neuroReport

NeuroReport 5, 949–953 (1994)

Reversible relocation of representational boundaries of adult rats by intracortical microstimulation

Friederike Spengler[CA] and Hubert R. Dinse

Institut für Neuroinformatik, Theoretische Biologie, Ruhr Universität Bochum, ND 04, Box 102 148, D-44780 Bochum, Germany

[CA] Corresponding Author

CORTICAL reorganization of somatosensory maps of adult rats is not restricted to central, already cutaneous zones. A few hours of intracortical microstimulation (ICMS) at the boundaries of the hindpaw representation generated plastic reorganization beyond these functionally defined representational borders by inducing new skin field representations in previously non-somatic cortical regions, from where low-threshold movements could be elicited. In this way, individually defined borders could be reversibly relocated over distances up to 800 microns, containing selectively skin field representations of the ICMS site. Response amplitude and latency characteristics of these newly induced cutaneous recordings sites resembled those recorded under control in the central representational zones. The results suggest that ICMS-induced plasticity acts across areal and modality borders by fast modulation of synapses in overlapping cortical and subcortical networks.

Key words: Somatosensory cortex; Motor cortex; Post-ontogenetic plasticity; Intracortical microstimulation; Receptive fields; Representational maps; Simultaneous recordings; Areal borders; Adult rats

Introduction

Neural systems organize behaviour according to the environmental constraints and conditions which change on a variety of time scales. Therefore, each system operating in such an environment must preserve considerable life-long adaptive capacities beyond the critical development period. Over the past few years, post-ontogenetic functional plasticity was observed in the cortex of many adult species—after circumscribed damage to peripheral receptor arrays of the skin, cochlea or retina, the input-deprived cortical areas are occupied by the representations of neighbouring sensory fields immediately or after weeks and months of recovery.[1–7] Remodelling of sensory representations following behavioural training, classical conditioning and prolonged natural sensory stimulation suggests that cortical maps and receptive fields are subject to modification by use.[8–13] Several hours of intracortical microstimulation (ICMS) has been shown to be highly effective in studies of short-term plasticity, offering the advantage of investigating in acute experiments the capacities and constraints of functional plasticity in the adult sensory and motor cortex, regardless of effects from the sensory periphery and the ascending pathways.[14–19]

So far, post-ontogenetic plasticity was studied by imposing plastic changes within a cortical zone, whose neurones were already part of an established and therefore active and drivable sensory representation. In this study, using an ICMS-protocol to evoke plastic changes,[15,16,18,20] we address the question of quickly emerging cortical reorganizations across predefined, functional representational boundaries of primary somatosensory cortex into adjacent motor cortex which, prior to the experiment, lacked postsynaptic activity driven by tactile stimulation.

Materials and Methods

A total of 39 rats were studied, which were anaesthetized with urethane (20% in Ringer's solution, 1.5 g kg^{-1}, i.p.) and held under urethane anaesthesia during the entire course of the experiment). For details of the experimental procedures see Refs 16 and 18. Treatment of all animals was within the National Institutes of Health Guide for Care and Use of Laboratory Animals (Revised 1987). In brief, after unilateral opening of the skull, the dura was removed and the cortex covered with silicon oil. Magnified video images of the brains were used to mark penetration sites and to reconstruct cortical topographic maps. The terms 'rostral' and 'caudal' are used to refer to the directions on the cortical surface. Action potentials were extracellularly recorded from small clusters containing 2 to 4 neurones at depths of 600 to 800 microns using glass micro-electrodes (1–2 MΩ) filled with 3 M NaCl and stored on computer as TTL pulses. A mechanical stimulator was used to apply computer controlled tactile stimuli of 8 ms duration at 1 Hz. For quantitative analysis, post-stimulus-time-histograms (PSTHs) were compiled following tactile stimulation at the receptive field (RF) centres averaged over 32 trials. Neurone responses were analysed for peak latency (time between stimulus onset and time of maximal response), response amplitude (maximal firing rate) and

