p > 0.8$). Nonetheless, the two groups differed in the time spent to explore the gap (Vibrissa-crossers: 1770 ms; Nose-crossers: 1202 ms; ANOVA, $p < 0.01$), and trial duration (Vibrissa-crossers: 7.7 s; Nose-crossers: 4.2 s; ANOVA, $p < 0.0005$) when successful trials were compared.

Animals were trained to gap cross in either a vibrissa-dependent or nose-dependent fashion, however both groups were able to perform the task with their spared vibrissa-alone, nose-crossers had the opportunity to use touch receptors around the nose. In the test session, rats trained at vibrissa distances were presented with some trials where the gap was set to a nose-distance to determine whether they used their vibrissa alone or in combination with their nose. On the other hand, animals trained at nose distances throughout the training sessions were never tested at vibrissa-only distances. Instead their tendency to use the spared vibrissa in the task was coded.

The nine animals trained at nose distances received 174 trials in the test session. In 62% of the trials they relied on a combination of the spared vibrissa together with the other tactile receptors around the nose to contact the target platform. However, in the rest of the trials they collected sensory information through the spared vibrissa alone (Figure 12a). Animals trained to gap cross at vibrissa distances used the touch receptors around nose in 21% of the trials when they were faced with short-gap distances (compare with 62% for nose-crossers) (see Figure 12b). The differential use of the spared vibrissa in gap-crossing for the two groups will be relevant to subsequent chapters when we report differences and similarities in the cortical representation of the spared vibrissa for the two groups.

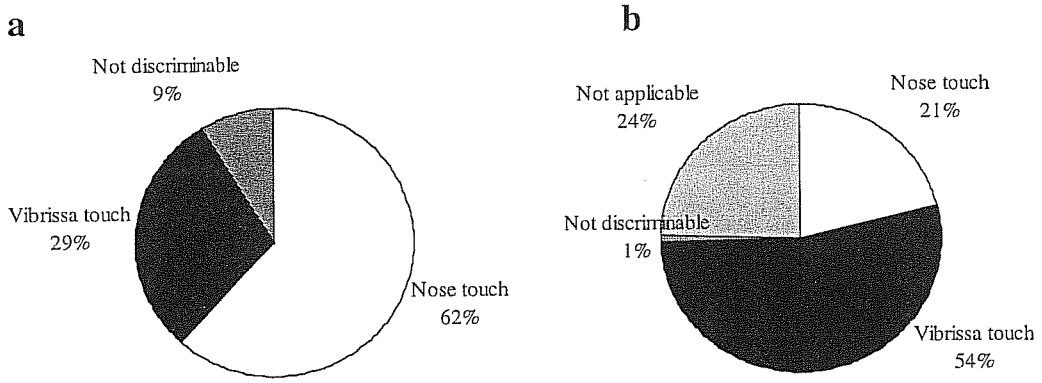


Figure 12. Animals in both groups were able to perform the task using vibrissa-alone or together with other sensory-tactile information (see the text for details). **(a)** Nose-crossers, which used nose plus spared vibrissa to perform in the task **(b)**Vibrissa-crossers.

DISCUSSION

The number or quantity of receptors used in a perceptual learning task

We have studied the rat vibrissae system in a vibrissae-dependent and nose-dependent task to reveal the dynamics of vibrissa use when the vibrissa is used alone or together with other touch receptors in a perceptual learning task. The study had two distinct, yet complimentary groups of subjects. We trained the first group of rats to use their single spared vibrissa in the gap-crossing task and, by introducing trials with a wide gap, we forced them to rely on vibrissal information. Later, we subjected them to a test session during which they could use the spared (trained) vibrissa in most trials. We trained the second group of rats in the gap-crossing task, but this time at nose distances (vibrissa-independent version of gap-crossing task) where animals always had the opportunity to collect sensory information using touch

receptors around nose together with the spared vibrissa. Analysis of the temporal components of vibrissa-use showed that, whether or not the spared vibrissa was used together with other tactile-sensory receptors around the nose, the rat took an equal amount of time to sample the target platform.

This behavioral finding was unexpected. In general, we need to collect sensory information about our immediate environment before deciding whether to perform a motor act. Similarly, rats, in the gap-crossing task, had to collect tactile information about the availability of the target platform before performing the gap cross. If some fixed quantity of sensory information were needed to decide whether or not to gap-cross, increasing the number of tactile receptors sampling the 'target' would be expected to reduce the sampling time required before performing the motor act. However, the results of the present study did not support this notion. We found that animals trained in the nose-dependent version of the gap-crossing task, collected sensory information about the target platform for as long as the animals trained to use their single vibrissa to perform the task.

The results, nonetheless, supported the idea that some 'decision making center' in the brain, which we will not attempt to define or identify, requires some fixed quantity of tactile information and can utilize whisker or nose information equally efficiently.

If one part of the study intended to describe how rats collect sensory information about a target object, the other intended to identify their sensory modality in performing the task. We explored this by subjecting the animals which were trained at vibrissa-distances to trials at nose distances, in one group, and in the other one, measuring which sensory-tactile organ the animals preferred to use to perform the task in the test session. Animals trained in the vibrissa-dependent version of the task were able to perform the task when the target platform was presented at nose distances. One argument, however, about using these trials to establish whether the animal could use some form of sensory information other than that with which it was trained might be that, in the nose trials, the animals were able to use vibrissal sensation. Hence they can recall the information by just considering the sensory information coming from the single vibrissa, and thus not exclusively from the other touch receptors activated in nose-distance trials. Therefore we coded vibrissa-use preference of animals trained in the nose-dependent version of the task. Results of this analysis showed that only in 61% of the all trials did they prefer to use the nose; in many trials, even at short gap distances, the rats crossed immediately after making whisker contact, without requiring "confirmation" from a nose touch. In the rest of the trials, they gap-crossed using only their sole vibrissa to collect sensory information about the target platform, just as if they were crossing at a vibrissa-dependent distance. This result indicates that even if the animals used information coming from several

tactile organs throughout training sessions, they could still perform the task using a subset of the sensory organs trained – the single spared whisker.

Vibrissa use during exploration

Another result of the present study is that rats acquire a strategy to perform a task, and do not modify the strategy even if the sensory information changes qualitatively and quantitatively. This result is in agreement with Carvell and Simon's earlier work which showed that rats first pause at the end of the platform and spend several hundred milliseconds sampling the target platform (if available) a number of times. The number of vibrissa contacts, frequency of vibrissa movements, and moreover, whisking pattern of the rat may differ between subjects and behavioral tasks (Carvel and Simons, 1990, 1995). However, as our results suggest, duration of the sampling does not change as a function of number of tactile receptors used to collect sensory information.

In summary, the results suggested that within a single sensory modality (tactile in this case), sampling the very same target with a wide array of sensory receptors, or just a small subset (the single vibrissa) does not affect performance. Results from the present study further suggest that information gained using a large number of sensory receptors could be recalled by a subset of those 'trained' organs. A final observation is relevant to the subsequent chapters. If the sensory capacities that are gained or sharpened during gap-cross training are related to the corresponding cortical functional modification, we would predict the cortical plasticity in nose-crossers and vibrissa-crossers to be, by and large, very similar. This conclusion comes from the fact that the analysis of behavior indicated that rats in both groups learned much of the same information; for example, rats in both groups learned to sample the opposite platform with a single vibrissa, and detect or recognize the target with sufficient certainty to initiate the motor action. Nose-crossers often supplemented the tactile message with "nose information", but on many trials made contact with the intact vibrissa alone. Thus, they acted much like vibrissa-crossers, and our expectation was to find a qualitatively similar shift in cortical organization.

Chapter 3. CORTICAL PLASTICITY AFTER 13 DAYS OF ALTERED VIBRISSA USE IN THE HOME CAGE OR IN A PERCEPTUAL LEARNING TASK

INTRODUCTION

One current issue in cortical functional organization is the character and mechanisms of the representational plasticity. Starting from Kalaska and Pomeranz (1979) a number of studies have shown that cortical sensory representations undergo plastic changes after chronic alterations in sensory organ use (Kalaska and Pomeranz, 1979; Merzenich *et al.*, 1983; Diamond *et al.*, 1994), nerve damage (reviewed in Kaas *et al.*, 1983), preferential use of parts of the sensory apparatus (Jenkins *et al.*, 1990), intracortical microstimulation (Recanzone *et al.*, 1992b) as well as sensory learning (Recanzone *et al.*, 1992a, 1993). Independently from the experimental protocol used, the studies seemed to lead to a common outcome: *increased* representation of the part of the sensory organ whose activity was favored. For example, when a single vibrissa or vibrissa pair is spared (all others removed either by plucking or trimming), the cortical representation of the spared vibrissa(e) expands (Fox, 1992; Diamond *et al.*, 1993, 1994; Lebedev *et al.*, 2000). Recently, however, studies began to question this principle. Daniel Polley and associates reexamined this phenomenon using optical imaging of intrinsic signals (Polley *et al.*, 1999). They were able to study individual rats repeatedly over the course of a 28-day long period during which a single vibrissa was spared on one face side, and found that the barrel cortex representation of the spared vibrissa expanded. In a second group of rats, they asked whether the changes seen after use of spared vibrissa in an active exploration were similar to the changes found after home cage vibrissa use, described above. In this second group of rats vibrissae were trimmed as before, but rats were given a total of 14 min (in 7 sessions of 2 minutes each) of exposure to a novel environment. The remarkable finding was that the representation of the spared vibrissa *shrunk* in comparison to its size before trimming. Although the available data suggest that vibrissae representations in cortex are modified when rats are exposed to a novel environment, there is no published study showing that similar changes may be found after rats are trained in learning task in which the success depends sensory information collected using a spared-vibrissa.

A second central issue is to identify the components of the sensory system which are modified. In classical studies, it has been suggested that representational plasticity in the nervous

system is expressed predominantly at the cortical level (Merzenich *et al.*, 1984). The evidence for this supposition came from a number of studies on monkeys. For example, Merzenich and colleagues (1983) showed that two representational maps of the monkey hand in somatosensory cortex, in areas 3b and 1, undergo different (i.e. continuous versus discontinuous) types of reorganization after median nerve section, although the two maps receive collateral inputs from common thalamic centres (Clark and Powell, 1957; Lin *et al.*, 1979). More direct evidence came from the finding that, after peripheral nerve section, large-scale sprouting occurs in the horizontal intracorticalconnectivity of the primary somatosensory cortex but not in the thalamo-cortical projections (Florence *et al.*, 1998; but also see Jones and Pons, 1998).

Further indications in support of an intracortical site of plasticity came from electrophysiological studies in the rat vibrissa system (Fox, 1992, 1994; Diamond *et al.*, 1994). These studies, using vibrissa sparing and vibrissae-pairing paradigms, consistently showed that the representation of a preferentially utilized vibrissa or vibrissae-pair in the barrel field increases. Analysis of the latency of responses to deflections of "new" whisker inputs suggested that the expansion of the spared receptors' representation resulted from the increased effectiveness of intracortical excitatory connections, not because of changes within subcortical centers or because of augmented connectivity between thalamus and cortex (Fox, 1994; Wallace and Fox, 1999; Lebedev *et al.*, 2000).

Several groups have recently questioned this tenet. Faggin *et al.* (1997) showed in the rat vibrissa system that shortly after focal application of local anaesthetic in the vibrissa-pad, representations of vibrissae in both cortical and subcortical loci are concurrently enlarged. An additional reason to doubt the concept of purely intracortical plasticity came from investigations of the plasticity of the digit representations of monkey (reviewed in Jones, 2000), which showed that not only cortex but also subcortical loci of the somatosensory axis express plasticity after long-term denervation of an upper limb (Jones and Pons, 1998). Nonetheless, the involvement of the subcortical structures in representational plasticity seems to be a function of the severity of the peripheral manipulation (Florence *et al.*, 1998; Jones and Pons, 1998; also see Merzenich, 1998).

The third important issue concerns laminar differences in cortical plasticity. Neocortex (which we will refer to simply as "cortex") is a six-layered structure that has orderly input-output relationships with the rest of the brain. The six laminae are generally classified in three distinct sets and differ from each other in several respects. In the rat barrel field, for example, input from the principal thalamic nucleus (VPM) enters cortex at the level of the granular layers, which primarily consist of stellate cells. The granular layers are equivalent to layer IV (the barrel proper)

together with the lower part of layer III. Neurons in the granular layers have very strong local interconnections, and project to both supragranular and infragranular layers (Keller, 1995). Infragranular layers (at the level of Vb) also receive direct input from VPM (Armstrong-James *et al.*, 1992; Lu and Lin, 1993, Keller, 1997). Neurons in the supragranular layers horizontally distribute the information that they receive from the granular and infragranular layers to the neighboring cortical columns (Nieuwenhuys, 1994; Keller, 1997; Staiger *et al.*, 2000). Infragranular layers, on the other hand, mainly project to the more distant cortical areas (Keller, 1997), to the supragranular layers, and subcortical structures (Nieuwenhuys, 1994) – they are the main output system of the cortex (Staiger *et al.*, 2000; Schubert *et al.*, 2001).

The layers of cortex differ not only in terms of differential afferent and efferent relationships, but also in the responses to neurotransmitters and neuromodulators. For example, Kyriazi *et al.* (1998) showed that neurons in different layers of cortex give distinct responses to the application of $GABA_A$ receptor antagonists. Neurons in all layers show increased evoked response magnitudes and response durations upon blocking $GABA_A$ receptors. This release from inhibition, however, is least effective in the infragranular layers, perhaps because of the modest number of $GABA_A$ receptors on these neurons (Land *et a.*, 1995). The infragranular layers also contain a lower number of GABAergic neurons than do the granular and supragranular layers (Li and Schwark, 1994).

Nongranular layers contain higher amounts of synaptic zinc than the granular layers (Land and Akhtar, 1999). Synaptic zinc is co-localized with glutamate in the synaptic vesicles and therefore is believed to be a marker for glutamatergic neurons. Although the number of glutamatergic neurons is higher in the nongranular layers, blocking their activity is more effective in the granular layers. Rema and colleagues (1998) studied plasticity after 5 days of vibrissa-pairing during which 500 μM D-AP5 was applied continuously using an osmotic mini-pump. Blocking NMDA receptors reduced but did not completely block the plasticity (the shift in the D1/D3 bias in evoked responses) seen in the supragranular layers. NMDA receptor block, nonetheless, blocked the vibrissa-pairing plasticity in the granular layers in the same animals, suggesting that vibrissa-pairing plasticity may depend on different cellular mechanisms in different layers of the cortex.

Neurons in different layers of cortex also vary in their responses to application of neuromodulators. Lamour *et al.* (1988) showed that the increase in evoked response amplitude after acethylcholine (ACh) application was largest in the supragranular and small in the infragranular layers (especially in layer Vb). A more drastic difference between the layers was

seen after noradrenaline (NA) application. Armstrong-James and Fox (1983) showed that application of NA at low concentrations resulted in inhibition of the evoked responses in supragranular layer neurons, but excitation of evoked responses in infragranular layer neurons.

Thus, the layers of barrel cortex differ in anatomical connectivity, density of neurotransmitter systems, and responses to application of neuromodulators. These observations are consistent with (and perhaps contribute to) laminar differences in normal receptive fields. This was first investigated by Chapin (1986) who showed that the responsiveness of a barrel cortex neuron to sensory stimulation was a function of the neuron's laminar location. Granular layer neurons had smaller receptive fields than did nongranular layer neurons. In the extragranular layers average receptive field was larger than in the supragranular layer neurons (Chapin, 1986). However, there is not general agreement about the receptive field size of extragranular neurons. Armstrong-James and Fox presented data showing that, although the largest receptive fields are found in the infragranular layers, as originally suggested by Chapin, the sizes of the receptive fields in the supragranular and granular layers do not differ (Armstrong-James and Fox, 1987).

A key observation concerns laminar differences in plasticity. Diamond *et al.* (1994) showed that when all but two vibrissae were trimmed for 24 h, evoked responses to the deflection of each of the two spared vibrissae increased in nongranular, but not in granular layers. Moreover, Glazewski and Fox (1998) showed that, when all vibrissae but one were trimmed for eight days responses evoked by previously clipped vibrissae decreased in the supragranular layers much more than they did in the granular layers. As yet, no study has used electrophysiological methods to probe the effects of single vibrissa sensory experience on the infragranular layers. However, 2-deoxyglucose data suggest that when a vibrissa is spared for 3 weeks, its representation expands in infragranular layers (Kossut *et al.*, 1988), whereas the deprived vibrissae's representations are suppressed (Skibinska *et al.*, 2000). In this study, we explored these three issues by studying the change in neural activity after a number of behavioral alterations in vibrissa use. The results of the experiments are organized in three sections. In the first, we report the role of behavioral use of a vibrissa in shaping the cortical plasticity in supragranular layers. In the second section, we try to identify the sites of the plasticity. And in the third, we explore the impact of vibrissa use in vibrissa representations in the infragranular layers.

As outlined in the Introduction, an earlier study suggested that the exact features of the changes in the representations of sensory organs depend upon the type of use of the sensory organ (Polley *et al.*, 1999). However, limitations in temporal and spatial resolutions of intrinsic optical imaging applied did not allow the authors to make a strong argument about the extent, and the

nature (thalamocortical vs. intracortical) of the representational changes after alterations in the sensory organ use.

In this study, we tried to overcome these problems using 100-channel UTAH electrodes to electrophysiologically study the change in the cortical representation of vibrissae and receptive fields of the cortical neurons after differential use of a spared vibrissa. We studied cortical plasticity first in the supragranular layers of barrel cortex after rats used their spared vibrissa (i) in the home cage alone ("home cage" group), (ii) in a learning paradigm in which rats learned to use the spared vibrissa alone to perform a tactile task ("vibrissa-crossers"), and (iii) a learning task in which success depended upon the sensory activity coming from the spared vibrissa together with the other touch receptors around the nose ("nose-crossers"). We further compared the change in the vibrissae representations after these behavioral modifications with the vibrissae representations in experimentally naïve animals.

RESULTS

Eight groups of animals (5 animals/group) were studied in this part of the thesis (see Methods and Table 1).

				Gap Cross Training	
Groups	**Number of Rats**	**Recording Site**	**Vibrissae Trimming***	**At vibrissa distances**	**At nose distances**
Naive	10	SG / IG	None	None	None
Homecage	10	SG / IG	Day 1-13	None	None
Vibrissa-crossers	10	SG / IG	Day 1-13	Day 2-13	None
Nose-crossers	10	SG / IG	Day 1-13	None	Day 2-13

Table 1. Experimental groups. (*Day on which vibrissae trimmed designated as Day 1; SG= supragranular layers, IG= infragranular layers).

Three of the four behavioral modifications included 13 days of vibrissa-trimming which spared D2 vibrissa on the right side of the face (so called vibrissa-sparing protocol). Two of the groups that received vibrissa-sparing protocol also received gap-cross training (for details, see Chapter 2). In order to measure the change in the neural responsiveness following these

behavioral modifications, we carried out electrophysiological recordings under urethane anesthesia (Chapter 2). Data collected during the electrophysiology session was used to evaluate representations of vibrissae, magnitude of the evoked responses, and receptive field of barrel cortex neurons in the supragranular and infragranular layers.

Section I

In this first section, we investigated the role of differential usage of vibrissa in neural plasticity in the supragranular layers. There are several reasons to choose the supragranular layers to trace neural plasticity – most importantly, the supragranular layers are believed to be the most plastic laminae in the neocortex (Fox, 1992; Diamond *et al.*, 1994).

REPRESENTATIONAL PLASTICITY IN THE SUPRAGRANULAR LAYERS

Vibrissa representation naive animal

The rat has about 35 mystacial vibrissae and the same number of barrel-columns in the barrel field of the primary somatosensory cortex. Length/thickness of vibrissae change depending upon vibrissa position, and the size of the barrel size changes in a correlated manner. Because of this intrinsic variability, it was important to select a set of whiskers and related barrel-columns which were similar in size. We studied the representations of the eight D2-neighboring vibrissae together with D2 itself. The barrel-columns of these 9 whiskers are similar in size.