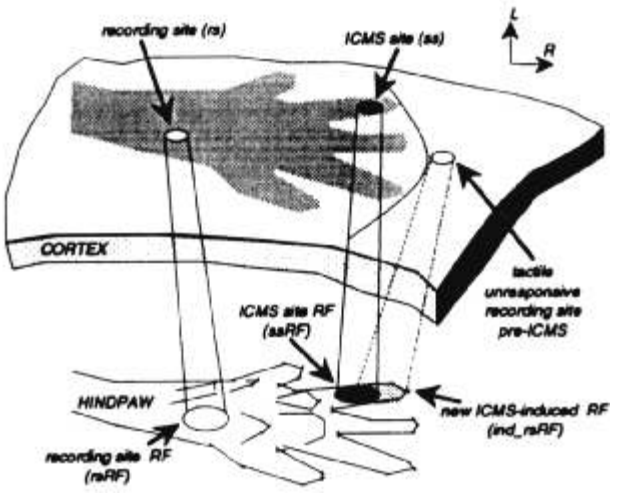

FIG. 1. Schematic illustration of the experimental ICMS protocol. ICMS was delivered at the rostral perimeter of the hindpaw representation at a so-called microstimulation site (ss), whose corresponding RF was denoted as ssRF. The other penetration sites were called recording sites (rs) with corresponding rsRFs. Tactile stimulation to the skin was delivered either at the RF of the recordings site or at the RF of the microstimulation site.

response peak mass (number of spikes during response peak).

ICMS was delivered with 13 pulses of 6 μA, 0.2–1 ms duration in 40 ms trains delivered at 1 Hz at a so-called microstimulation site (ss), whose corresponding RF was denoted as ssRF. The other penetration sites were called recording sites (rs) that had corresponding rsRFs (see Fig. 1). Tactile stimulation to the skin was delivered either at the RF of the recording site or at the RF of the microstimulation site. When the rsRFs were stimulated at the non-corresponding RF of the microstimulation site, a sensitivity difference was obtained that could be used to quantify the effects of ICMS (cf. Figs 4 and 5). Newly induced RFs were denoted as ind_rsRFs and were usually tested at the RF of the microstimulation site. For mapping, cells that responded to just visible skin indentations were classified as cutaneous. Cells responding either to high threshold stimuli, joint movements or deep inputs were classified as noncutaneous. Penetrations were usually placed 100 to 200 μm apart, which allowed a precise definition of the spatial extent and topography of the hindpaw representation and its borders. In addition, the cortical region along and beyond the rostral border of the hindpaw representation was mapped following a standard microstimulation protocol to evoke motor responses at recording depths of about 1500 microns using currents between 10 and 60 μA.[20] We observed low-threshold movements including toe flexion and ankle extension. Typical motor responses were video-taped for documentation.

Results

Under normal conditions, maps of the rat SI hindpaw representation are characterized by small, low-

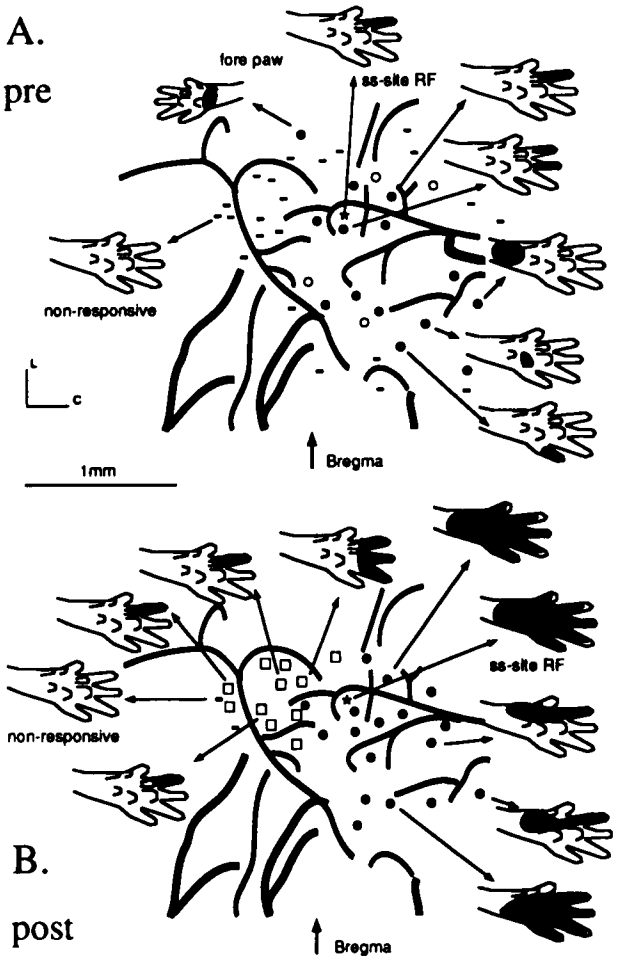

FIG. 2. Control map (a) of the hindpaw representation defined in rat primary somatosensory cortex based on a reconstruction of an enlarged brain photo. Black lines represent blood vessels, penetration sites are marked. Selected receptive fields (RFs) are drawn on sketches of the hindpaw. Dark points indicate cutaneous, open circles indicate noncutaneous responses, and open squares indicate newly induced cutaneous sites. Bars indicate locations where cells could not be driven by sensory inputs. For ICMS, a so-called microstimulation site RF (ssRF) on digit 2 was selected. After 4 h of ICMS, the border region and the central hindpaw representation were remapped (b). In the experiment shown, new skin representations containing the ssRF emerged up to 500 μm beyond the rostral boundary, while recording sites further rostrally maintained their unresponsiveness to tactile stimulation.

threshold, cutaneous receptive fields, located on single digits, pads or parts of the heel (Fig. 2a), defining a fine-grained topographic representation over a total cortical surface of about 1.5 mm^2. Application of 2–4 h of ICMS near the rostral perimeter of the hindpaw representation caused an expansion of the representation beyond these initially defined borders (Fig. 2b). Relocations of skin field representations into cortical zones of former non-somatic responsiveness were observed in all experiments ($n = 33$), but were variable in the spatial extent and overall shape. Shifts of the boundary between somatosensory and motor cortex ranged between 300–800 microns.

In the example shown, the border of the area of cutaneous responsiveness was relocated up to 500 μm, which was demonstrated by recording sites that reflect

newly emerged receptive fields similar to those at the microstimulation site, namely the skin field on digit 2 (Fig. 2a and b). ICMS-induced new receptive fields close to the microstimulation site were enlarged, showing low-threshold characteristics, and comprised skin sites on multiple digits, always including the microstimulation site RF. More rostrally located recording sites outside the zone of ICMS-effectiveness maintained their unresponsiveness to cutaneous stimulation. After ICMS, the microstimulation site RF was increased by integration of the surrounding inputs. Similarly increased skin fields were found at recording sites close to the microstimulation site, revealing a distance-dependent, directed enlargement towards the control microstimulation site RF with the tendency to comprise it (Fig. 2b). Accordingly, the fine-grained topography of the hindpaw is replaced by a representation of multiple skin sites, dominated by the representation of the microstimulation site RF. This was quantitatively assessed by analysing RF overlap. Data from eight rats were pooled, in which the microstimulation site was located similarly at about 200 μm caudally from the rostral extent of the hindpaw representational border. Before ICMS, the rostral border can be identified according to the sharp decline of RF overlap, which after ICMS is shifted rostrally and shows a distribution similar to that for the caudal direction (Fig. 3).