It has been shown using UTAH multi-electrode arrays that activity in the barrel field starts as early as 5 ms (interquartile range 6-12 ms) after vibrissa-deflection, continues to expand and reaches an offset at about 50 ms (44 ms ± 15 ms; mean ± STD) after stimulus onset (Petersen and Diamond, 2000). Hence we estimated the maximum spatial extent of the vibrissa-evoked activity by calculating the number of electrodes giving a higher number of spikes in the 50 ms interval post-deflection compared to the 50 ms pre-deflection duration. Figure 1 summarizes the results of this comparison in experimentally naive animals.

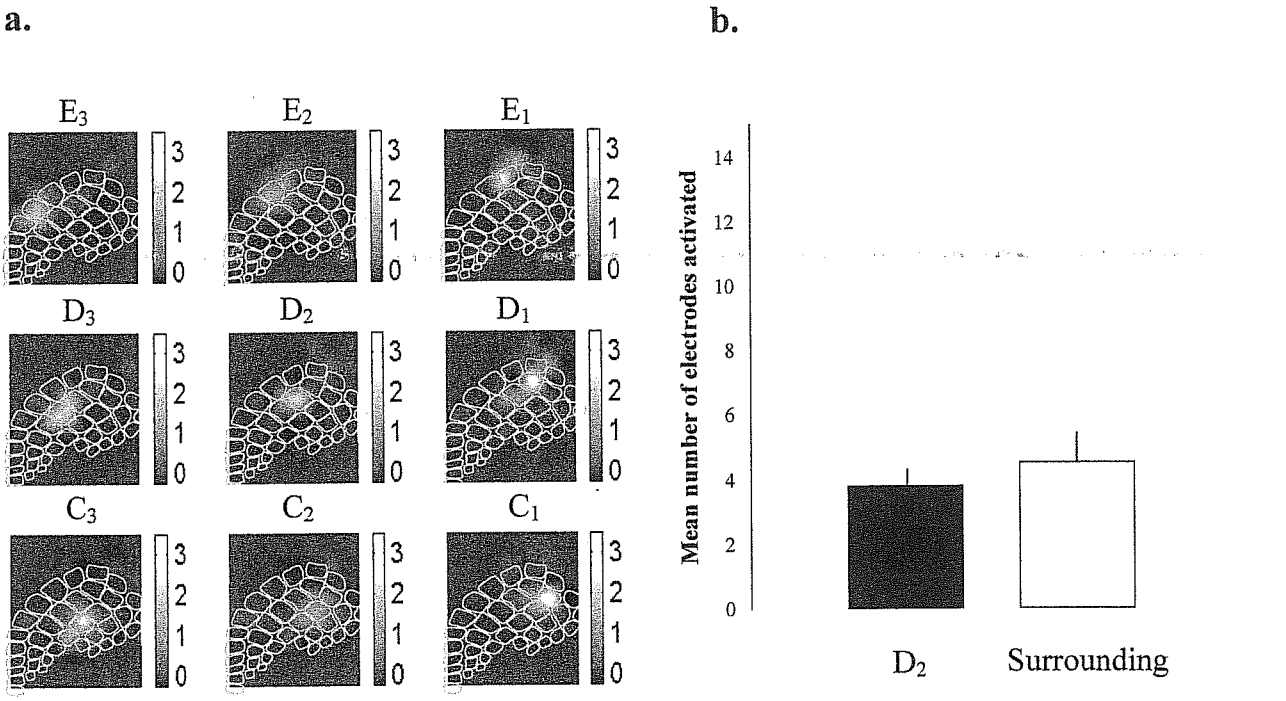


Figure 1. Representations of neighboring vibrissae were comparable in naïve animals in the studied 3 X 3 vibrissa grid. **(a)** An example of within-animal comparability of the neighboring vibrissae representations. A sketch of the barrel field was fit onto the representational maps of the studied vibrissae. **(b)** Mean number of electrodes activated by D2 and surrounding vibrissae deflections (Mean ± STD).

The number of electrodes activated by a given vibrissa in the 3 X 3 grid studied was 4.5, on average. There was no significant difference between representations of D2 (n = 5; 3.8 ± 1.5 electrodes; mean ± std.) and surrounding (n = 40; 4.5 ± 2.3 electrodes; mean ± std) vibrissae (One-Way ANOVA, $p < 0.47$). It is important to note that the number of electrodes activated after naive vibrissa deflection in this study was smaller than the previous published data (see below for discussion).

Effect of vibrissa-sparing in home cage on cortical representation

To study the effect of vibrissa-sparing alone, a second group of animals had all vibrissae clipped, except their right D2 (Described in detail in Chapter 2). Since these animals were left in the homecage and received no training, we call this the 'homecage' group. After 13 days of sparing we studied the representation of vibrissae C1-C3, D1-D3, E1-E3 in the supragranular layers of the barrel cortex using the same methods as in the previous section.

In all rats (n=5) in the home cage group, the representation of the spared whisker (D2) was significantly larger than those of the cut vibrissae (One-way ANOVA, $p < 0.005$; Figure 2a). On average, D2 deflection evoked significant neural activity in 14.6 ± 2.9 electrodes; cut vibrissae, on the other hand, evoked significant neural activity in just 8.7 ± 3.9 electrodes (Figure 2b).

Between-group comparisons showed that the representation of vibrissa D2 after home cage experience was significantly larger than in naïve animals (14.6 versus 4.5 electrodes ;Tukey-HSD, $p < 0.0005$). But not only the D2 representation expanded in homecage group: in fact, the representations of surrounding (cut) vibrissae were significantly larger than those of the corresponding vibrissae in naive animals (Tukey-HSD, $p < 0.0001$), although smaller than that of the spared, D2 vibrissa (One-way ANOVA, $p < 0.005$).

We further asked whether, in the home cage group, the representation of the D2 vibrissa in comparison to cut vibrissae was different from the difference between the representations of D2 and surrounding vibrissae representations in the naïve animals. Factorial analysis of variance on the representation sizes (i.e. number of activated electrodes) of spared and cut vibrissae showed that the spared-cut difference in the home cage group was significantly different ($F=21.320$; $df= 15$; $p < 0.01$) from that in the naïve animals (see Figure 2c).

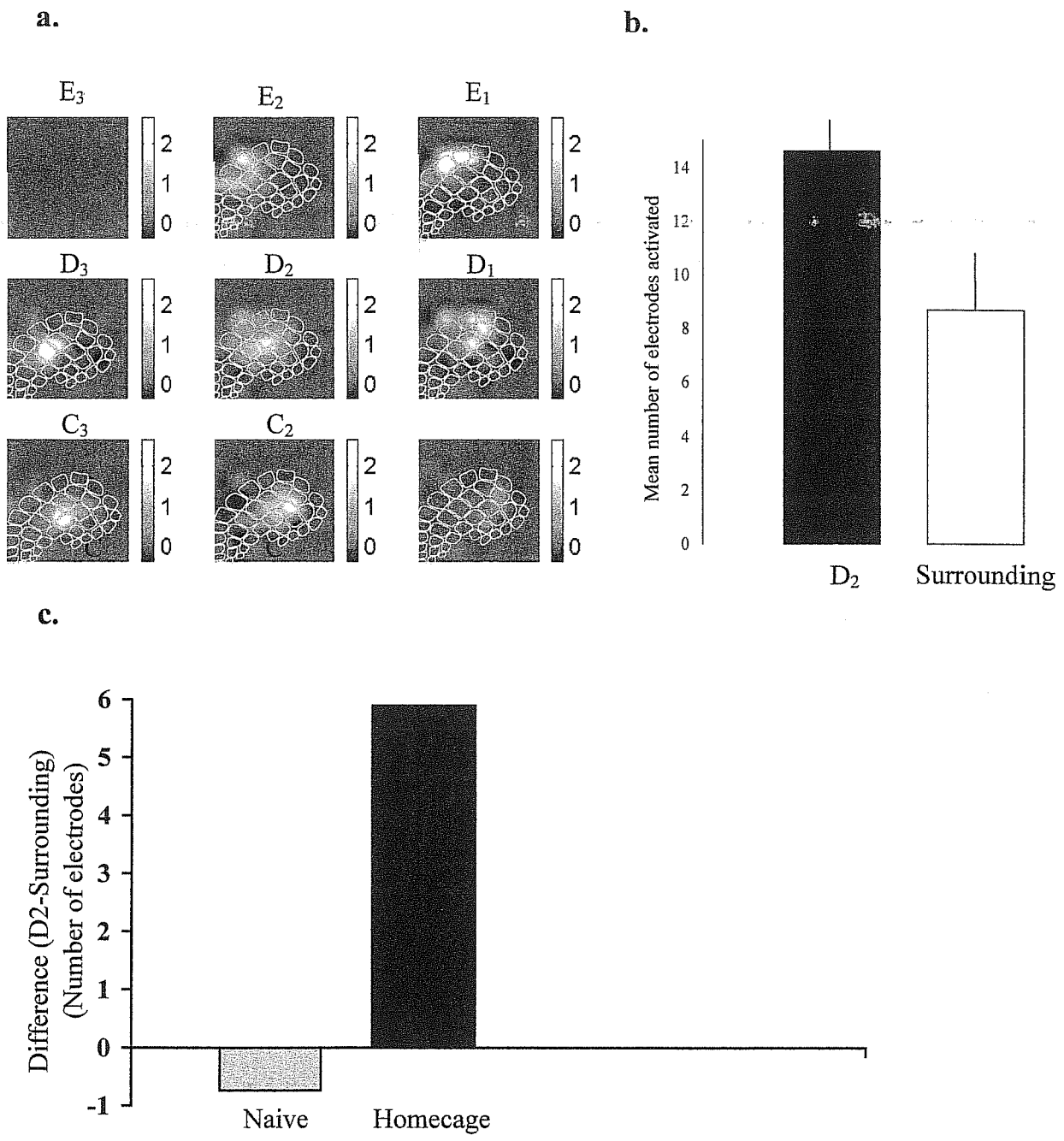


Figure 2. Barrel cortex plasticity in the homecage group. **(a)** A typical example of the representational maps of the spared (D2) and cut vibrissae. Note that the representation of the cut vibrissae increased in comparison to the vibrissae representations in naïve animals. **(b)** Mean number of activated electrodes plotted for D2 and cut vibrissae deflections (Bars STD). **(c)** Between-group analysis showed that increased representation of the D2 vibrissa in comparison to cut vibrissae was significantly different from the differences of the representations of the D2 and surrounding vibrissae in the naïve animals.

In summary, 13 days of vibrissa-sparing caused a global expansion in the representation of all vibrissae, spared and cut, in supragranular layers. The expansion was greater for the spared vibrissa.

Vibrissae representation in vibrissa-crossers

In order to test whether the nature of vibrissa usage affects cortical representation, we trained a third group of rats on a behavioral task for which information gathered by D2 vibrissa was crucial (see Chapter 2 for details). Rats in this group, called the "vibrissa-crossers," received 13 days of vibrissa sparing exactly as for the homecage group; representations of the 9 vibrissae were studied as above.

The representations of the five trained (D2) and 35 cut vibrissae were mapped in this group. The striking result was that representation of the trained vibrissa was confined to a territory of 8.0 ± 5.2 electrodes (mean ± STD) and was *not* significantly larger than the D2 representation in the naïve animals (Tukey-HSD, $p > 0.05$). *Moreover, the D2 representation in vibrissa-cross rats was smaller than that in the homecage group* (8 versus 14.6 electrodes; Tukey-HSD, $p < 0.005$).

Deflection of cut vibrissae in the vibrissa-crossers, on the other hand, evoked significant neural activity in 6.4 ±5.0 electrodes, which was not significantly different (One-Way ANOVA, $p > 0.51$) from the trained vibrissa (See Figure 3). Between-group comparison showed that the size of the cut vibrissae representations in the gap-crossers were not significantly different from those in the naïve animal ($p > 0.05$). The size of the representation of the cut vibrissae after training at vibrissa-distances was also significantly smaller than the representations of the cut vibrissae in the homecage group (Tukey-HSD, $p < 0.0001$).

In summary, representations of both the spared and cut vibrissae were smaller in gap-cross trained rats than in home-cage rats, and the representation of the D2 was comparable to that in naive animals.

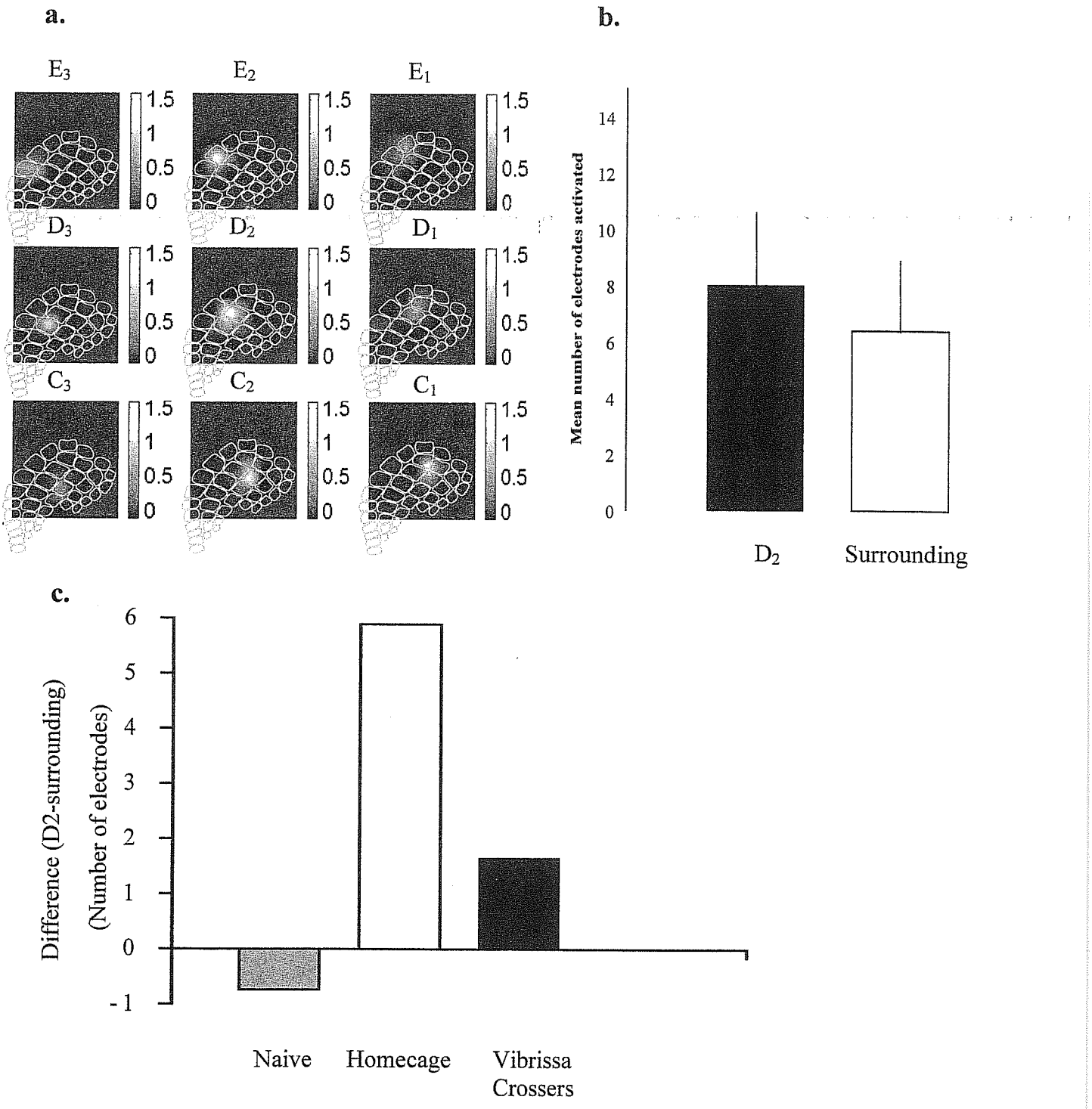


Figure 3. Vibrissae representations in subjects trained to gap-cross at vibrissa-distances. **(a)** Typical example of the change in size of the representations of trained and cut vibrissae. **(b)** Mean number of electrodes activated by D2 and the cut vibrissae (bars, STD). **(c)** Between-group analysis showed that the size of the cortical vibrissae representations in vibrissa-crossers did not differ from experimentally naïve animals, but was significantly smaller those in the homecage group (Factorial Analysis of Variance, $p < 0.01$).

We further compared the change in the representations of the trained and cut vibrissae by calculating the difference between number of electrodes activated after the two types of vibrissae deflected (see Figure 3c). In vibrissa-crossers, vibrissa D2 activated on average 1.6 electrodes more than the cut-vibrissae ($p > 0.51$). Between-group comparison showed that this difference (D2 versus cut vibrissae) was not significantly larger than that seen in the naïve animals (Factorial Analysis of Variance, $F=2.790$; $df= 15$; $p > 0.05$), but it was significantly smaller than the difference found in the homecage group (Factorial Analysis of Variance, $F=8.685$; $df= 15$; $p < 0.01$). This leads to a second observation: the spared/cut vibrissa imbalance – in favor of the spared vibrissa in home cage rats – appears to have been counteracted by the gap-crossing experience.

Vibrissae representations in nose-crossers

To understand these results in more detail, we attempted to isolate the role of single vibrissa use in the gap-cross training on cortical vibrissae representations. A fourth group of rats received vibrissa sparing precisely as above. In addition, they were trained on the same gap-crossing task as the vibrissa-crossers, with the difference that the gap distance was set at a maximum of 6 cm, permitting rats to use both the nose and vibrissae in order to perform the task. We call this group 'nose-crossers'. These rats thus received very similar experience to the vibrissa-crossers, but were not forced to rely on information from the D2 vibrissa.

For comparison with the vibrissa-trained group, it is helpful to recall that nose-crossers used the intact whisker alone (much like the rats in the vibrissa-trained group) on approximately 40% of trials. If plasticity of the cortical vibrissa representation depends closely on the way in which whiskers are used to collect sensory data, one would predict that cortical representations in the nose-trained group would be similar to those in the whisker-trained group, but with some tendency to resemble the homecage group. For the five rats in this group, in total, five spared (trained) and 35 cut vibrissae representations were mapped. Deflection of spared vibrissae evoked significant responses at 9.2 ±2.8 electrodes, whereas that of cut-vibrissae activated 5.9 ±3.1 electrodes (see Figure 4a-b). The difference was significant (One-Way ANOVA, $p < 0.05$). Compared to the homecage group, the extent of the activity evoked by the trained-vibrissa was significantly smaller (Tukey-HSD, $p < 0.005$). The comparison between the D2 vibrissa in the nose-crosser (9.2±2.8 electrodes) and naïve (3.8 ± 1.5 electrodes) groups was marginally significant after Bonferroni corrections ($p < 0.00828$; after Bonferroni correction $\alpha = 0.00833$).

This suggests that training in gap-crossing task at vibrissa, but not nose, distances reduced the spatial extent of the vibrissa-evoked activity.