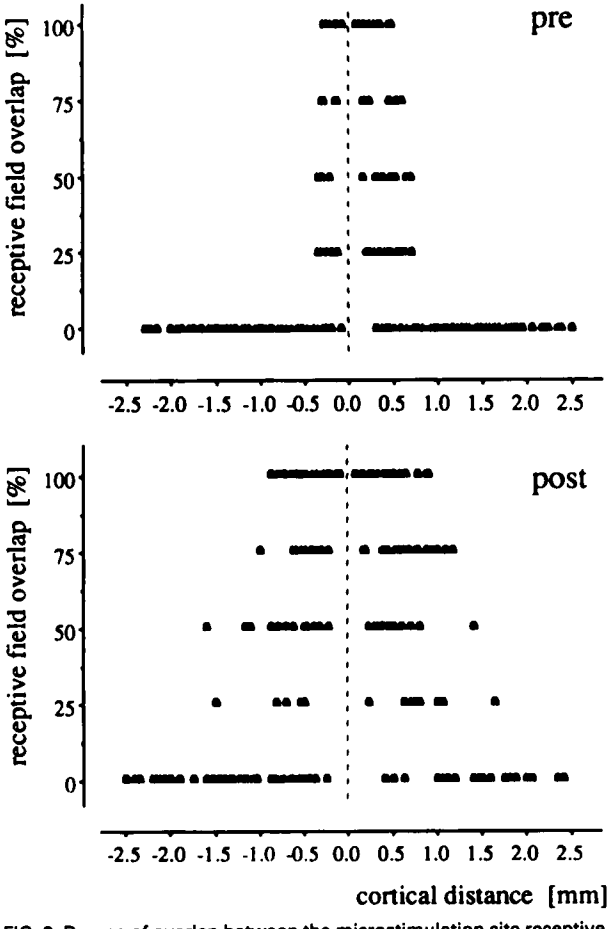

FIG. 3. Degree of overlap between the microstimulation site receptive field and the receptive fields of each recording site as a function of cortical distance before and after ICMS. Overlap is classified according to 0, 25, 50, 75 and 100%. Location of the microstimulation site is zero, recording sites located rostrally from the microstimulation site are negative, those located caudally are positive. Recording sites deviating mediolaterally are projected onto a virtual rostrocaudal axis. Data shown are from eight rats, in which the microstimulation site was located similarly at about 200 μm caudally from the rostral extent of the hindpaw representational border, which before ICMS can be identified according to the sharp decline of RF overlap. After ICMS, the border region is shifted rostrally and shows a distribution of RF overlap similar to that for the caudal direction.

In order to quantify the changes of response characteristics of the newly induced RFs, we simultaneously recorded neurone responses within the newly induced zone as well as inside the central core zone with two independent microelectrodes (62 pairs). This procedure offers the advantage that the response of one neurone can be used to calibrate the response strength of another neurone in terms of relative excitability.[18] As a rule, the overall neurone responses in the newly induced cutaneous recording sites resembled those recorded under the control in the central representational zones. However, they usually had slightly longer latencies, lower firing rates and broader response peaks (Figs 4 and 5). In detail, the mean pre-ICMS latencies for recording sites were 4 ms longer when stimulated at the ssRF than those following rsRF stimulation. After ICMS, latencies became fairly similar, independent of the location of stimulation. In the newly induced skin representations, however, latencies were about 2 ms above the average post-ICMS values. Pre- and post-ICMS firing rates were about the same for ss and rs, but recording sites stimulated at the ssRFs before ICMS showed on average 70% lower firing rates. After ICMS, the newly induced RFs had on average 50% lower firing rates than the mean rs recorded in the central representational zone. The width of the response peaks was quantified by the ratio of maximal firing rates and peak mass. In general, the newly induced RFs had broader peaks (10–50%) than all pre- and post-ICMS RFs. All mean values were tested for significance using an unpaired t-test. Significant differences ($p < 0.01$) are marked by asterisks in Fig. 4.

Possible non-specific effects of the procedure were tested and ruled out in six sham stimulation control experiments in this series and in two other animals in a previous series,[16] in which the entire protocol was followed with the exception that no current was passed through the electrode. Mapping was conducted under single-blind conditions, i.e. without the experimenter being aware of the stimulation conditions. As to the time course of these effects, we recently described that initial effects in normal ICMS experiments can be detected after 15 min, but reach maximum after 2 to 3 h.[18] Here, we studied the time constants of reversibility, which could be assessed in four rats. In these experiments, the described relocation of representational borders was fully reversible 6 to 8 h after termination of ICMS.

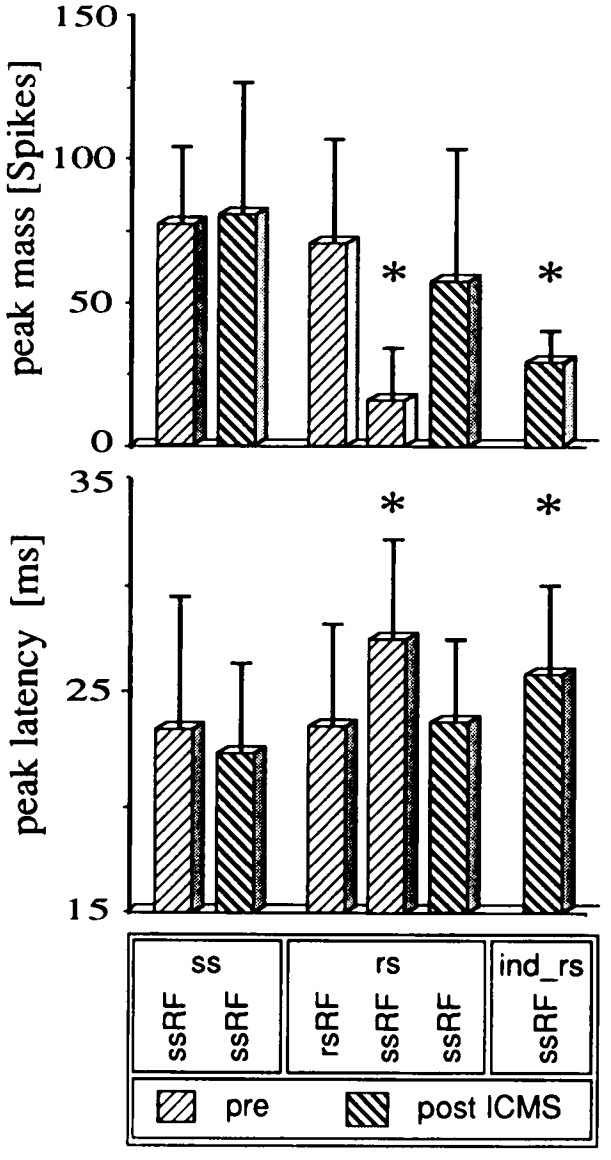

FIG. 4. Mean and standard deviations of peak masses (top) and peak latencies (bottom) of neurons of the ICMS sites (ss), recording sites (rs) and newly induced sites (ind_rs) for tactile stimulation applied at the RFs of the ICMS sites or the RFs of the recording sites before and after ICMS.

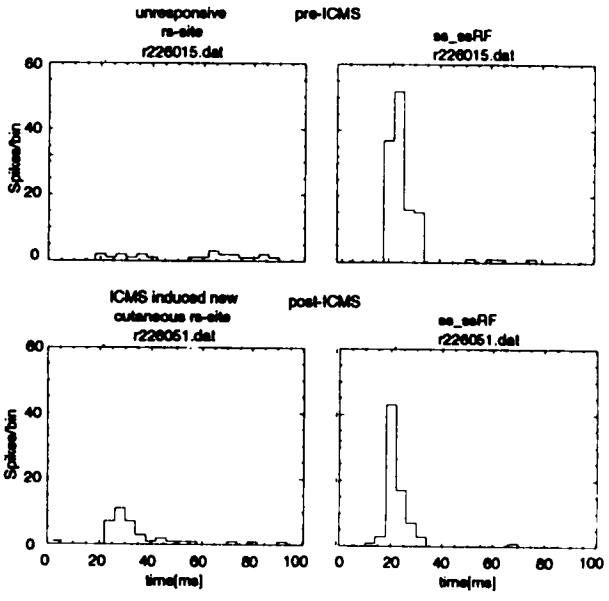

FIG. 5. PSTHs following tactile stimulation before and after ICMS for an ICMS site and a newly induced RF. The recording site of this newly induced RF was under control unresponsive to tactile stimulation.