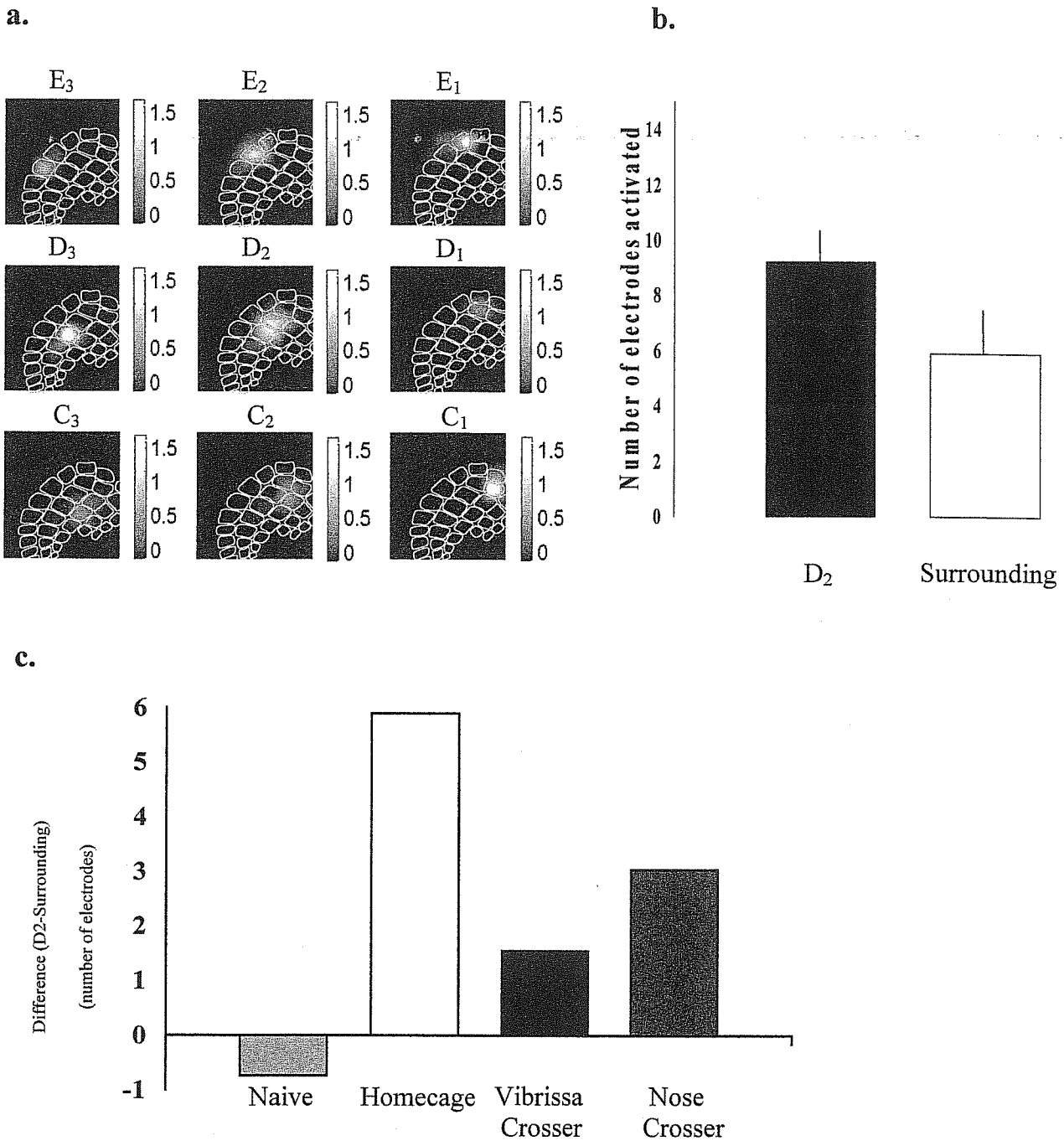


Figure 4. Vibrissae representations in nose-crossers. **(a)** An example of the change in representational size of trained and cut vibrissae. **(b)** Mean number of electrodes activated by D2 and cut vibrissa (bars, STD). **(c)** Between-group analysis showed that nose-crossers differed neither from the homecage group nor from vibrissa-crossers group, but did differ from experimentally naïve animals (Factorial Analysis of Variance, $p < 0.05$).

There was a trend (though not statistically significant) towards expansion of the spared vibrissa in these nose-trained rats in comparison to the D2 vibrissae representation in naive animals (9.2±2.8 vs. 3.8 ±1.5 electrodes, Tukey-HSD, $p > 0.05$).

Between group comparison of cut-vibrissae representations showed a similar pattern. The spatial extent of the neural activity elicited by cut-vibrissae deflection (5.9 ± 3.1) was smaller (Tukey-HSD, $p < 0.0001$) than that elicited by cut-vibrissae deflection in the homecage group (8.7 ± 3.9 electrodes), but was not different than naive (4.6 ± 2.2 electrodes) or the vibrissa-crossers (6.4 ± 5.0) (One-Way ANOVA, $p > 0.05$).

We further calculated the difference scores in every subject (number of electrodes for D2 minus number of electrodes for cut vibrissae) and classified the data according to the experimental group as described earlier (Figure 4c). Factorial analysis of variance by group showed that vibrissa representations in nose-crossers were more like those in the homecage group ($F=3.9$; $df=15$; $p > 0.05$) than the naïve control group ($F=6.9$; $df=15$; $p < 0.05$). The two trained groups (nose-crossers and vibrissa-crossers), however, did not differ from each other ($F=0.9$; $df= 15$; $p > 0.05$).

CHANGE IN THE MAGNITUDE OF EVOKED ACTIVITY

Until now, we described the change in the spatial extent of the evoked activity after differential vibrissa use. In order to determine whether the magnitude of the evoked activity was modified, we calculated the number of spikes evoked within 50 ms interval after vibrissa deflection and substracted from the background activity. Background activity was defined as number of spikes registered within 50 ms period before the stimulus onset.

In naïve animals, vibrissa deflections evoked on average 3.4 ± 1.9 spikes. Evoked activity upon D2 vibrissa deflection (3.7 ± 1.1 spikes) did not differ from that evoked upon surrounding-vibrissa deflection (3.4 ± 2.0 spikes; One-Way ANOVA, $p > 0.76$). In the homecage group, on the other hand, D2 vibrissa-evoked activity (10.7 ± 3.2 spikes) was significantly greater than that evoked by surrounding vibrissae (6.9 ± 3.9; One-Way ANOVA, $p < 0.05$). Furthermore, in the homecage group, the number of spikes elicited by stimulation of vibrissa D2 was significantly greater than in the naïve group (Tukey-HSD, $p < 0.005$). Vibrissa-sparing increased the amount of activity evoked by surrounding (cut) vibrissae in the homecage group as compared with surrounding vibrissae-evoked activity in the naïve animals (Tukey-HSD, $p < 0.0001$).

As for the analysis of the spatial activity, in the vibrissa-crossers, in the animals trained at vibrissa-distances there was no statistically significant difference between the evoked activity elicited by the trained vibrissa (5.5 ± 3.1 spikes) and surrounding-cut vibrissa (4.2 ± 3.6 spikes) (One-Way ANOVA, $p < 0.46$). Moreover, the amount of neural activity evoked by the trained vibrissa was not different from the D2 vibrissa-evoked activity in naïve animals (One-Way ANOVA, $p > 0.05$). The amount of activity was, however, significantly smaller than that evoked by spared-vibrissa deflection in the home-cage group (Tukey-HSD, $p < 0.005$). This observation, like those concerning the spatial extent of activation, support the notion that gap-cross training caused a shrinkage in the representation of the D2 vibrissa compared to the that in the homecage group.

When the neural activity evoked by cut vibrissae was compared between groups, it was found that the difference between the homecage group and vibrissa-crossers was preserved: cut-vibrissae deflections in the homecage group evoked more spikes than the cut-vibrissae deflections in vibrissa-crossers (Tukey-HSD, $p < 0.0001$). Evoked activity after cut-vibrissae deflections in vibrissa-crossers was not different from those in naive rats (One-Way ANOVA, $p > 0.05$). Overall the barrel cortex functional representations resembled those in naive rats more than they resembled homecage rats.

Within 'nose-crosser' animals, the spared-vibrissae evoked more spikes (10.0 ± 3.1 spikes) than did the cut vibrissae (4.4 ± 2.6 spikes; One-Way ANOVA, $p < 0.0001$). D2 vibrissa deflection in nose-crossers evoked more spikes than in naïve animals (Tukey-HSD, $p < 0.005$). However, the response to vibrissa D2 in nose-crossers was statistically indistinguishable from that in the other two groups. Comparison between groups concerning the activity evoked by cut vibrissae yielded similar results: after animals were trained in the gap-crossing task at nose distances, deflections of cut vibrissae evoked significantly more spikes in naïve animals (Tukey-HSD, $p < 0.0001$), although the response magnitude for deflections of cut-vibrissae were comparable in all other groups.

We further studied the change in magnitude of the evoked activity after vibrissae deflections by calculating the difference between the number of spikes elicited by D2 and surrounding vibrissae deflections and making between group comparisons using factorial analyses of variance. The results are summarized in Figure 5.

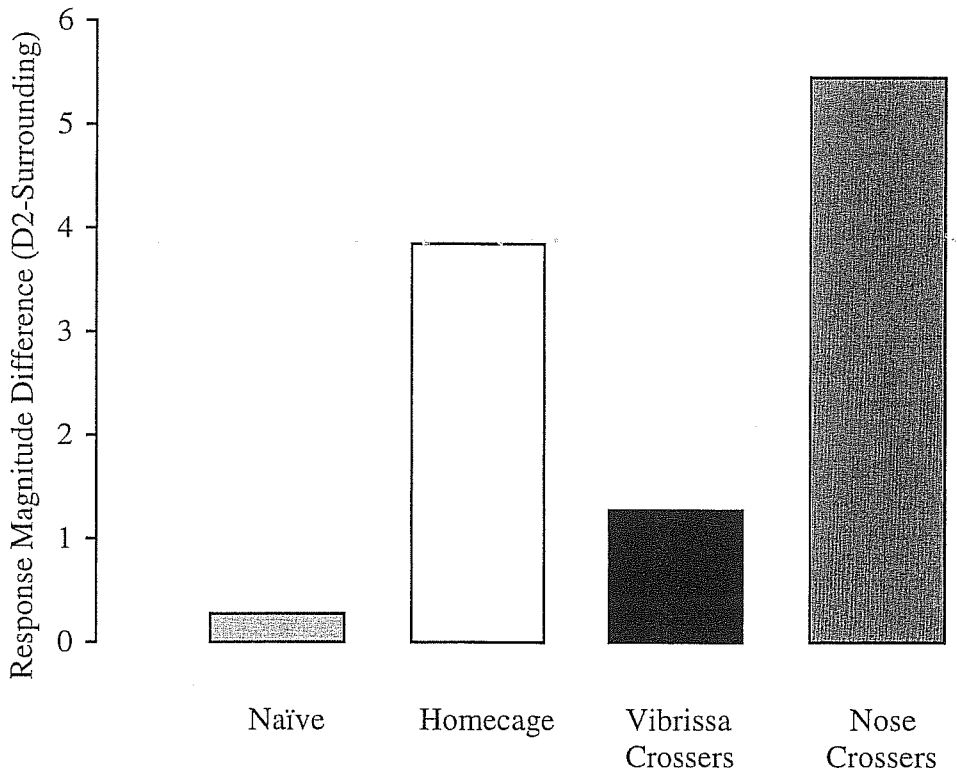


Figure 5. Group comparisons in terms of difference between D2 and surrounding vibrissae evoked activity. (See the text for details.)

When difference scores on the response magnitudes were compared, animals in the homecage group ($F=6.709$; $df= 15$; $p < 0.05$) and nose-crosser group ($F=14.118$; $df=15$; $p <0.01$) but not vibrissae-crossers ($F= 0.531$; $df= 15$; $p > 0.05$) significantly differed from the naive group (Factorial analysis of variance). Again, the vibrissa-crossers seem to have a cortical topography closest to that of normal, naive rats. Difference scores in vibrissa-crossers were significantly different from those in nose-crossers ($F=9.175$; $df=15$; $p <0.01$).

RECEPTIVE FIELD PLASTICITY

We analyzed neural activity in 153 neuronal clusters in 15 rats of three experimental groups (see Table 2 for the distribution of the neural clusters per experimental group). In addition to the groups which received experimental manipulation in vibrissae use for 13 days, a group of naive rats were studied. A total of 37 clusters, 11 of which was from D2 column, was studied in this group. All neural clusters of interest were located in the columns: D1, D2, and D3.

Recording site	Duration of vibrissa sparing	Homecage	Vibrissa-crossers	Nose-crossers
Supragranular Layers	13 days	62 (25, 37)	53 (14, 39)	38 (16, 22)

Table 2. Distribution of the recorded neural clusters as a function of experimental group. Values in front of the parentheses are the total number of neuronal clusters recorded in a particular experimental group. Values in parentheses are the numbers of clusters recorded from D2 and Dcut (D1 or D3) columns, respectively.

For all groups that received vibrissa-trimming, D2 was the spared vibrissa. Two kinds of controls were used to examine the effect of gap-cross training and home cage experience in the size of receptive fields: (1) between-group comparisons against naive rats, and (2) within-group comparisons between the receptive fields of D2 neuronal clusters compared to D1/D3 neuronal clusters. The stimuli in the receptive field mapping consisted of single vibrissa mapping for 60 trials at 1 Hz. Invariably, vibrissae C1-C4, D1-D4, E1-E4, γ and δ were deflected in every animal.

CHANGE IN THE SIZE OF RECEPTIVE FIELD IN THE SUPRAGRANULAR LAYER NEURONS AFTER 13 DAYS OF VIBRISSA-SPARING

Neurons in naive rats had receptive fields containing, on average, 2.9 ± 1.4 vibrissae. Data from D1 and D3 columns were pooled and referred to as Dcut (Diamond et al., 1993). There were no significant differences in receptive field size between the D2 column (3.0 ± 1.2) and the Dcut columns (2.8 ± 1.4; Student's t-test, $p < 0.92$). In the home cage group, however, receptive fields of neurons in columns D2 (4.0 ± 1.9) and Dcut (4.2 ± 1.8) increased in comparison to those in naïve animals (see Figure 6; One-Way ANOVA, $p < 0.001$). The difference between D2 and Dcut receptive field sizes was not significant (Student's t-test, $p > 0.62$).

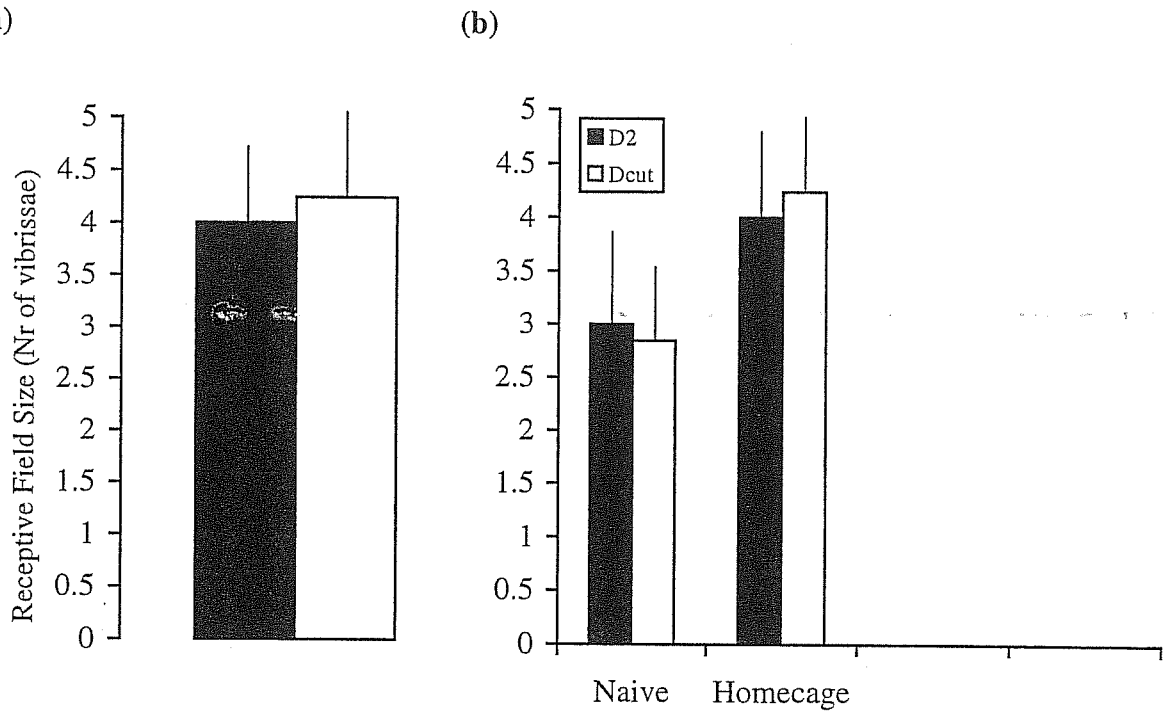


Figure 6. Change in the receptive field size in the homecage group. **(a)** Within homecage group between D2 and Dcut comparison. **(b)** Between group comparison in terms of receptive field sizes of D2 and Dcut

Two of the four groups were trained in the gap-crossing task. In vibrissa-crossers, receptive fields in columns D2 as well as in Dcut columns expanded, but *not* significantly, in comparison to the receptive fields in naive rats. On average, the receptive field of D2 neurons was 3.8 ± 1.1 vibrissae, whereas that for Dcut neurons was 3.6 ± 1.4 vibrissae (see Figure 7a). There was no significant difference within vibrissa-crossers, between vibrissa type (trained versus cut vibrissae) comparisons in this group (Student's t-test, $p > 0.59$). Between group comparisons showed that receptive field sizes in vibrissa-crossers were comparable to those in the homecage group (Student's t-test, $p > 0.5$).

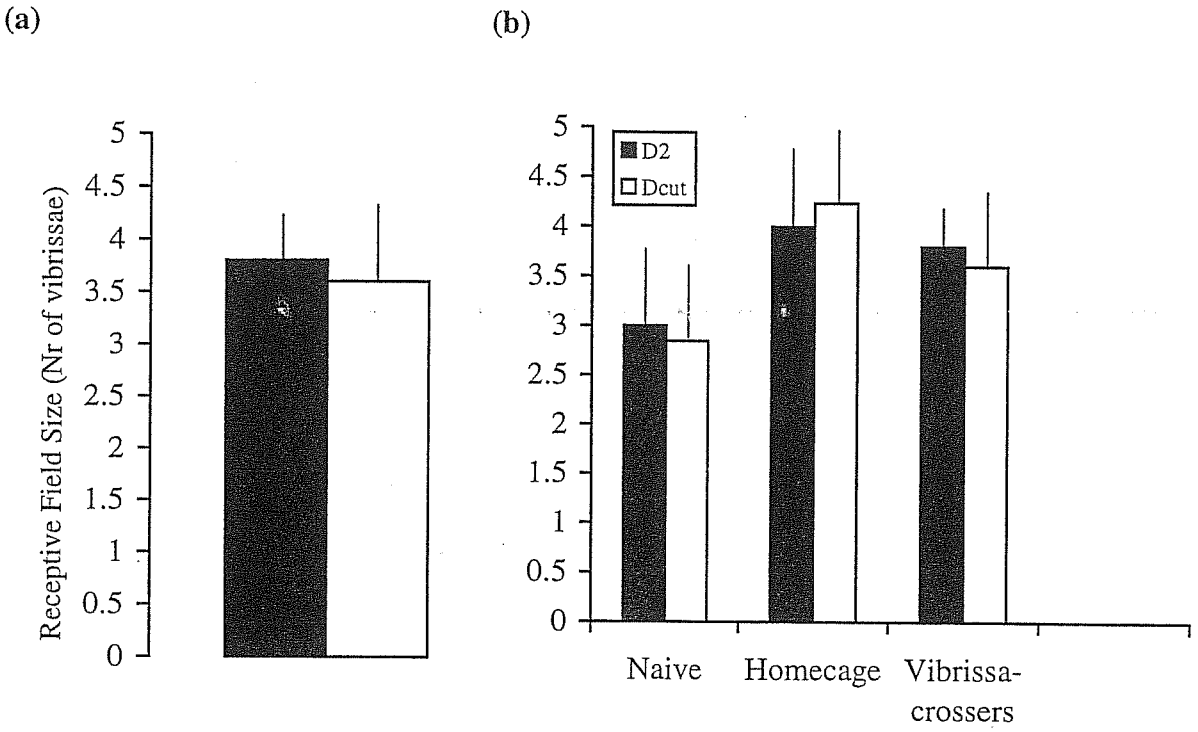


Figure 7. Change in the receptive field in vibrissa-crossers. **(a)** Mean receptive field size comparison for D2 and Dcut neurons in vibrissa-crossers. **(b)** Between group comparisons in terms of receptive field sizes of D2 and Dcut neurons.