Discussion

These results demonstrated that neurones, which under control conditions are not responsive to any type of cutaneous stimulation, can be transformed into tactile responsive neurones following a protocol of a few hours of ICMS, thus relocating the border of a cortical representational zone into the adjacent motor representation. Accordingly, the presence of specific postsynaptic activity driven by peripheral sensory stimulation like that described for the central core representations appears not to be essential for generating plastic reorganization.

The present study extends the reorganizational variables that have so far been reported during ICMS-induced plasticity such as map changes,[14–16,18] changes of RF size and RF overlap,[15–18] changes of correlated activity[18] and changes of functional aspects of RF transfer functions,[19] by introducing relocations of areal borders.

The rapid time-scale of ICMS-induced reorganization and the aspect of reversibility support the hypotheses of fast modulations (here in the minutes to hours range) of synaptic efficiency in dynamically maintained networks, resulting in the formation of new representational neuronal groups or assemblies.[21] Therefore, anatomical changes such as dendritic and axonal sprouting or synaptic proliferation appear rather unlikely.

The use of anaesthetics in plasticity-related experiments might interfere with plasticity effects, as it is known for ketamine, which blocks NMDA receptors. We used urethane because of its long-lasting action thus enabling a stable state of anaesthesia and its little depressive effects on respiration and cardiovascular action. Although urethane was reported to interfere with the GABAergic and adrenergic systems, our sham experiments ruled out that many hours of urethane anaesthesia might affect cortical representations by altering RF size. Moreover, the effects of RF changes we observed under urethane were comparable with those described earlier using barbiturates.[16,18]

Although ICMS must be regarded as an artificial tool, its usage for stimulation of directionally selective neurones in MT visual cortex during a motion detection task changed the animals' judgements towards the direction of motion encoded by the stimulated neurones, indicating that ICMS may directly influence behaviour.[22] As to the spatial extent of current spread, a 10 μA pulse of cathodal current was reported to acti-

vate neurones within 80 to 100 microns around the stimulation site in primate motor cortex.[20] Using a temporal pairing paradigm of natural tactile stimulation (PPTS) in experiments performed in rat SI hindpaw representations, we have demonstrated changes of RFs and representational maps to occur within a few hours[13] which are comparable with those obtained in the ICMS experiments. In addition, these PPTS experiments revealed relocations of borders between hindpaw and other skin representations as well as between SI and the adjacent motor cortex.[13] In monkeys, after several months of selective tactile discrimination training, cortical reorganization was reported to cross boundaries of submodalities in areas 3a and 3b.[23]

The hindlimb including the hindpaw representation in rat somatosensory cortex is morphologically described as a sensorimotor overlap zone, the target of somatic sensory and efferent motor neurones from the ventrobasal and ventrolateral thalamic nuclei.[24] To generate relocation of representational borders by ICMS, the underlying networks of overlapping cortico-cortical and thalamo-cortical axons, projecting into that overlap zone and spreading across areal borders must be assumed to be directly or indirectly connected. It seems conceivable that the morphology of the underlying neurones, the asymmetry of arborization and the variation of synaptic density account specifically for the spatial extent and the shape of cortical reorganization and the magnitude of the influences of the microstimulation site. Further neuroanatomical studies are important to unravel the nature of the representational border zones. As motor responses are usually recorded in layer V, while new cutaneous responses were seen to emerge in deep layer III and in layer IV, it cannot be excluded that this sensorimotor overlap is restricted to different cortical layers.

In spite of all the recent evidence for plastic-adaptive capacities, systems must possess some inherent generic stability in order to perform stable operations despite continuous changes in the environment. Therefore, one should expect a trade-off between modifiability warranting sufficient flexibility on the one hand, and stability enabling the system to achieve a minimum of invariance on the other. Hence, the limit of about 800 microns as encountered earlier[16,18] might reflect a meaningful constraint of short-term plasticity that could provide this stability of processing.