In total, 38 neuronal clusters were studied in nose-crossers. Receptive fields in column D2 (average 4.1 ± 2.4 vibrissae) and Dcut (4.8 ± 2.8 vibrissae) did not differ significantly (Figure 8a; Student's t-test, $p > 0.41$). Nor did either value differ from the corresponding values in the home-cage ($p > 0.5$), or vibrissa-crossers groups ($p > 0.08$). Nonetheless, they were significantly larger than those for the naive group (Figure 8b ; One-way ANOVA, $p < 0.001$)

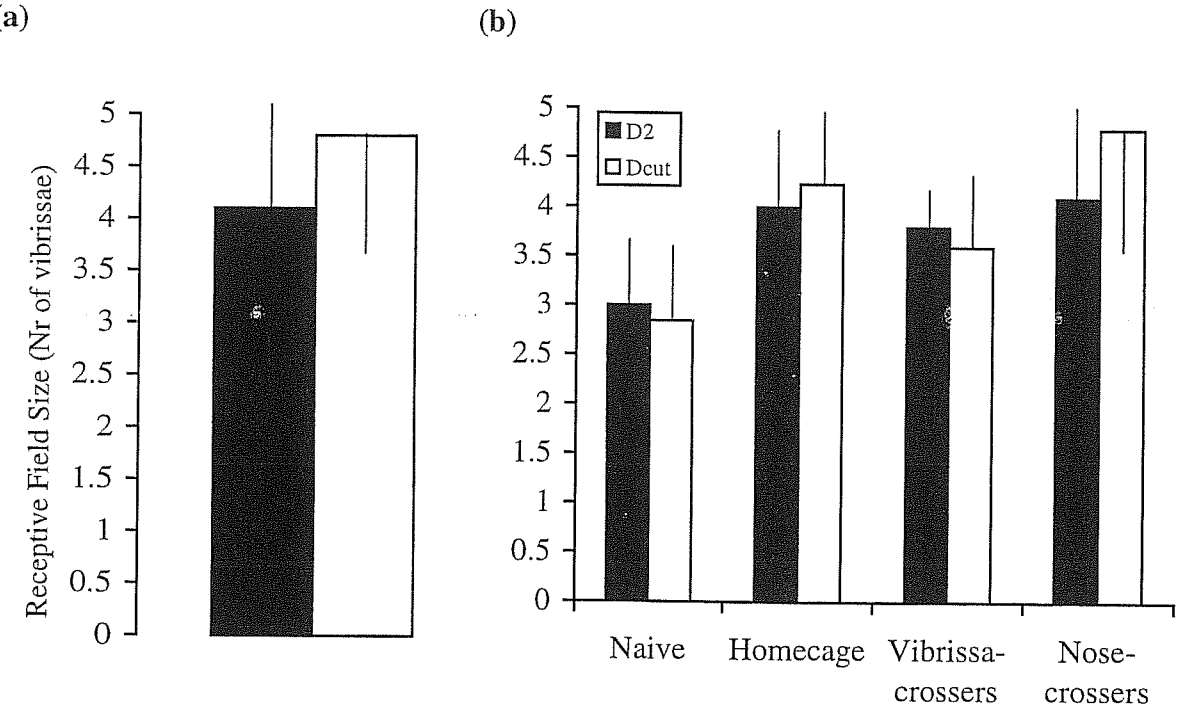


Figure 8. Change in the receptive field size in nose-crossers. **(a)** Receptive field size comparison between D2 and Dcut neurons, within nose-crossers. **(b)** Between group comparisons receptive field size of neurons in D2 and Dcut columns in all groups.

SUMMARY

In this section, we examined whether the nature of cortical plasticity in supragranular layer neurons depends on the nature of vibrissa usage. Three different measurements of cortical plasticity (representation of vibrissae, amount of activity evoked elicited by vibrissa deflections, and the receptive fields of barrel cortex neurons in the supragranular layers), suggested the effects of vibrissa use on cortical plasticity. With vibrissa-sparing alone (homecage group) representation of the spared vibrissae expanded in comparison to naïve animals. The amount of vibrissa-evoked activity, and the extent of the receptive field also increased. However if the animals were trained in the gap-crossing task at vibrissa-distances (vibrissa-crossers), both the representation of vibrissae, the amount of the vibrissae-evoked activity and the size of the receptive field all were reduced compared to the homecage group and the nose-crosser group, but were not different from those in the naïve group. These results are discussed below.

SECTION II
SITES OF PLASTICITY

Neurons in the supragranular layers of the barrel field show alterations in response properties as a function of vibrissa usage. At what level of the vibrissa-to-barrel system do these changes take place? Two possibilities are (i) subcortical (including thalamocortical synapses) and (ii) cortical. According to theory of subcortical the change, plastic changes should be manifested in the very earliest responses (about 8 ms) elicited by vibrissa deflections. According to the theory positing pure cortical plastic changes , changes neural responses should be manifested only in the entire response (0-50 ms). Thus, to determine loci of the modifications described in the previous section, we calculated the spatial extent of statistically significant response at the response onset (8 ms) and compared the four groups described above.

For both naive animals and vibrissa-crossers, the spatial extent of the neural activity evoked by D2 did not differ from that of cut vibrissa (see Table 3; One-way ANOVA, $p > 0.05$). However D2-evoked activity was more widespread than that evoked by cut vibrissae in both the homecage ($p < 0.001$) and nose-crosser ($p < 0.05$) groups (One-Way ANOVA).

	Naïve	Home Cage	Vibrissa-crosser	Nose-crosser
D2	2.2 ± 0.8 (n=5)	6.2 ± 1.8 (n=5)	3.2 ± 1.1 (n=5)	4.2 ± 2.2 (n=5)
Surrounding	2.5 ± 1.3 (n=40)	3.3 ± 1.7 (n=35)	2.4 ± 1.3 (n=35)	2.2 ± 1.7 (35)

Table 3. The spatial extent of the vibrissa evoked activity within 8 ms after stimulus onset. Values are mean ± std (n=number of vibrissae studied). See the text for details and statistical interactions.

Between group comparisons of D2 vibrissa representation showed that when a vibrissa was spared and used only in a familiar environment (i.e home cage) but not in the gap-crossing task (ie as in nose-crossers and vibrissa-crossers), the areal extent of the initial cortical responses increased in comparison to that of naïve animals (Table 3; Tukey-HSD, $p < 0.01$). However in the

homecage group, cut-vibrissa deflections did not evoke significantly more widespread initial activity in comparison to the naive group. This suggests that the increased initial activity in the cortex was not simply due to a global change in the excitability of the cortical neurons.

In the vibrissa-crossers there was no difference in the spatial extent of the initial evoked activity at 0-8 ms time course after vibrissae deflections in comparison to naïve animals (Table 3; One-Way ANOVA, $p > 0.05$). However when the vibrissa-crosser group was compared to animals in the homecage group, it was found that D2 deflections in the vibrissa-crossers evoked activity in a smaller area than it did in the home cage group (3.2 ± 1.1 and 6.2 ± 1.8, respectively; Tukey-HSD, $p < 0.01$)

To compare the changes seen after homecage experience versus those seen after training, we calculated the difference between the activity evoked by D2 and surrounding vibrissa and performed between group factorial analysis of variance. The results showed that within-animal alterations (i.e. vibrissa trimming) in the size of vibrissa representation in the home cage group and nose-crossers were significantly larger that the changes seen in the naïve animals (Vibrissa-spared vs. naïve : $F=22.980$; $df=15$; $p < 0.01$; Nose-trained vs. naïve: $F=9.704$; $df=15$; $p < 0.01$). However, animals that received gap-cross training at vibrissa-distances did *not* differ from the naïve animals ($F=2.749$; $df=15$; $p > 0.05$).

In summary, the results of this analysis indicate that after 13 days of vibrissa-sparing experience in the home cage, the activity evoked by D2 vibrissae deflection in the supragranular layers of the barrel field increases in comparison to that in both naïve animals and vibrissa-crossers. This suggests that thalamocortical connections strengthened in the home cage but not in the vibrissa-crosser group. This enhanced connectivity is specific to the D2 vibrissa in the home cage group, given that cut vibrissae in the home cage group did not show any facilitated initial responses. The results of this analysis suggest that pathways of the reorganizational modifications following vibrissa-sparing depends to some extent on how the spared vibrissa is used.

SECTION III
PLASTICITY IN THE INFRAGRANULAR LAYERS

Motivated by the anatomical, biochemical, and electrophysiological differences between laminae reviewed in the introduction to this chapter, we asked what sort of functional changes the infragranular layers might show following 13-d of altered sensory experience. Since the infragranular layers are the main output from barrel cortex, these findings should help us understand how the rest of the brain "sees" barrel cortex modifications. These observations might also provide insights into whether cortical columns act as functional units during sensory learning, or whether the different layers are modified in particular ways.

Methods and stimuli applied in this section were as described above, except that arrays were implanted into infragranular layers (>1100 μm from the pial surface) (See Chapter 2 for details).

Representational Plasticity

In naïve animals (n=5), the spatial extent of vibrissa D2-evoked activity (2.0 ± 1.0 electrodes) was not significantly different from that evoked by surrounding vibrissae (2.4 ± 1.6 electrodes; $p > 0.5$). After 13 days of vibrissa-sparing (homecage group), there was no significant difference between the D2 (8.6 ± 1.9 electrodes; n =5) and cut vibrissa (7.4 ± 3.7 electrodes; n=37) representations ($p = 0.3$; Figure 9a). Vibrissa D2 did activate a larger area compared to the naïve group ($p < 0.001$). Deflection of the cut vibrissae also evoked activity in a larger area in the infragranular layers compared to the naïve group ($p < 0.0001$; Figure 9b).

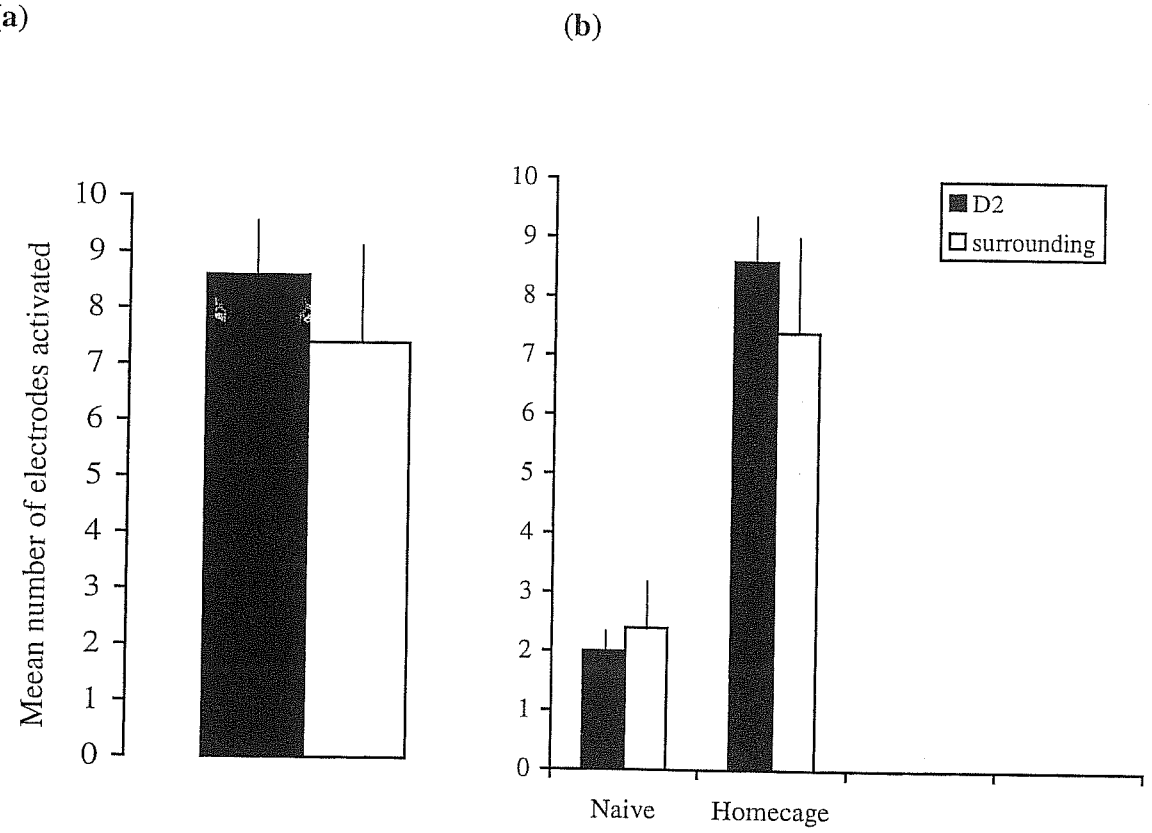


Figure 9. Cortical plasticity induced by vibrissa-sparing for 13 days in home cage (Homecage group). **(a)** Within-group between vibrissa type (D2 versus cut vibrissae) comparison in terms of mean number of electrodes activated (bars, STD). **(b)** Between-group comparisons for both D2 and cut vibrissae elicited activity (bars, STD).

Comparisons within-group, between vibrissae type (i.e. trained vs surrounding-cut vibrissae) showed that, in the groups that received gap-cross training, representations of the trained vibrissae (D2) were no larger than those of surrounding-cut vibrissae. In vibrissa-crossers, the trained vibrissa activated 4.6 ± 2.2 electrodes, whereas surrounding vibrissae activated 5.1 ± 3.6 electrodes. The difference was not significantly different ($p = 0.65$) (Figure 10a). The D2 representation in vibrissa-crossers was significantly larger than in the naïve group ($p < 0.03$), but significantly smaller than in the homecage group ($p < 0.008$; Figure 10b).

After gap-cross training at vibrissa-distances, surrounding-cut vibrissa deflections, on the other hand, activated about 5 electrodes. The spatial extent of the activity in the vibrissa-crossers group was significantly larger than that in the naïve group ($p < 0.0001$) but was significantly smaller ($p < 0.006$) than that in the homecage group (see Figure 10b).

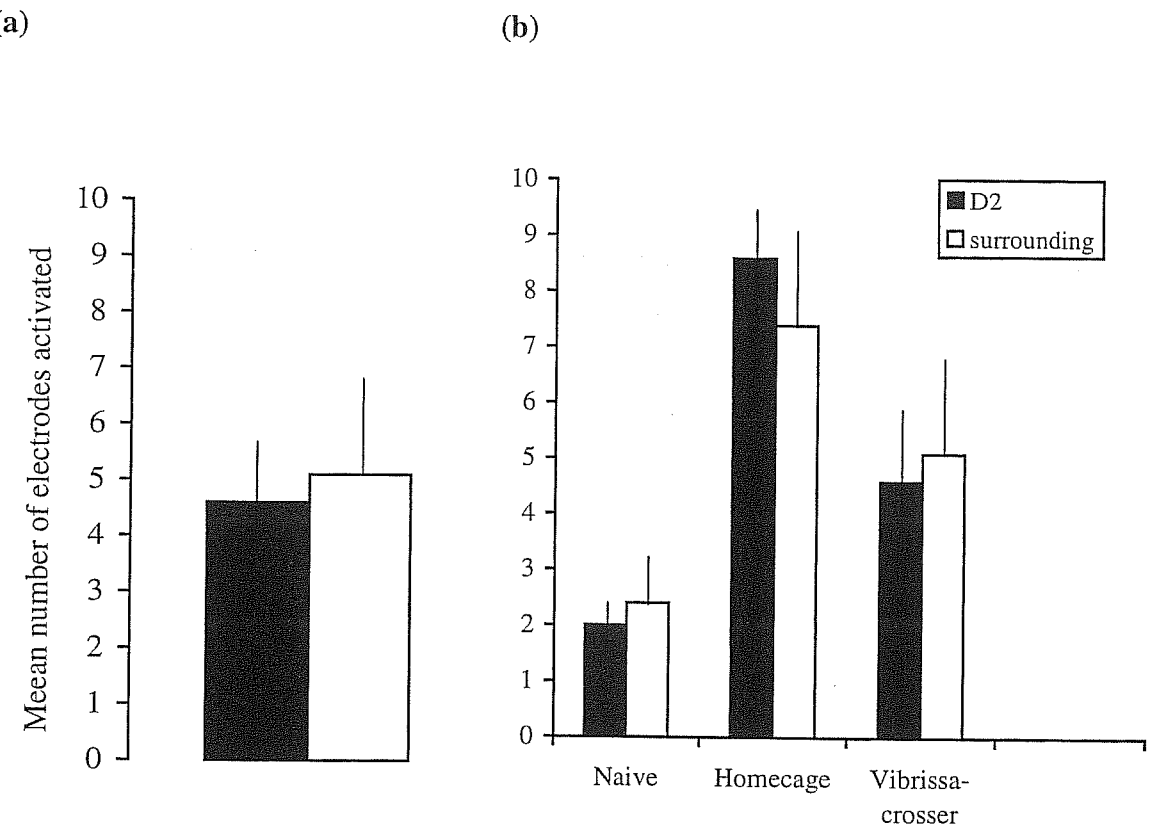


Figure 10. Representational changes after gap-crossing training in vibrissa-crossers. **(a)** Within-group between vibrissa type comparison. **(b)** Between-group comparisons as a function of mean number of electrodes activated (see text for details).

As in the other three groups, in nose-crossers, representations of D2 (6.2 ± 3.7 electrodes; n=5) and the surrounding-cut vibrissae (4.4 ± 3.3 electrodes; n=36) did not differ significantly ($p < 0.36$; Figure 11a). However, the spatial extent of the D2 representation was significantly larger than that in naive group ($p < 0.03$), and indistinguishable from that in the homecage group ($p>0.21$) and the vibrissacrossers ($p=0.12$) (Figure 11b). A similar pattern was found for the surrounding-cut vibrissae. Representation of the surrounding-cut vibrissae expanded after gap-cross training at nose distances in comparison to the naive group ($p<0.0001$) but was smaller than the corresponding representation in the homecage group ($p <0.0005$). The nature of the training, thus, did not significantly affect the representation of the surrounding-cut vibrissae (Figure 11b).

These results suggest that plasticity in infragranular layers follows different rules than it does in supragranular layers. In supragranular layers, the single intact vibrissa showed a greatly

enlarged representation after homecage experience, leading to an "imbalance" between the D2 representation and the surrounding vibrissae's representations. In contrast, in the infragranular layers the single intact vibrissa did not shown an enlarged representation (in comparison to the surrounding/cut vibrissae) in the homecage group, allowing the barrel cortex output to maintain a balance between the intact and the clipped vibrissa.

In common with the supragranular layers, gap-cross training caused a diminished representation of both the spared (trained) and the clipped vibrissae. The effect was similar for vibrissa-crossers and nose-crossers.