It is an open question whether the underlying cortico-cortical and thalamo-cortical networks are directly or indirectly coupled to generate relocation of representational borders. Highly interconnected networks alone as typical for cortices might provide sufficient lateral spread of information without involving discrete long-range horizontal connections of axon collaterals. Such a system of predominantly dynamically maintained cortical processing might provide a neural basis of lifelong adaptational mechanisms that allow macroscopic reorganization during learning and the acquisition of skills.

Conclusion

A few hours of intracortical microstimulation resulted in significant relocations of the borders between somatosensory and motor cortex of adult rats. These results extend plastic processes beyond central aspects of representational maps. The short time scale and the aspect of reversibility provide further evidence for the widespread existence of fast (here minutes to hours range) plastic synapses within highly interactive cortical and subcortical networks across areal and modality borders.

References

1. Merzenich MM, Nelson RJ, Stryker MP*et al. J Comp Neurol* **224**, 591–605 (1984).
2. Pons TP, Garraghty PE and Mishkin M. *Proc Natl Acad Sci USA* **85**, 5279–5281 (1988).
3. Robertson D and Irvine DRF. *J Comp Neurol* **282**, 456–471 (1989).
4. Eysel UT. *Nature* **299**, 442–444 (1982).
5. Kaas JH, Krubitzer LA, Chino YM *et al. Science* **248**, 229–231 (1990).
6. Calford MB and Tweedale RD, *Nature* **332**, 446–448 (1988).
7. Gilbert CD and Wiesel TN. *Nature* **356**, 150–152 (1992).
8. Weinberger NM, Ashe JH, Metherate R *et al. Concepts Neurosci* **1**, 91–132 (1990).
9. Scheich H. *Curr Op Neurobiol* **1**, 236–247 (1991).
10. Recanzone GH, Merzenich MM, Jenkins WM *et al. J Neurophysiol* **67**, 1031–1056 (1992).
11. Recanzone GH, Schreiner CE and Merzenich MM. *J Neurosci* **13**, 87–103 (1993).
12. Pascale-Leone A and Torres F. *Brain* **116**, 39–52 (1993).
13. Godde B, Spengler F and Dinse HR. In: Elsner N and Heisenberg H, eds. *Neurogenetics–Brain–Behavior.* Stuttgart, New York: Thieme, 1993: 140.
14. Nudo RJ, Jenkins WM and Merzenich MM. *Somatosen Mot Res* **7**, 463–483 (1990).
15. Dinse HR, Recanzone GH and Merzenich MM. In: Eckmiller R, Hartmann G and Hauske G, eds. *Parallel Processing in Neural Systems and Computers.* Amsterdam: Elsevier, 1990: 65–70.
16. Recanzone GH, Merzenich MM and Dinse HR. *Cerebral Cortex* **2**, 181–196 (1992).
17. Bedenbaugh PH, Maldonado PE and Gerstein GL. *Soc Neurosci Abstr* **18**, 346, (1992)
18. Dinse HR, Recanzone GH and Merzenich MM. *NeuroReport* **5**, 173–176 (1993).
19. Spengler F and Dinse HR. In: Elsner N and Richter DD, eds. *Rhythmogenesis in Neurons and Networks.* Stuttgart, New York: Thieme, 1992: 129.
20. Stoney SD, Thompson WD and Asanuma H. *J Neurophysiol* **31**, 659–669 (1968).
21. Edelman GM and Mountcastle VB. *The Mindful Brain.* Cambridge: MIT Press, 1978.
22. Salzman CD, Britten KH and Newsome WT. *Nature* **346**, 174–177 (1990).
23. Recanzone GH, Merzenich MM and Jenkins WM. *J Neurophysiol* **67**, 1057–1070 (1992).
24. Neafsey EJ, Bold EL, Haas G *et al. Brain Res Rev* **11**, 77–96 (1986).

ACKNOWLEDGEMENTS: We gratefully acknowledge the support of the Institut für Neuroinformatik. We thank Professor W. von Seelen for fruitful discussions and Professors Drs A. Aersten, U. Eysel, R. Necker and C. Schreiner for critical comments on earlier versions of the manuscript.

Received 4 January 1994; accepted 15 January 1994