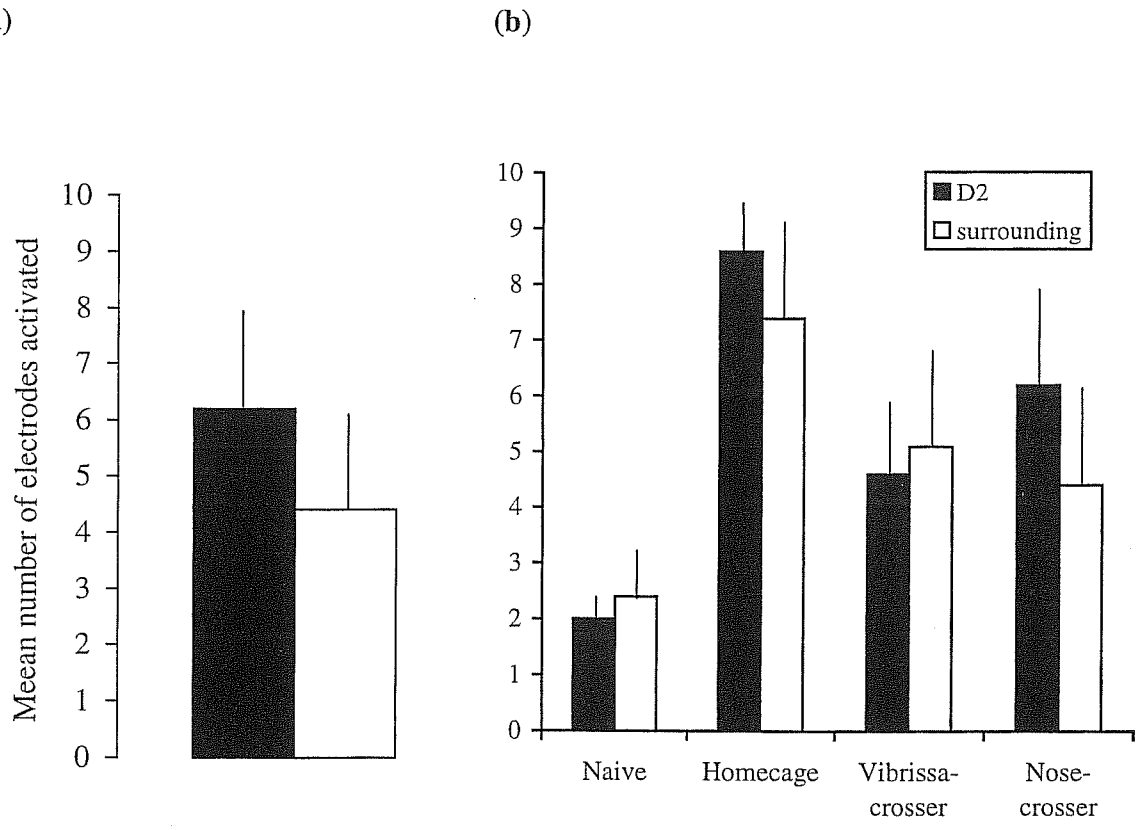


Figure 11. Cortical plasticity in nose-crossers. **(a)** Within-group between vibrissa type comparison. **(b)** Mean number of electrodes activated after D2 and surrounding-cut vibrissae deflections are plotted as a function of group (see text for details).

CHANGE IN THE MAGNITUDE EVOKED RESPONSE MAGNITUDE

In the infragranular layers, vibrissa deflection in the naïve animals evoked about 1.5 spikes per stimulus (n=41). There was not any significant difference between the magnitude of

the response evoked by D2 (1.5 ± 1.4 spikes/stimulus; n=5) and the surrounding vibrissae (1.4 ± 1.2 spikes/stimulus; n=36) in the naïve groups ($p = 0.39$; See Figure 12). In homecage group, D2-evoked responses (9.0 ± 2.5 spikes/stimulus; n=5) were significantly potentiated compared to those of surrounding-cut vibrissae (6.1 ± 4.0 spikes/stimulus; $p < 0.026$) and by all vibrissae in the naive group ($p < 0.0005$) (See Figure 12). Similarly, activity evoked by surrounding-cut vibrissae in the homecage group was statistically greater than that in the naive group ($p < 0.0001$).

In the vibrissa-crosser group, trained (3.5 ± 2.3 spikes/stimulus) and the surrounding-cut vibrissae (3.6 ± 3.0 spikes/stimulus) did not differ significantly in terms of number of spikes evoked after vibrissa deflection ($p > 0.47$). This D2 versus surrounding-cut vibrissae similarity is reminiscent of the analogous result for cortical representation size. We further compared the magnitude of the evoked response for the trained-vibrissa with that for the spared-vibrissa in the homecage group and for all vibrissae in the naïve group. The results showed that, although there was a tendency towards increased spiking activity after training, the amount of neural activity registered after trained-vibrissa deflections was not different from that registered after D2 deflection in the experimentally naïve animals ($p > 0.07$). Nonetheless, D2-evoked activity in trained rats was significantly less than D2-evoked activity in the homecage group ($p < 0.005$; See Figure 12). When the cut vibrissae-induced activity was compared between groups, it was found that the difference between the homecage group and the vibrissa-crossers was preserved: cut-vibrissae deflections in the homecage group evoked more spikes than the cut-vibrissae deflections in the vibrissa-crossers ($p < 0.005$). The difference between the evoked activity after surrounding-cut vibrissae deflections in the vibrissa-crosser group, and those in the naïve group was significant ($p < 0.005$) suggesting facilitation in the evoked responses after training.

The last group of animals was trained at nose-distances (Nose-crossers). Within this group, vibrissa D2 evoked more spikes per trial (5.3 ± 2.8 spikes/stimulus) than the surrounding-cut vibrissae did (3.2 ± 2.7 spikes/stimulus; See Figure 12). Nonetheless, the difference between the activity evoked by the two types of vibrissae did not reach statistical significance ($p = 0.087$). D2 vibrissa deflection generated more spikes than did corresponding vibrissa in the naive group ($p < 0.05$), but fewer spikes than those in the homecage group ($p < 0.05$). The two gap-cross trained groups (vibrissa-crossers versus nose-crossers) did not differ from each other in terms of number of spikes elicited by D2 deflection ($p > 0.15$). Comparison of the activity evoked by cut-vibrissae in different experimental groups yield similar results: in nose-crossers, deflections of the cut vibrissae evoked significantly more spikes than those in the naive group ($p < 0.001$), but fewer

spikes than the corresponding vibrissae in the homecage group ($p < 0.001$). As in the D2-evoked activity, the two gap-cross trained groups did not differ from each other ($p > 0.28$).

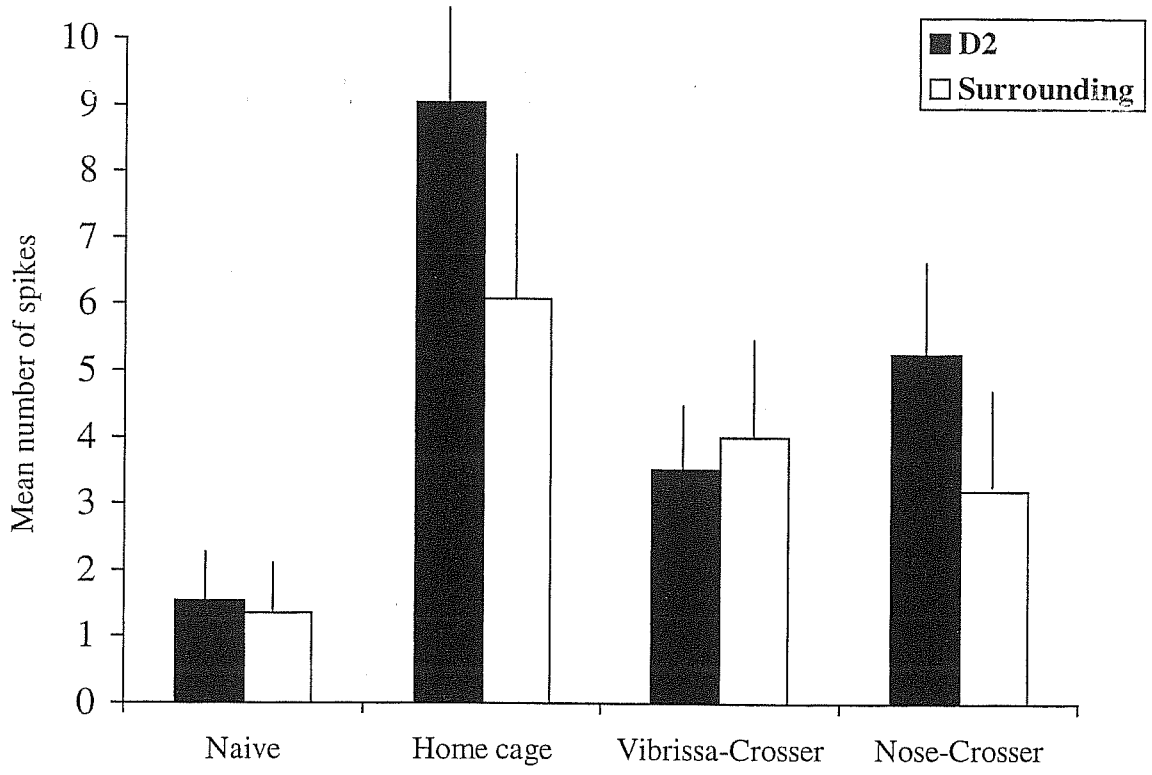


Figure 12. Change in the number of spikes evoked after vibrissa deflections between experimental groups (See text for details).

CHANGE IN RECEPTIVE FIELD SIZE

We analyzed neural activity in 223 neuronal clusters in 15 rats (see Table 4 for the distribution of the neural clusters per experimental group). An additional 30 clusters of neurons were studied in 5 naïve rats as a control. As in the first section, receptive field sizes of neurons in barrel-columns D1, D2, and D3, were studied. The stimuli used to quantify receptive field size were single vibrissa deflections of C1-4, D1-4, E1-4, γ and δ for 60 trials.

Recording site	Duration of vibrissa sparing	Homecage	Vibrissa-crosser	Nose-crossers
Infragranular Layers	13 days	103 (35, 68)	66 (20, 46)	54 (24, 30)

Table 4. Distribution of the recorded neural clusters as a function of experimental group. Values in front of the parentheses are the total number of neuronal clusters recorded in a particular experimental group. Values in parentheses are the numbers of clusters recorded from D2 and Dcut (D1 or D3) columns, respectively.

CHANGE IN THE SIZE OF RECEPTIVE FIELDS OF INFRAGRANULAR LAYER NEURONS AFTER 13 DAYS OF VIBRISSA-TRIMMING

In infragranular layers, receptive field of the neurons in the naïve group contained, on average, 1.6 ± 1.0 vibrissae. There was no significant difference between D2 (1.7 ± 0.6) and neighboring D1 plus D3 (1.6 ± 1.2) neurons' receptive fields (Student's t-test, $p > 0.66$). Following 13 days of vibrissa sparing experience in the home cage (homecage group), on the other hand, receptive fields of neurons in column D2 (4.4 ± 1.9) and columns Dcut (4.3 ± 1.8) increased in comparison to those the naïve group (see Figure 13; One-Way ANOVA, $p < 0.0001$). The difference between D2 and Dcut receptive field sizes within the homecage group was insignificant, (Student's t-test, $p > 0.93$).

Gap-cross training at vibrissa-distances (vibrissa-crossers) increased the receptive field of the D2 (3.3 ± 1.5) and Dcut (3.8 ± 1.7) neurons in comparison to those in the naïve group (Figure 13; One-Way ANOVA, $p < 0.0001$). This enlargement was still less than that seen in the homecage group (Tukey's HSD, $p < 0.0001$). When the receptive field sizes were compared within group between vibrissae type it was found that the training did not significantly modify the receptive fields of neurons in column D2 compared to neurons in Dcut columns (Student's t-test, $p > 0.21$). A similar pattern in the receptive field organization was also found in the nose-crosser group. In other words, the average receptive field of neurons in column D2 (3.2 ± 2.0 vibrissae) was *not* different than that in Dcut (3.8 ± 1.9 vibrissae) (Student's t-test, $p > 0.22$). Receptive fields were larger after gap-cross training at nose distances in comparison to receptive fields in naïve rats (One-Way ANOVA, $p < 0.0001$). Receptive fields in nose-crossers were still smaller than those in the home-cage groups (Figure 13; One-Way ANOVA, $p < 0.0001$). The two groups

which received gap-cross training, on the other hand, did not differ in terms of receptive field size (One-Way ANOVA, $p > 0.05$).

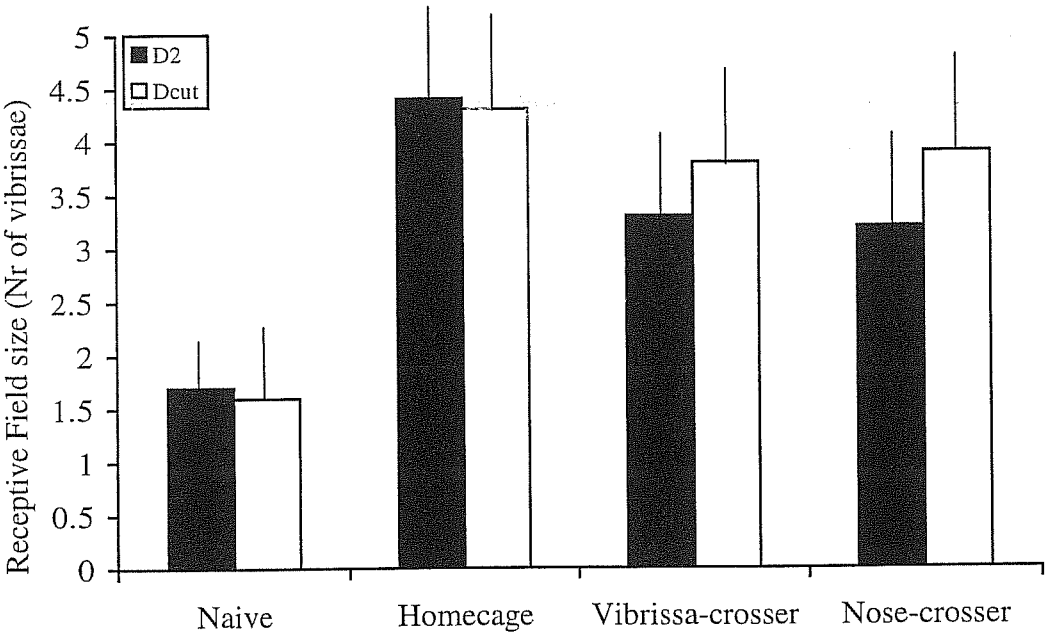


Figure 13. Change in the receptive field size after 13 days long alterations in the vibrissa use as studied in the infragranular layer neurons.

In summary, the results showed that following 13 days of vibrissa-trimming in the homecage, the representation of the spared vibrissa increased significantly in comparison to experimentally naïve vibrissae representations. Moreover amount of the evoked responses after spared-vibrissa deflection, and the size of the receptive field of the spared-vibrissae neurons increased. But if the spared vibrissa was trained in the gap-crossing task the representation of the trained vibrissa shrunk, evoked of the vibrissa-evoked activity reduced, and the size of the receptive field of the 'trained column' decreased in comparison to the spared-vibrissae used only in the homecage. The results suggested that infragranular layers of the barrel cortex are capable of showing representational changes upon alterations of the vibrissa use, and the spatial extent of the change depends upon how the spared sensory receptors are used.

DISCUSSION

In this study we examined change in neuronal activity in barrel cortex elicited by vibrissa deflections after several different modifications in vibrissa usage. Three different methods were utilized to quantify the cortical plasticity. In the first part of the discussion we discuss the results on representational plasticity using spatial extent and magnitude of the evoked responses. In the second part, we discuss the data on receptive field plasticity.

REPRESENTATIONAL PLASTICITY

Representational plasticity has long been recognized to a large extent as increased representation of that portion of a sensory organ which is spared after a surgical operation or is used for sensory learning. Our data show that this does not always hold. The functional representation of a spared vibrissa increases when it is used in the home cage, or in the gap-crossing task but decreases if the spared vibrissa is employed in a perceptual-learning task.

The plastic changes reported here are unanticipated. All but one of the previous studies documented an increased representation of the receptors that are favored after surgical operations or sensory training. However, our data together with those of Polley and colleagues' suggest that spared organ representation increases only if the animal uses its spared organ in a familiar environment (i.e.home cage), but decreases if the spared organ is used alone or together with other tactile organs around the nose in an active exploration task (Polley *et al.*, 1999) or in a perceptual learning task (see results). Although the two studies are comparable in several ways, there are significant differences.

Polley et al. studied the representation of the spared-vibrissa after animals explored a novel environment for a very brief period of time (in 6-7 sessions for 14 min in total) throughout a 28-d period using the spared-vibrissa on one face side, and all vibrissae on the other side of the face. Having prolonged vibrissa-sparing period, limited amount of sensory organ training in an active-exploration task and limited means (i.e. one face side had all its vibrissae intact) of vibrissa-sparing allowed Polley and colleagues to question the role of vibrissae use in an exploration task to modify the nature of the plastic modifications in the cortex. In contrast, we studied the change in the representations of the spared- and cut-vibrissae after 13-d of unilateral single vibrissa experience and 12 training sessions presented daily for about 10 min everyday. This training and sparing protocol led us to question nature of the representational change in the spared and cut

vibrissae after the animals learned a perceptual task using their spared vibrissa following an extensive training period.

There are numerous differences between the results documented in the literature, and the ones presented here. Most previous studies employed only home cage experience (Merzenich *et al.*, 1983, 1984; Fox, 1992, 1994; Diamond *et al.*, 1993, 1994). Some other studies, on the other hand, employed learning tasks (Jenkins *et al.*, 1990; Recanzone *et al.*, 1992). We, on the contrary, studied representational plasticity both with animals exposed to a familiar environment alone (i.e. home cage) and with animals exposed to the home cage environment and to a novel environment where the animal was required to learn a perceptual task. Thus, whatever plasticity processes were evident in trained rats are likely to have been superimposed (linearly or nonlinearly) on the modifications that occurred in the homecage rats.

Another difference between past studies and the present one concerns the time course of the altered sensory experience. Plasticity has been studied across many different time courses. It is well-known that after invasive procedures, modifications in the responsiveness of a neuron can start as early as a few minutes after denervation (Calford and Tweedale, 1991; Faggin *et al.*, 1997). After innocuous changes, cortical modification has been found as early as 1 day after vibrissae-trimming (Diamond *et al.*, 1994). Although differential time courses could explain the differences in the maximum extend of the sensory organ representation, they can't account for the bi-directional plasticity (i.e. increased or decreased representation of a spared, or trained-vibrissa, respectively).

PATHWAYS OF PLASTICITY

In order to be able to assess the loci of functional reorganization, we studied the spatial extent of the evoked activity after spared and cut vibrissae deflections at short latency. Comparison of D2 vibrissa-evoked activity between groups showed that extent of the evoked activity in the vibrissae-spared group was larger than in other groups. The presented data could not elucidate the mechanism of such enlargement (see Chapter 5 for a detailed discussion of possible mechanisms). However, our data prove that this effect is specific to the spared vibrissa, given that when cut-vibrissae representations were compared between groups no difference was found.

RECEPTIVE FIELD PLASTICITY

We examined the receptive fields of neurons in the D2 and Dcut vibrissae and quantified change in receptive field organization after various types of alterations in the vibrissa-use. We have shown that receptive field of a barrel column neuron increases after vibrissa-sparing experience in the home cage. This result is far from being original. Firstly, Merzenich *et al.* (1983) showed that following median nerve sectioning, receptive field organization changes. The nature of the change depends upon the duration of the altered use of the sensory organ. Immediately after the denervation of the somatosensory cortex, or application of a local anesthetics neurons are shown to have increased receptive fields (Merzenich *et al.*, 1983 ; Calford and Tweedale, 1991 ; Faggin *et al.*, 1997). Enlarged receptive field starts to shrink as early as 11 days after denervation, approaches to normal values in the naïve animals around 22 days, and yet befit normal values at about 144 days after the initial denervation (Merzenich *et al.*, 1983).

Results reported in this study agree readily described time span of the receptive field plasticity. We showed in the supragranular layer that receptive field of a neuron which had undergone to single vibrissa experience for 13 days significantly increased, and yet if the vibrissa-sparing continued for an additional 13 days it moderately shrunk and became insignificantly larger than the receptive field of experimentally naïve neurons (see Chapter 4).

One important finding is that the extent of the plastic change in receptive field organization depends upon how a vibrissa is used after it has been spared. Our data suggested that if a spared vibrissa used in a task in which success depends upon the sensory information coming from the spared vibrissa (i.e. gap-cross training at vibrissa distances), receptive field of the trained vibrissa becomes statistically indistinguishable from the naïve receptive fields. Nonetheless, if an animal is trained in the very same learning task but this time in distances where spared vibrissa might be used together with other touch receptors around the nose, receptive field size enlarges and becomes statistically larger than the naïve neurons' receptive fields.

Receptive fields of the infragranular layer neurons showed somewhat different modifications after aforementioned modifications. Independent from the type of training that the animals received receptive field of the trained and untrained neurons significantly increased in comparison to the naïve neurons' receptive field sizes. If the animals were subjected to the vibrissa-sparing protocol without any training, receptive fields of the 'spared' and 'cut' neurons increased in size in comparison to those in naive and training groups.

Gap-cross training and the type of the training affect the receptive field organization of the barrel field neurons differently in different layers of the cortex as shown by between group comparisons. Nevertheless training, per se, does not affect the organization of the receptive field. In order to question, the role of training and sparing in the receptive field organization, we compared the receptive field sizes of the D2 and Dcut neurons in every group studied, and found that receptive fields of the D2 neurons, in none of the groups studied, is not significantly different than those of Dcut neurons, suggesting that modifications studied in the present study is because of a global change effecting D2 as well as Dcut neurons upon vibrissa-sparing and/or training.

Laminar differences in the receptive field organization

Armstrong-James and Fox (1987) earlier found that receptive field of the barrel field neurons change in a laminar dependent manner. As they reported, barrel cortex neurons have multi-vibrissae receptive fields, which allow individual sensory neurons to collect sensory information from several vibrissae. Armstrong-James and Fox found that supragranular layer neurons have receptive fields extending to 3.1 vibrissae, whereas neurons in the infragranular layers have larger receptive fields giving statistical spiking response to deflection of 6.1 vibrissae. We, on the other hand, report in the current study that under our experimental conditions supragranular layer neurons have receptive fields consisting of 2.9 neurons and infragranular layer neurons have receptive fields somewhat smaller (1.6) than those of superficial layers. Although there is a firm match in terms of receptive field size of the supragranular layer neurons between the current study and that of Armstrong-James and Fox (1987), there is a noteworthy difference between the two study when infragranular layer neurons compared. One of the most imported differences between the two studies is that we recorded from clusters of neurons whereas Armstrong-James and Fox studied single neurons in the barrel field. However this difference is unlikely to be a candidate to explain the different results we got. If cluster recording leads any difference in the receptive field size, it's likely to be an overestimation of the receptive field size, given that a few neurons having different receptor field will tend to broaden the receptive field of the clusters, studied. We have no data directly comparing receptive field changes in cluster and single neuron recordings. However, readily published data suggest that in the primary somatosensory cortex, area 3b, of the monkey cortex, receptive field sizes of the multi and single-unit recording do correspond (Sur, 1980; also see Recanzone *et al.*, 1992c).

Both sets of data reported by Armstrong-James and Fox (1987) and the current study were collected under urethane anesthesia using a mechanical stimulator that allowed researchers to

delivery repeatable vibrissa deflections over the prolonged durations of vibrissa stimulations. Given the comparability of the two methodologies and the similarity in the supragranular layers neurons' receptive fields in the two studies, it is unlikely that the difference in the infragranular layer neurons is because of the factors discussed above.

One aspect of the receptive field evaluation, which might have caused the difference between the two studies, could be the 'cut-off' value for statistical significance. We described a neuron giving statistical response to any given vibrissa deflection if the neuron yields significant response at a $p < 0.01$ level in the Wilcoxon-signed rank test. Having smaller cut-off values should theoretically turn weaker responses out to be significant, and so broaden the receptive field of the neurons. This is especially important in infragranular layer neurons where surrounding 'weak' responses are twice as large those seen in the supragranular layers (Armstrong-James and Fox, 1987).

METHODOLOGICAL CONSIDERATIONS

There are limiting factors in generalizing the results obtained in this study. One of them is that we studied the neural activity under anesthesia. The brain state under anesthesia is different than that of in awake, undrugged animals. Most importantly not all the components of functional circuitries of the brain might be active under anesthesia. For example, during the gap-cross training, it is highly likely that several sub-cortical structures functional in attention and motivation are as active as the primary somatosensory cortex. Moreover extensive feedforward and feedback connections between sensory systems and those structures related with reward processing might have different pattern of connectivity under anesthesia, suggesting that the activity studied under anesthesia may be different than that of in the awake, undrugged animals.

We studied multi-unit activity elicited by vibrissa deflections. Although, the number of the cells recorded per electrode should not affect the spatial extent of vibrissa representation, it may cause overestimation of the number of spikes recorded. Therefore results outlined here are true for multi-unit recording under urethane anesthesia.

In summary, present study suggest that the nature, extend, and pathways of the cortical plasticity depends upon how vibrissae are used. Moreover, the mechanisms governing the changes in the neural responsiveness changes as a function of the cortical laminae studied.

Chapter 4. CORTICAL PLASTICITY AFTER 26 DAYS OF ALTERED VIBRISSA USE IN THE HOME CAGE OR IN A TACTILE LEARNING TASK

INTRODUCTION

In the previous chapters, we quantified the change in the cortical representations of the spared and cut vibrissae in supra and infragranular layers after various conditions of vibrissa use. The main finding was that after 13 days of spared-vibrissa experience in the home cage, the cortical representation of the single intact whisker (and, to a lesser extent, the cortical representation of the surrounding, clipped whiskers) expanded considerably in comparison to that in naïve rats with all whiskers intact. In contrast, after 13 days of spared-whisker experience *accompanied by gap-cross training*, the size of the cortical representation of both the single intact whisker and the clipped whiskers was similar to that in naïve rats. Cortical reorganization differed for the two groups of gap-cross trained rats (vibrissa-crossers and nose-crossers): the resemblance to naïve rats was greatest for vibrissa-crossers, meaning that the final form of the cortical whisker representation was determined not just by the general environment (tactile training) but also by the specific use of the vibrissae.

The previous observations were consistent with two possible processes controlling the reshaping of the cortical representation of trained vibrissae. First, it is plausible that gap-cross training acted to counter any sort of topographic reorganization of cortex. Although it would be pure speculation to propose possible mechanisms whereby gap-cross training might "freeze" the cortical representation in its initial form (similar to that of naïve rats), it is clear that such a mechanism would prevent the representational expansions that would otherwise be induced by the homecage experience. It is important to note that, in this model, gap-cross training does not cause an active reorganization, but instead passively "shunts" the reorganization that would occur with homecage experience alone. A second possibility is that gap-cross training might provoke an active process of reorganization. Thus, while experience in homecage were acting to expand the representation of the spared (and surrounding) whiskers, tactile training would act to oppose the reorganization. Again, the potential underlying mechanisms are not certain, but it is clear that the active maintenance of cortical topographic order (the second hypothesis) differs fundamentally from the passive maintenance of the current map (the first hypothesis).

In the present chapter, we aimed to distinguish between these two hypotheses. The design of the experiments was to use two groups, both of which were left with a single vibrissa

in their homecage for 13 days. From Chapter 3, we know that at this point of the experiment, rats in both groups would possess expanded cortical representations of the clipped vibrissae and, in particular, the spared vibrissa. After 13 days, one group was left in its homecage with the single spared vibrissa for another 13 days (so called 'homecage' group)– these rats underwent no tactile training. This group served to reveal how barrel cortex was modified when the 13-day period used in Chapter 3 was prolonged to a 26-day period of single-whisker experience. The second group, after the first 13-day period of single-vibrissa homecage experience, began a 13-day period of gap-cross training at vibrissa-distances (so called 'vibrissa-crossers'). If the first model given above is correct, then gap-cross training will block, or "short-circuit" any further reorganization, and the cortical vibrissae representations of the second group should be like those in the 13 day spared-vibrissa homecage group described in Chapter 3. On the other hand, if the second model is correct, then gap-cross training will act to *restore* cortical topography, reversing the modifications present after 13-days of homecage experience and rendering the cortical representations like those in naïve rats – namely, with sharper, more spatially orderly vibrissa maps.

Besides shedding light on whether the cortical reorganization found in trained rats in Chapter 3 resulted from an active reimposition of topographic order, these experiments also had the goal of allowing us to make more precise suppositions about the mechanisms governing the reorganization of cortex. One mechanism which may govern reorganizational plasticity is the balance between excitation and inhibition in the network; this balance might be upset by a change in the sensory receptor use. The preceding chapters showed that the representation of a spared-vibrissa differed in a number of ways depending on whether (i) the animal used it exclusively in its home cage or, (ii) the animal used the intact vibrissa to perform a specific tactile distance detection or object detection task. At the network level, increased representation of the spared vibrissa (homecage group) might reflect facilitated responsiveness of the neurons in the deprived cortex to spared vibrissa deflections, while dimished representation of the trained vibrissa (in vibrissa-crossers and nose-crossers) might reflect increased inhibition in the cortex to the spared-vibrissa deflections.

Change in the balance between inhibition and excitation has been commonly proposed as a model to explain cortical plasticity. For example, in the vibrissae-to-barrel pathway, Faggin *et al.* (1997) showed that shortly after blocking vibrissal sensation by application of a local anesthetic, neurons whose sensory input was deactivated by the anesthetic started to respond to the stimulations of the intact vibrissae. Reorganization after such short periods is usually attributed to release from inhibition in the deprived area of cortex, given the fact that structural changes accompanying change in the sensory receptor use are not expressed in such

time courses

Change in the balance between inhibition and excitation in the cortex also has been suggested after longer periods of altered vibrissa usage. Lebedev *et al.* (2000) found that after 3.5 days of vibrissae-sparing, representations of the paired-vibrissae increased in spatial distribution. Although a widened representation of *intact* vibrissae could derive from activity-dependent enhanced lateral excitatory connections, additional evidence suggested that one of the mechanisms of reorganization might be disinhibition: neurons in deprived columns showed an enhanced response to *clipped* vibrissae. Activity-dependent enhancement of excitatory inputs from clipped vibrissae would not be expected, therefore decreased inhibition in the deprived columns was the preferred explanation.. Lastly, in the previous chapters, we presented evidence showing that release from inhibition in the cortex seems to be a part of the reorganization process in the supragranular layers of the somatosensory cortex: similarly to Lebedev *et al.* (2000), we noted that clipped vibrissae representations increased.

Increased inhibition, on the other hand, has recently been introduced as a mechanism that could contribute to critical period plasticity in the visual cortex (Hensch *et al.*, 1998; Fagiolini and Hensch, 2000; also see Feldman, 2000) and acquisition of auditory space maps in the external nucleus of the inferior colliculus (Zheng and Knudsen, 1999).

Earlier work showed that the cortical representation of a vibrissa increased if the spared vibrissa was used solely in the rat's familiar environment, but shrunk if the spared vibrissa was used to explore a novel environment (Polley *et al.*, 1999). In our hands, the spared vibrissa representation was smaller when used in gap-crossing than when used in the homecage (Chapter 3). In theory, there might be two mechanisms that could yield such "bi-directional plasticity" in the representation of a spared sensory receptor. One of these is increased inhibition in the deprived cortex in response to the spared vibrissa, provided that this vibrissa is used in the novel environment. Increased inhibition was put forward to explain bi-directional plasticity in the barrel field (Polley *et al.*, 1999). The other is maintenance of the balance between the deprived and spared vibrissa at the pre-deprivation stage: the novel experience may "shunt" any mechanisms which would otherwise modify the cortical representations (our first hypothesis, described above). In order to distinguish between these two possible mechanisms, we designed an experiment to further explore the representation of the spared vibrissa after 26-day home cage experience. As mentioned above, we hypothesized that if the mechanism which keeps the representation of the vibrissa restricted during gap-crossing is increased inhibition in the deprived cortex, then the representation of a spared vibrissa should decrease and become indistinguishable from the representations of the surrounding-cut vibrissae when 13 days of homecage spared vibrissa experience is followed by gap-cross

training. If the mechanism which keeps the representation of a trained vibrissa comparable to the cut-vibrissae representations is simply the maintenance of whatever state exists at the onset of training, then the enlarged representation of the spared vibrissae present after 13 days of homecage spared vibrissa experience should remains so even if it is followed by gap-cross training.

In the present study, therefore, we trained animals in the gap-crossing task for 12 sessions across 13 days after they received 13-day long vibrissa sparing experience in their home cages and compared the representational changes in the trained vibrissa with surrounding-cut vibrissae. An additional group of animals which underwent 26-day vibrissae sparing has been included in the study to exclude the possibility that the change seen after training is simply is a result of prolonged vibrissa-sparing, independent of the training.

RESULTS

CHANGE IN THE SPATIAL EXTENT OF VIBRISSA-EVOKED ACTIVITY

Vibrissa representation in supragranular layers after 26 days vibrissa sparing in the homecage

As in the previous chapters, we studied evoked responses to 9 vibrissae, specifically D2 together with the 8 surrounding vibrissae.

We first describe the 'home cage' group, which underwent the 26-dayvibrissa sparing experience. In all of the animals studied (n=5, see Figure 1), representation of the spared vibrissa increased in comparison to the surrounding/cut vibrissae representations (Student's t-test, $p < 0.05$). The representation of the spared D2 vibrissa (7.4 ± 2.3, n=5) was also significantly larger than the representation of the D2 vibrissa in the naïve group (3.8 ± 1.5, n=5) (Student's t-test, $p < 0.05$). Nonetheless, size of the representation of the cut vibrissae in the 26-day home cage group (4.4 ± 2.4, n=35) did not differ from the size of the representation of intact vibrissae in naïve vibrissa rats (4.5 ± 2.2, n=40 Student's t-test, $p > 0.41$).

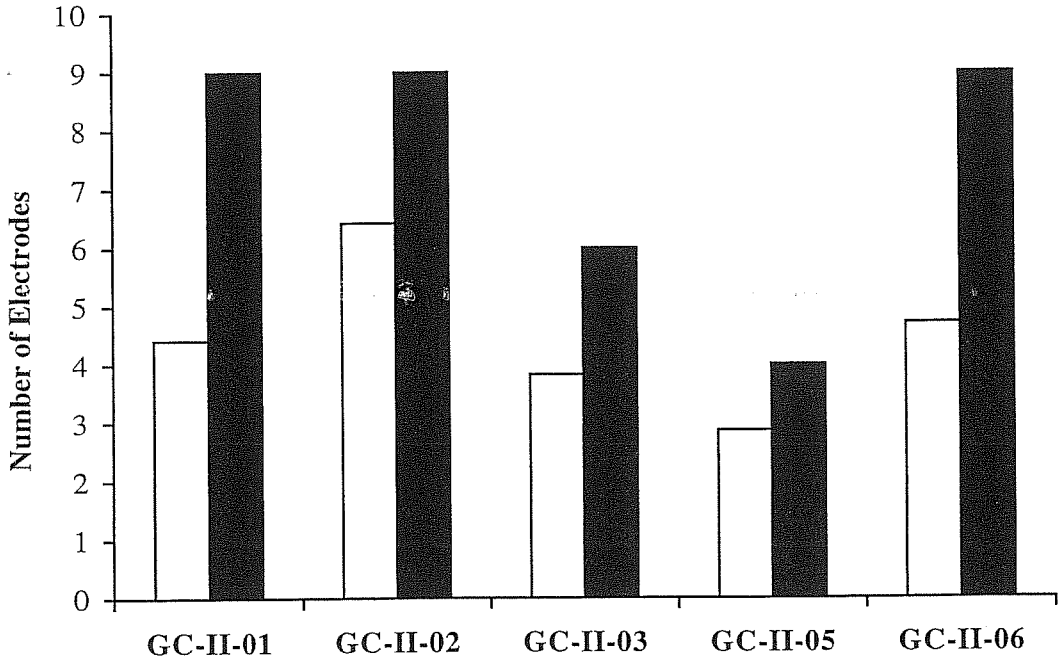


Figure 1. Representation of a vibrissa increased (black columns) after 26 days of vibrissa sparing in home cage in comparison to the surrounding (cut) vibrissae (white columns) in all animals studied.

Vibrissa representation in the supragranular layers after 13 days of vibrissa-spared homecage experience followed by 12 sessions of gap-cross training

The second group that underwent 26 days of vibrissa-sparing also received 12 sessions of gap-cross training at vibrissa-distances during the last 13 days of vibrissa sparing. In comparison to that of the surrounding-cut vibrissae, would the spared vibrissa representation remain expanded, as it was after 13 days of vibrissa-sparing, or would it diminish in size and magnitude? The representation of the trained/spared vibrissa was not significantly different from the representation of the surrounding/cut vibrissae in all animals of this group (Student's t-test, $p > 0.48$, see Figure 2). *The key observation is that the size of the representation of vibrissa D2 in this group (6.4 ± 2.9 electrodes, n=5) was not significantly different from that in experimentally naïve control animals* (Student's t-test, $p > 0.05$). Nonetheless, training in the gap-crossing task altered the spatial extent of the cut vibrissae-evoked activity. Cut-vibrissae representations (6.4 ± 4.1 electrodes, n= 38) in the 26-day vibrissa-crossers were significantly

larger than the vibrissa representations in experimentally naïve animals (Student's t-test, $p < 0.01$), and were also larger than the cut vibrissae representations in the 26-day homecage group (4.4 ± 2.4, n=35; Student's t-test, $p < 0.01$). Although cut-vibrissae deflections activated a larger area of cortex in the vibrissa-crossers than in the homecage group, the two groups did not differ in the size of their D2 vibrissa representations (Home-cage = 7.4 ± 2.3 electrodes; Training = 6.4 ± 2.9 electrodes; Student's t-test, $p > 0.05$).

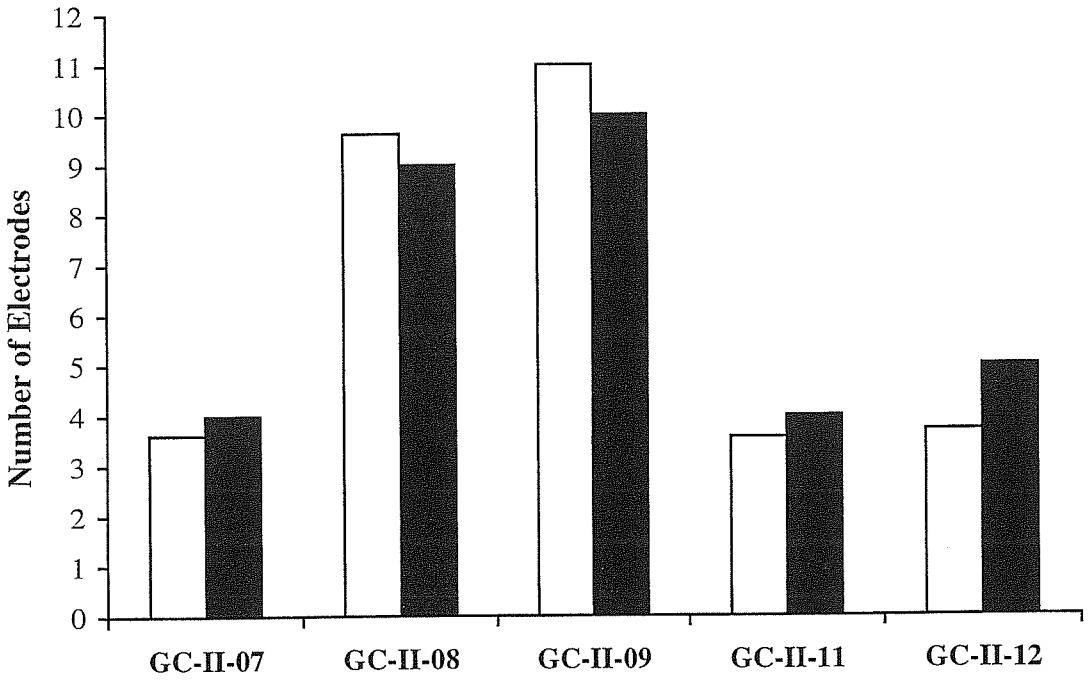


Figure 2. Representation of the intact vibrissa after 26 d home cage experience is *not* larger than that of the surrounding (cut) vibrissae if the spared vibrissa is used in the gap-crossing task. (D2 vibrissa: Filled, Surrounding vibrissae: white columns)

13 days of homecage experience or 13 days of vibrissa-sparing together with gap-cross training at nose distances shifted the balance between representations of the neighboring vibrissae, favoring the spared vibrissa. In order to find out whether the longer duration of vibrissa sparing would generate an unbalanced representation of spared and cut vibrissae and, if so, whether training in the gap-crossing task at vibrissa-distances would counteract the change, we compared the difference between D2 and surrounding vibrissae representations in each group employing factorial analysis of variance. This comparison allowed us to 'filter-out' the between animal variance caused by unavoidable factors (different electrode arrays, different lot of anesthetics etc.). The comparisons showed that the difference in the

D2/surround representations in the vibrissa-crossers was statistically indistinguishable from the difference in the D2/surround representations in naïve animals ($F=0.391$, $df=14$, $p > 0.05$). The difference in the D2/surround representations in the homecage group, on the other hand, was statistically significant in comparison to that in naïve animals ($F = 7.511$; $df = 14$, $p < 0.05$), suggesting that the representational balance between neighboring vibrissa was altered following 26-day vibrissa sparing to favor the intact vibrissa. However, training the intact vibrissa in the gap-crossing cancelled the difference in the extent of the spared and cut-vibrissae evoked activity.

CHANGE IN THE MAGNITUDE OF THE VIBRISSA EVOKED ACTIVITY

In addition to the spatial extent of the neural activity evoked by vibrissae deflections, we studied the change in the response magnitude -- the number of spikes recorded in the 50 ms time window after stimulus onset minus the number of spikes in the 50 ms prestimulus window.

As reported earlier, we found that vibrissae in experimentally naïve rats evoked 3.4 ± 1.9 spikes (mean ± std; $n = 45$). Moreover, there was no difference between the number of spikes evoked by D2 (3.7 ± 1.1, $n = 5$) and surrounding vibrissae (3.4 ± 2.0, $n = 40$) (Student's t-test, $p > 0.3$). After 26 days of homecage experience, on the other hand, this balance between spared and cut vibrissae evoked activity was altered, leading to a more pronounced response to the spared vibrissa (Student's t-test, $p < 0.005$; also see Figure 6). Increased responsiveness to the spared vibrissa in comparison to the cut vibrissae did not occur because of increased responsiveness to spared vibrissa deflections, but because of decreased responsiveness to surrounding vibrissae (2.3 ± 1.7, $n = 35$; Student's t-test, $p < 0.005$) in comparison to the naïve vibrissa evoked activity.

Thus, homecage experience with spared D2 vibrissa significantly modified the balance between the activity evoked by D2 and surrounding-cut vibrissa. Yet this effect was plastic and reversed by training the animal in the gap-crossing task at vibrissa distances. Then, evoked responses after trained and cut vibrissae deflections were statistically indistinguishable from each other (Student's t-test, $p > 0.4$; Figure 3).

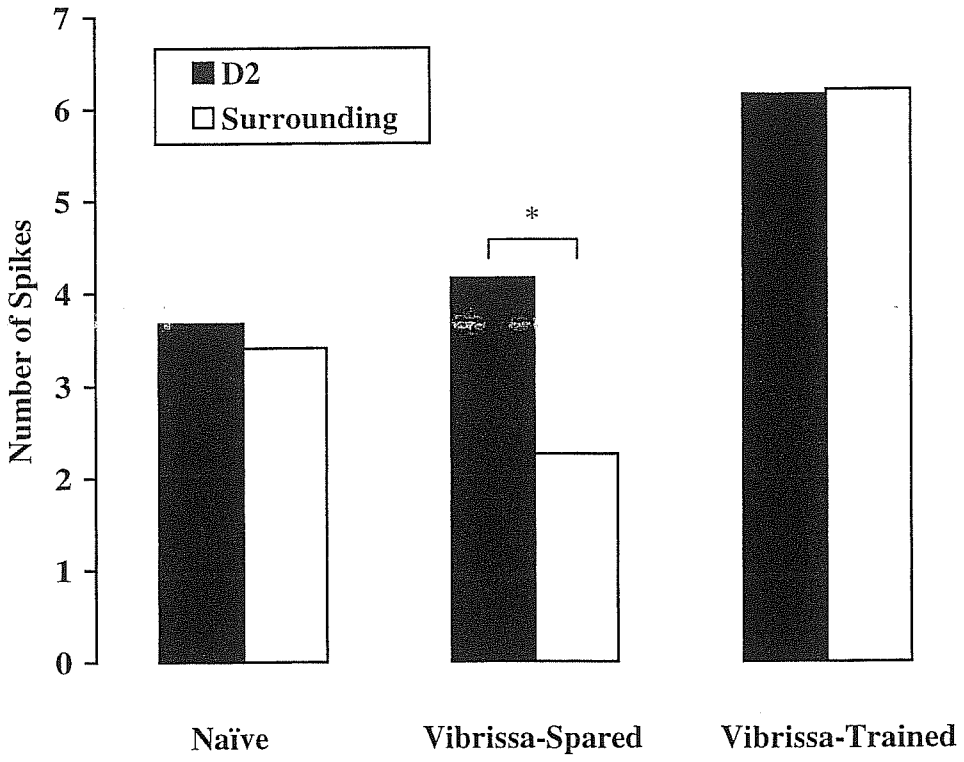


Figure 3. Between and within group comparisons. Only vibrissa-spared group showed difference between vibrissa-type within experimental group ($p < 0.005$). Please see the text for further details.

Between-group comparisons for D2 and surrounding vibrissae showed that, in comparison to activity evoked by "naïve" vibrissae, activity evoked by cut vibrissae, but not by D2 vibrissa, was modified after homecage experience as well as by gap-cross training (Analysis of Variance, $p < 0.0001$). The number of evoked spikes evoked by cut-vibrissae deflection was significantly lower after homecage experience (2.3 ± 1.7), in comparison to that after training at vibrissa-distances (6.2 ± 5.0). Moreover, activity evoked by cut-vibrissae deflections in the vibrissa-crossers was also larger than that evoked by naïve vibrissa deflections (3.4 ± 2.0).

RECEPTIVE FIELD PLASTICITY AFTER 26 DAYS OF ALTERED VIBRISSA USE

Another objective of the present study was to probe the plastic changes in the receptive field organization as a function of the vibrissa-sparing duration and type of vibrissa use. In order to pursue these questions, we studied the receptive field organization of neurons in D2 and surrounding Dcut (D1 and D3) columns at the level of supragranular layers.

As outlined in the previous chapter, neurons in naïve rats have receptive fields including, on average, 2.9 ± 1.4 vibrissae. The preceding analysis uncovered no significant difference in receptive field size between the neuronal clusters in column D2 (3.0 ± 1.18) and surrounding columns (2.85 ± 1.43; $p < 0.92$).

After 26 days of vibrissa-spared experience in the home cage, the average receptive field size of neurons in column D2 was 3.0 ± 2.2 vibrissae. Although neurons in Dcut columns had slightly larger receptive fields (4.4 ± 1.8 vibrissae), the difference was insignificant ($p > 0.18$). The expansion in the Dcut neurons' receptive fields – but not D2 neurons' receptive fields ($p > 0.14$) – was significant in comparison to the naïve neurons' receptive fields ($p < 0.001$).

The receptive field of D2 neurons in the vibrissa-crossers consisted of 5.0 ± 2.3 vibrissae, on average. Dcut neurons, on the other hand, had receptive fields with 4.6 ± 2.4 vibrissae. The small difference between the two values was not significant ($p > 0.61$). Nonetheless, the average receptive field size for the two types of neurons was significantly larger than the receptive fields of the neurons in naïve rats ($p < 0.001$). There was no effect of the training in the receptive field size in the 26 days vibrissa sparing groups ($p > 0.05$).

EXPERIENCE-DEPENDENT RECEPTIVE FIELD PLASTICITY: A COMPARISON BETWEEN 13-DAY AND 26-DAY VIBRISSA SPARING AND TRAINING

In this section, we wanted to meta-analyze the receptive field plasticity seen after 13-day as well as 26-day long periods of altered vibrissae use. Previously reported data are compared in Figure 4.

Supragranular layer neurons normally have receptive fields including about 3 vibrissae. When the animals were subjected to vibrissa-spared experience for 13 days, whether or not they concurrently received gap-crossing training at vibrissa distances, receptive fields in columns of the spared/trained and cut vibrissae increased. Enlargement of the receptive field was about 43% after homecage vibrissa-sparing and 26% after gap-cross training at vibrissa distances. When the animals which received 13 days of vibrissa-sparing (13-day homecage) without training continued to receive the vibrissa-sparing experience for another 13 days with (26-day vibrissa-crossers) or without (26-day homecage) gap-cross training, receptive field sizes were modified. After the 26-day long vibrissa-sparing experience in home cage, the receptive fields of the D2 neurons decreased towards the size found in naïve rats. Training the animals in the gap-crossing task at vibrissa distances, however, led an increase in the receptive field size (see Figure 4). This increment was significant in comparison to receptive fields in

naïve and 13 days vibrissa-training, but not vibrissa sparing, groups (Tukey HSD, $p < 0.001$).

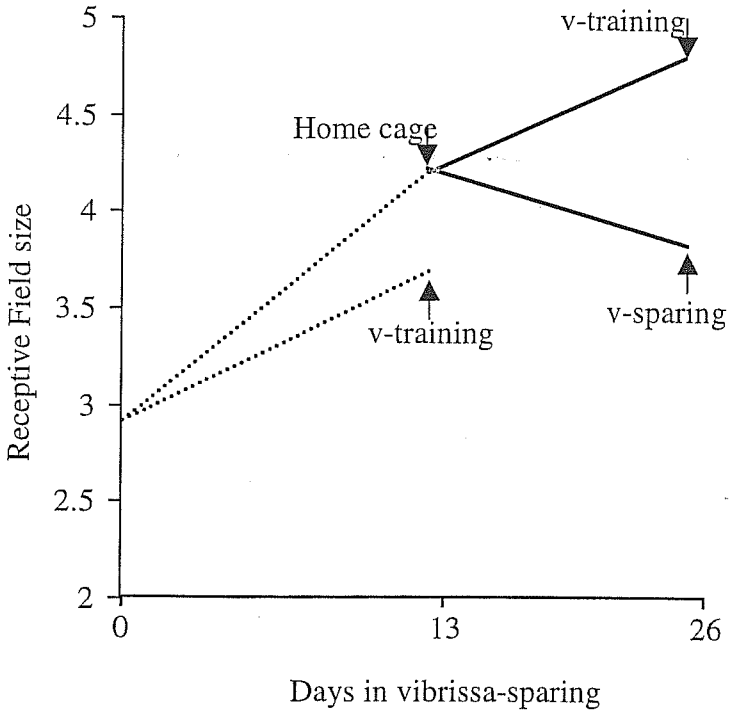


Figure 4. The change in receptive field size as a function of vibrissa-sparing duration and the type of vibrissa use. After 13 d aysof vibrissa sparing, increase in the receptive field size was larger if the animal was not trained but solely left in the home cage. After 26d of vibrissa sparing on the other hand if the animal was trained in the last 13d of the sparing, receptive field sizes increased in comparison to the animals who were not trained, but received vibrissa-sparing experience in their home cages (see the test for details).

We further compared the change in the receptive field size after 13 and 26 days of altered vibrissa use. There was no significant change after 26 days of homecage experience in comparison to 13 days of homecage experience. Nonetheless, the general tendency in the data was to show shrinkage in the receptive field size towards the values found in naïve animals (Table 1).

Vibrissa-crosser groups revealed a different pattern. There was a robust and statistically significant increase in the receptive field size if the animals were trained for 12 sessions thru the last 13 days of the 26 days vibrissa sparing. Receptive field size for neurons in columns of D2 vibrissa but not Dcut vibrissae increased in the 26-day group in comparison to the animals which received 12 sessions of gap-cross training at vibrissa distances (Table 1).

		Vibrissa Sparing		Vibrissa Training	
	Naive	13-d	26-d	13-d	26-d
Average	2.9 ± 1.3	4.2 ± 1.8	3.8 ± 2.0	3.6 ± 1.3	4.7 ± 2.3*
D2 only	3.0 ± 1.2	4.0 ± 1.9	3.0 ± 2.2	3.8 ± 1.0	5.0 ± 2.3*
Dcut	2.8 ± 1.4	4.2 ± 1.8	4.4 ± 1.8	3.6 ± 1.4	4.6 ± 2.4

Table 1. Raw data for between group comparisons. The size of the receptive field increased in 26d vibrissa-crossers in comparison to the 13-day training group. Asterisk denotes significant difference at $p < 0.05$ in comparison to the naïve animals.

DISCUSSION

Change in the cortical sensory representations has long been defined as an increased representation of the spared receptors. We earlier showed that the sensory representation does not *always* shift in a way that delegates an increased territory to the spared receptors – the nature of the sensory cortical representation depends not only upon presence or absence of sets of sensory receptors, but also upon how the receptors are used. In this study, we wanted to know whether a cortical region that had undergone a plastic change (as a result of 13 days homecage experience, in our case) is further modifiable. If it is modifiable, we hoped to learn something about the network mechanisms underlying this plasticity. We had hypothesized that the mechanism of the change in the representation of a spared vibrissa after training could be better understood if we trained animals to use a vibrissa which had been spared for a time sufficient to allow an expansion of its cortical representation.

When a vibrissa was spared for 13 days in the home cage, its cortical representation increased in comparison to that of surrounding cut vibrissae. This asymmetry was lost if the animal used this spared vibrissa in the vibrissa-dependent (but not nose-dependent) gap-crossing task (see Chapter 3). We therefore trained animals to use their sole vibrissa in learning to gap-cross at vibrissa-distances (vibrissa-crossers) after they had already received 13 days of vibrissa-sparing in the home cage. The results showed that representation of the spared and subsequently trained vibrissa did not significantly differ from that of the cut-vibrissae representations within the same rats. This suggests that the way the cortex reorganizes after vibrissa training is not to simply maintain the existing relationship between trained and surrounding vibrissa – this model would have been supported if there remained significant differences between trained and surrounding vibrissae representations -- but to

actively regulate the representations of the trained and cut vibrissae to keep them in balance.

Representational plasticity and functional topography

Keeping the trained and cut-vibrissae representations similar to each other might serve to maintain functional topography. When a localized region of the body's sensory sheet is stimulated, primarily its topographically matched representation, and to a lesser extent the neighboring regions, are activated. This functional projection has been proposed to be a fundamental component of perception (Kaas, 1997). One way to test this hypothesis is to train a restricted set of receptors with repeated application of a stimulus, and to test the change in the response to stimuli presented to the trained locus together with one of its neighboring sensory receptors. Such a study has been done in the visual system. Cave and Zimmerman (1997) asked human subjects to fixate on a single point to ensure repeated stimulation of a restricted part of the visual field by visual stimuli. Then, they tested subjects with randomly introduced visual distractors near the target. Directed attention diminished with lateral distance from the target, as indicated by the fact that the deficit in target detection increased when the distractors were presented close to the target. Another study suggesting that functional topography in the sensory systems has a role also in retrieval of the learned information, is performed by Harris and colleagues (1999). They trained rats to perform the gap-crossing task after trimming all but one vibrissa. After animals reached the learning criterion they were tested on the gap-crossing task, but this time with a 'prosthetic' vibrissa, placed one or two rows or columns from the trained vibrissa. Subjects could use the prosthetic vibrissa immediately only if it were attached to the trained, but not to any other, vibrissa's stump.

These studies suggested that functional topography in the sensory maps has a role in perception and in information retrieval processes. However, they did not speak to the neural mechanisms by which topography is maintained. We now show that keeping the representations of the trained and untrained sensory receptors in equilibrium might act to reinstate functional topography in the somatosensory system after a spared vibrissa is used in a learning task.

Mechanisms to form functional topography

The home cage experience caused a representational imbalance between spared and cut vibrissae, and the equilibrium was restored by training the animals in the gap-crossing task at vibrissa-distances. In theory, there are two ways to reinstate the relationships between neighboring sensory representations after learning: inhibiting evoked responses to trained vibrissa deflections or facilitating evoked responses to surrounding vibrissae deflections. To

find out which of the two actually takes place, we compared the number of electrodes activated after trained and spared vibrissae deflections to ask whether training, *per se*, causes a shrinkage in the representation of the spared vibrissa. Another comparison was made between naïve and cut vibrissa evoked activity in vibrissa-crossers to examine whether being exposed to the training environment, without any active use of the cut vibrissae in the task, yields some change in the representation of the cut vibrissae. The results suggested that the equilibrium in the representations of the trained and surrounding vibrissae after 26 days of vibrissae sparing during which 12 sessions of training was delivered in the last 13 days of vibrissa-sparing is a result of increased response to the cut-vibrissae deflections rather than depressed response to the trained vibrissa deflections.

An excited state of neural activity upon cut-vibrissae deflections may reveal the mechanism behind alterations in the vibrissae reorganizations in the current paradigm. However, it can not be generalized to explain the representational balance between trained and surrounding cut vibrissae after 13 days of home cage experience during which animals were subjected to 12 sessions of vibrissa-training. In the previous chapter, we showed that the representation of cut-vibrissae increased after vibrissa-trimming, independently from how the spared vibrissa was used, whereas the representation of a spared vibrissa decreased if it was used in the gap-cross training task. This trend was not observed in the current experiment with 26-day groups. Thus it is possible that mechanisms of the plastic changes after 13 and 26 days of alterations in the vibrissae use might be different.

Universality of the equilibrium between the representations of the trained and cut vibrissae

One important question is how global the effects described here are. In other words, might the active maintenance of the size and magnitude of representations of trained and surrounding vibrissae also be found in other types of learning that involve barrel cortex? Let us assume that information collected through a vibrissa has two functions, specifically to identify the 'where' and 'what' features of external objects. In the case of gap-cross training at vibrissa-distances, the spared vibrissa is the only vibrissa that could be used to collect information about the availability of the target platform. Because the training involves repeated exposures of the animal to the apparatus, one might expect that all *what* information collected through the vibrissa except that which indicates the presence or absence of the platform might be irrelevant, given that the *what* information was invariant throughout the training sessions. If the *what* information was modified, one could expect that a larger territory of cortex would be involved with the processing: larger territory translates to a greater

number of activated neurons and, presumably, faster and more reliable processing. If true, one might predict that training on a task in which a spared vibrissa was used to collect *what* information about the object explored (for example after roughness discrimination task), a trained vibrissa's representation may increase rather than decrease. In a distance detection task, which whisker was stimulated might be essential to the completion of the task; in these conditions, spatial information may be maintained in the cortex, at the cost of the expansion in the representation of the spared vibrissa.

Changes in the receptive field size as a function of vibrissa-trimming duration

One other objective of the present study was to examine the changes in the receptive field size as a function of vibrissa-sparing duration. As described above, when a vibrissa was spared for 13 days in the animal's home cage, the receptive fields of the spared as well as cut vibrissae increased in size. As the vibrissa-sparing duration increased, nonetheless, enlarged receptive fields shrunk towards the normal values. After 26 days vibrissa sparing, the receptive field shrank to the values seen in the naïve rats. Training in the gap-crossing task at vibrissae distances dampened this reduction. When animals which had received 13 days vibrissa-sparing in home cage were trained in the gap-crossing task for 12 sessions, no subsequent decrease in the receptive field size occurred. This potentiation in the receptive field size seems because of a global excitation, given that trained as well as cut vibrissae receptive fields increase concurrently. But whatever the mechanism of this potentiation, one observation coming from this study is that training in a perceptual learning task facilitates the receptive field reorganization which would otherwise be depressed during the 26 day long vibrissa-sparing experience in home cage.

In summary, results of the present study showed that it is possible to induce further modifications in barrel cortex which had been already undergone a modification. In other words, even in cortex is not in its baseline state (normal home cage experience with all vibrissae intact), training an animal's sole vibrissa in a vibrissa-dependent learning task rebalances the functional topography between trained and cut vibrissa in trained animal When compared to the training effect in previously naïve cortex, this mechanism constitutes a new way of reorganizing vibrissal representations.

POSSIBLE NEURAL MECHANISMS OF CORTICAL PLASTICITY

Single-unit recordings in monkeys suggested that if a neuron loses its principal sensory input, it begins to respond to the stimulation of surrounding, intact portions of the sensory organ (Kaas *et al.*, 1983; also see chapter 1). However, several groups studying plasticity in the vibrissae-to-barrels neuroaxis found evidence that release from inhibition in the sensory-deprived neurons can be seen as early as minutes after a peripheral manipulation (Faggin *et al.*, 1997), and continues for days (Lebedev *et al.*, 2000).

Release from inhibition might be an initial step in cortical map reorganization, allowing deprived-neurons to give facilitated responses to the stimulation of the spared vibrissae (Lebedev *et al.*, 2000). In the present experiment, evidence consistent with a release from inhibition was found after 13 days of vibrissa sparing, as suggested by the widened representation of cut vibrissa, and the larger number of spikes evoked by these same vibrissae. Nonetheless, potentiation of the spared vibrissa responses (in comparison to naive animals) was observed only in the animals which used their spared vibrissa in their home cage, not in gap-cross trained animals. This suggests that, in addition to disinhibition, there was a potentiation mechanism acting to facilitate the responses to the spared vibrissa in the homecage group. Facilitation of responses to the spared-vibrissa was reduced if the animal was trained in the gap-crossing task at nose distances, and was essentially eliminated (i.e., indistinguishable from vibrissa representations in naïve rats) after training at vibrissa-distances. This suggests that there might be a mechanism that either suppresses already potentiated responses to the trained-vibrissae or keeps the trained-vibrissae responses from expanding.

In order to differentiate between different types of plasticity, we calculated the difference scores for the representations of cut and spared vibrissae. Because release from inhibition is a widespread phenomenon, it might be controlled by extra-barrel field structures. This suggests that disinhibition evident in the responses evoked by cut-vibrissae could also be present in the responses evoked by the spared vibrissa. Hence, difference scores should reveal the net potentiation in the spared-vibrissa responses. Comparison of the difference scores between groups showed that potentiation of the intact vibrissa responses was seen in the animals which used the vibrissa either in home cage, or in the gap cross training at short distances. Rats trained to gap cross at vibrissa-distances did not differ from naïve animals in the relationship between spared and cut vibrissae representations within animal (D2 and surrounding vibrissae in naive rats).

It is worthwhile to briefly review the cellular mechanism whereby "disinhibition" could alter the spatial extent and magnitude of cortical sensory responses. Microiontophoretic application of bicuculline (which blocks activity mediated by $GABA_A$ receptors) results in increased responsiveness of cortical neurons to sensory stimuli (Tremblay *et al.*, 1990). Post-deactivation effects of the intracortical inhibitory circuits are well studied in the receptive field reorganization in the primary somatosensory cortex. In their original studies, Dykes and his colleagues showed that the receptive field of the primary somatosensory cortical neurons undergoes expansion during application of GABAergic system inhibitors (Hicks and Dykes, 1983; Dykes *et al.*, 1984). In a similar set of studies, but this time in sedated rats, Kyriazi et al. (1996) showed that applications of baclofen and phaclofen significantly reduced or increased, respectively, 'weak' responses to vibrissa stimulations. These findings suggest that both $GABA_A$ and $GABA_B$ receptor mechanisms take a role in shaping neural responsiveness to sensory stimulation. Nonetheless, the most important support for the role of GABAergic system in modulating neuronal responsiveness following vibrissa-trimming comes not from electrophysiological studies but from immunohistochemical research which showed that vibrissa-trimming decreased GAD levels in the deprived columns. This suggests that vibrissa deprivation causes decreased activity of the inhibitory circuitry which in turn increases the excitability of the neurons after vibrissa deflections (Akhtar and Land, 1991).

PATHWAYS OF PLASTICITY

Another key question concerns the locus of plastic changes. Several previous studies argued that plastic changes in sensory representation are a result of increased intra-cortical connectivity between columns (Fox, 1992, 1994; Rhoades *et al.*, 1996; Wallace and Fox, 1999; Lebedev *et al.*, 2000). However other studies suggested that subcortical centers of the somatosensory axis might also show plastic modifications upon alterations in the sensory organ use (Nicolelis *et al.*, 1991; Faggin *et al.*, 1997; Melzer and Smith, 1996, 1998), subcortical changes have only been observed after direct receptor organ damage or application of local anesthesia to the periphery. In order to assess the loci of functional reorganization under our experimental conditions, we studied the spatial extent of the evoked activity after spared and cut vibrissae deflections at short latency. Comparison of D2 vibrissa-evoked activity between groups showed that the extent of the evoked activity in the home cage vibrissae-spared group was larger than in other groups. There are several possible mechanisms that may cause such expansion. Increased thalamocortical connectivity, reduced inhibition in the cortex, and changes in subcortical representations such as in the thalamic are just some of the possible ways

In principal, increased connectivity between loci of the brain could explain the spatial expansion in the vibrissa-evoked activity in the cortex. For example, there could be an increase in the extent of projections from each thalamic neuron to cortex, or an increase in the number of thalamic neurons that respond to vibrissal deflection. Barreloid neurons do not anatomically connect to each other, thus the latter assumption suggests that there must be a secondary relay center through which barreloid cells receive reverbatory connections (Crabtree *et al.*, 1998; also see Kaas and Ebner, 1998). We used electrophysiological but not anatomical techniques to describe the expansion of the spared vibrissa representation at short latencies. Thus we cannot firmly conclude that expansion in the spared vibrissa representation at short latencies is because of increased thalamocortical connectivity.

Another possible way to account for the increased spatial extent of vibrissa evoked activity is to posit a purely cortical modification. As we discussed above, release from inhibition is one of the modifications that can occur in the cortex following vibrissa deflections. Disinhibition may mean that, in effect, the net inhibitory influence converging on a neuron is reduced. In this case, the distance between a neuron's resting membrane potential and the spiking threshold might decrease. Thus, thalamic inputs to that yielded only subthreshold activity before vibrissa sparing could begin to cause the cortical neuron to fire.

LAMINAR DIFFERENCES IN CORTICAL PLASTICITY

Our results for the supragranular layers suggest that there are at least two different plasticity mechanisms, which together act to modify the representations of the spared and deprived vibrissae. One mechanism is a global excitation following sensory deprivation that leads to expanded representation of both spared and cut vibrissae. This topographically "nonspecific" excitation mechanism was in addition to a topographically specific augmentation in the representation of the spared vibrissa, evidenced by comparing representations of the spared and deprived vibrissae within each animal. However, when spared and/or trained vibrissae were compared with the surrounding cut vibrissae in infragranular layers, there was no difference in any of the groups that underwent vibrissa-sparing experience and/or gap-cross training. Since the changes were not specific to the "sensory history" of the vibrissa, they might be attributable purely to a global excitation – there was no evidence for any additional, selective potentiation of responses to the spared and/or trained vibrissae.

The spatial extent of the vibrissal representation might be related to decreased inhibitory modulation in cortex. Lowering the amount of inhibition could, therefore, effectively increase the excitation and increase the representation of the sensory receptors. Upon vibrissa deprivation GAD staining decreases in the cortex, especially in the

supragranular layers (Akhtar and Land, 1991). The asymmetry in GAD staining after vibrissa-deprivation suggests that release from inhibition following vibrissa-trimming is larger in the supragranular than the infragranular layers. This is consistent with our results, namely that the extent of the representational changes following vibrissa-sparing and training is larger in the supragranular laminae than in the infragranular layers.

Increased global excitation of the barrel cortex also might be due to the contribution of any of several neuromodulatory mechanisms which potentiate evoked activity or suppress inhibitory neurotransmitters. Evidence for increased excitation after application of a number of neuromodulators has been found in the primary somatosensory cortex. For example, application of acetylcholine (ACh) in rat primary somatosensory cortex enhances the activity evoked by sensory stimuli (Donoghue and Carroll, 1987). Furthermore, some cells respond to sensory input only in the presence of the ACh (Donoghue and Carroll, 1987; also see Metherate *et al.*, 1988). The main cholinergic input to the barrel field is from nucleus basalis (Mesulam *et al.*, 1983; Rye *et al.*, 1984; Baskerville *et al.*, 1993), which projects densely to both supra- and infragranular layers of the cortex – the same sites where we found globally potentiated evoked activity after vibrissae deflections. ACh, furthermore, is one of the mechanisms implicated in the plastic changes following vibrissa-pairing. When cholinergic input from nucleus basalis is lesioned, vibrissa-pairing plasticity does not develop in the barrel cortex (Sachdev *et al.*,1998). Moreover, changes in the neural firing pattern following a conditioning paradigm that includes sequential deflection of two vibrissae with a fixed intertrial interval do not develop if nucleus basalis is selectively lesioned using immunotoxins (Baskerville *et al.*, 1997).

How cholinergic modulation enhances the cortical response to sensory stimuli is not well understood. Nonetheless, the available data suggest that ACh potentiates NMDA-induced calcium flow into neurons (Auerbach and Segal, 1992). Given that NMDA receptors are denser in the supragranular layers compared to the infragranular layers of the cortex (Rema and Ebner, 1996), it is reasonable that blockade of NMDA receptor transmission hinders evoked activity in supra- but not infragranular layers of the primary somatosensory cortex (Fox *et al.*, 1989); in our experiments representational plasticity was larger in the supragranular layers than in the infragranular layers of the cortex, and .

The results of the present study show that independently from how vibrissae are used, their representations are larger in supragranular layers than in infragranular layers. What are the mechanisms behind the differential spatial extent of the evoked responses after very similar behavioral treatments? Besides the potential role of inhibitory modulation discussed above,

our data are consistent with two other candidate systems which may cause asymmetric representational changes in different laminae of the cortex. One candidate mechanism is a differential amount of excitation upon vibrissa deflections in different layers of the cortex. NMDA and AMPA receptor activation is crucial for the barrel cortex sensory processing (Armstrong-James *et al.*, 1993). In the barrel field, the distribution of the NMDA receptors is not even throughout the thickness of the cortex; rather, it is densest in the supragranular layers (Rema and Ebner, 1996). Because NMDA receptors have a key role in synaptic plasticity, their concentration in the supragranular layers (compared to infragranular layers) could make the former more readily modifiable.

The third way to account for the differential plasticity of the different layers might be the presence of neuromodulators in the cortex. The responses of neurons might be modified not only by the quantity of the neuromodulators available (Silito and Kemp, 1983; Metherate *et al.*, 1988; Bassant *et al.*, 1990), but also by the location of the neuron (Lamour *et al.*, 1988). For example, application of ACh increases the evoked responses in a laminae dependent manner (Lamour *et al.*, 1988). Magnitude of the evoked responses after an equal amount of ACh application is larger in the supragranular layers than infragranular laminae. One important question, then, is whether the laminae above and below the granular layers receive equal amounts of cholinergic input from the nucleus basalis: uneven distribution of the cholinergic input to different layers cortex could result in asymmetric representations of the input in different layers.. Cholinergic projections to the supra and infragranular layers of the cortex are comparable in density (Kristt, 1979; Lysakowski *et al.*, 1989).

One of the three mechanisms, or more likely a combination of several mechanisms mentioned above (GABAergic, Glutamatergic, Cholinergic), might account for different degrees of plasticity of vibrissae in different layers. Available data suggest that basal forebrain might be one of the crucial centers controlling sensory representations and plasticity (reviewed in Dykes, 1997). Basal forebrain neurons receive inputs from structures that are classically believed to be occupied in arousal and attention (Rasmusson *et al.*, 1994). More importantly, neurons in the basal forebrain region are active in awake, undrugged animals when they are in an environment which requires increased motivation, or when they are running in a goal-directed learning task (Richardson and Delong, 1986; Wilson and Rolls, 1990). The gap-crossing task is such a goal-directed task. Moreover vibrissa-trimming itself probably forces animals to attend to their sole vibrissa in a novel manner in order to collect information about their immediate environment. Hence both gap-cross training and vibrissa-trimming might be expected to induce increased basal forebrain activity. Increased activity in this region would in turn increase the release of ACh and GABA in the cortex (Dykes, 1997). As outlined earlier,

increased ACh availability in the cortex results in increased responsiveness of the cortical neurons to stimuli, and enlarged representation of the sensory receptors. Given the cholinergic modulation of the cortical evoked activity and the fact that basal forebrain GABAergic projections to the neocortex mostly terminate in GABAergic interneurons (Freund and Gulyas, 1991; Freund and Meskenaite, 1992), which in turn reduces the inhibition in the cortex, it is possible to argue that, following vibrissa-deprivation and gap-cross training, increased basal forebrain activity may cause global excitation in the cortical neurons.

Another key difference between supragranular and infragranular layer plasticity is that, following vibrissa sparing or gap-cross training at nose distances, representations of the spared vibrissae expanded in comparison with that of the surrounding (cut) vibrissae – but only in the supragranular layers, not in the infragranular layers. This effect cannot be explained by a global increase in the vibrissa evoked activity. Available data on activity dependent plasticity indicate that plastic changes require NMDA activity. The distribution of the NMDA receptors in different layers of the cortex is densest in the supragranular layers (Rema and Ebner, 1996). Having a larger amount of machinery available to generate activity dependent long-term plasticity in the supragranular layers might allow the cortex to exhibit such plasticity primarily in the supragranular layers. This hypothesis predicts that infragranular layers might also show activity-dependent plasticity after longer times of vibrissa-deprivation to assure that the machinery producing long-term changes in the representation of the spared organ is activated enough to produce the changes. The hypothesis, moreover, assumes that NMDA receptor subtypes in supra- and infragranular layers of the cortex are comparable and vibrissa-deflection evoked activity is generated using similar machinery.

INCREASED REPRESENTATION OF DEPRIVED VIBRISSAE

Another result of the present studies is that not only spared vibrissa representation but also those of deprived vibrissae increased in comparison to those in naive animals. Although this result is novel for the vibrissa representations, in other sensory systems similar findings have been reported, earlier. For example, in the visual system, after a focal lesion in the retina, primary visual cortex (V1) neurons that receive input from the lesioned part of retina reorganize their inputs and shift their receptive fields to the perilesion retina over the course of a few months (Gilbert *et al.*, 1990: Kaas *et al.*, 1991). Short term changes following retinal lesions are, to a large extent, restricted to the enlarged receptive fields of the V1 neurons whose receptive fields were in the lesioned retina (Gilbert and Wiesel, 1992). Such enlargement of the 'deprived' neurons' receptive fields is not specific to pathological conditions where a part of the circuitry is lesioned. Also after stimulation conditions which mimic the lesions by

masking out an area covering the receptive field of a V1 neuron, the receptive field size of the deprived neurons increases (Pettet and Gilbert, 1992).

These results suggest a global mechanism that operates in several sensory systems in a similar way following alterations in sensory inputs.

CONCLUSIONS

In this thesis we began by (i) quantifying the gap-cross behavior. Having thus determined that the sensory history of an individual rat's vibrissa could be documented, we set out to study cortical plasticity electrophysiologically , and asked (ii) whether extragranular layers of the cortex undergo modifications following 13 days of alterations in vibrissa use (i.e. vibrissa-sparing). After answering this question affirmatively, we went on to investigate (iii) whether forcing animals to use their spared-vibrissae in a learning task modifies the cortical plasticity, and (vi) whether different laminae of the extragranular layers differ in the plasticity they manifest after the very same vibrissa-use alterations. We, furthermore, examined (v) how cortical plasticity is altered after longer duration of modifications in vibrissa usage. The results of the studies suggested (in the order of chapters presented) that:

- The exact temporal parameters of the behavior are, to our surprise, not strongly dependent on whether several components of the sensory system sample the very same target object (i.e. nose alone, vibrissa alone, nose plus vibrissa).
- Information learned using a large number of sensory receptors could be recalled with input from a subset of those 'trained' receptors.
- The change in the Supragranular cortical representation of a vibrissa is conditional upon how a spared vibrissa is used during the sparing period.
- The representation of a spared vibrissa increases if it is used only in a familiar environment (i.e. the animal's home cage) throughout the vibrissa-sparing period. However, if the vibrissa is used in a perceptual learning task on which success depends on the activity of the spared vibrissa (i.e. the vibrissa-crosser rats), the representation of the spared vibrissa decreases in comparison to those of the rats with a single vibrissa intact in the home cage. If some other sensory receptors, in addition to the spared vibrissa, are trained in the task, the spatial extent of the spared vibrissa representation in this group does not change compared to those in vibrissa-crossers.

- There are at least two types of mechanism that may play a role in cortical plasticity. One is a global excitation affecting the spared and deprived vibrissae, and the other is a mechanism selectively facilitating spared vibrissa representations.
- Using the spared vibrissa in a familiar environment (home cage) and in the learning task, modifies the same neural circuitry in different ways, as suggested by expanded vibrissa representation of the spared vibrissa after home cage, but not learning, experience at short-latency epochs of cortical activity.
- The magnitude of the neural activity elicited by vibrissa deflection changes in a parallel manner to the vibrissa representations. The number of spikes evoked after vibrissa deflection increases after home cage use, but not after training on a vibrissa-dependent learning task.
- Different mechanisms affect the number of spikes evoked after vibrissa deflections. A global excitation causes increased spiking activity in neurons deprived of sensory input and neurons with sensory input spared. In contrast, selective facilitation in the number of spikes elicited by spared vibrissa might be a local phenomenon.
- Receptive field properties of cortical neurons (number of vibrissae projecting to individual neuron clusters) are modified after alterations in vibrissa use. These modifications are not completely predictable according tothose seen in vibrissa projections across cortex (number of neuron clusters receiving input from individual vibrissae). Most notably, mechanisms modifying the receptive field properties of cortical neurons affect not only the neurons in the columns of the spared vibrissa but also in the deprived vibrissa columns.
- The change in the vibrissal representations described earlier after home cage use and training experiences persists if the training and alterations in the sensory organ use are carried out after the cortex is already modified.
- Duration of the alterations in vibrissa-use significantly affects the mechanisms mediating cortical plasticity. After 13 days a global excitation, which may or may not be due to reduced inhibition (see below) accompanies a local excitation specific to spared vibrissa. The global excitation is depressed towards 'normal' levels if the alterations are continued for an additional 13 days in the home-cage. If the animals receive training on a vibrissa-dependent learning task, during the second half of this 26 day vibrissa sparing, further modifications occur in the level of excitation assessed by spatial extent and magnitude of evoked activity.
- A longer vibrissa-sparing period and type of vibrissa-use during this term also modifies the receptive field size. If the spared vibrissa is used to perform the task for 12 sessions throughout the second half of the 26 day vibrissa-sparing, receptive field size of neurons

increases as opposed to decreasing to the 'naive' neuron values in the animals which receive only home cage experience through the vibrissa-sparing period.

- Receptive field organization changes as a function of vibrissa-sparing duration. When a vibrissa is spared and the animal left in the home cage for 13 days, receptive field size of the neurons increase. If the animal is left in the home cage for an additional 13 days period with a single vibrissa, the receptive field of the neurons returns to a size similar to that of naïve neurons'.

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