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Associative pairing of tactile stimulation induces somatosensory cortical reorganization in rats and humans

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WE used a protocol of associative (Hebbian) pairing of tactile stimulation (APTS) to evoke cortical plastic changes. Reversible reorganization of the adult rat paw representations in somatosensory cortex (SI) induced by a few hours of APTS included selective enlargement of the areas of cortical neurones representing the stimulated skin fields and of the corresponding receptive fields (RFs). Late, presumably NMDA receptor-mediated response components were enhanced, indicating an involvement of glutamatergic synapses. A control protocol of identical stimulus pattern applied to only a single skin site revealed no changes of RFs, indicating that co-activation is crucial for induction. Using an analogous APTS protocol in humans revealed an increase of spatial discrimination performance indicating that fast plastic processes based on co-activation patterns act on a cortical and perceptual level.

Key words: Input statistics; LTD; LTP; Psychophysics; Receptive fields; Reinforcement; Representational maps; Somatosensory cortex; Synaptic plasticity; Two-point discrimination

Introduction

According to the Hebbian postulate, the temporal coincidence of neural events and thus the characteristics of the input statistics are hypothesized to be crucial parameters for the induction of changes in synaptic excitability.[1] This protocol has been extensively used in cellular studies of synaptic plasticity.[2–4] A similar degree of plasticity has been postulated to account for the alterability of cortical receptive fields and cortical maps, not only during the critical period of developmental, but also in adult nervous systems (postontogentic plasticity).[5–12] Several lines of evidence suggest the importance of correlated inputs in the induction of plastic changes.[13–15] In this study, we address the question of rapid *in vivo* cortical reorganization induced by associative (Hebbian) pairing of natural, i.e. tactile stimulation through temporally coherent co-activation patterns. To address the question of the relevance of plastic reorganization induced by pure variation of the input statistics without providing any reinforcement at a perceptual level, we initiated a parallel study to test in humans psychophysically the impact of an analogous APTS protocol by measuring spatial discrimination performance.

Material and Methods

A total of 30 rats, including four sham experiments, were anaesthetized with urethane (20% in Ringer solution, 1.5 g kg^{-1}, i.p.) and maintained under urethane anaesthesia during the entire experiment. Treatment of all animals was within the National Institution of Health Guide and Care for Use of Laboratory Animals (Revised 1987). In brief, after unilateral opening of the skull, the dura was removed, the cortex covered with silicone oil and an enlarged video image was taken to use the blood vessels as landmarks for mapping. Action potentials were extracellularly recorded from small clusters of 2–4 neurones at depths of 600–800 μm using glass microelectrodes filled with concentrated NaCl (1–2 MOhm) 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 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 100–200 μm apart, which allowed a precise definition of the spatial extent and topography of the hindpaw representation and its borders. In order to study quantitatively changes of RF organization, we modified the response plane technique[16,17] by recording neuronal responses to computer-controlled tactile stimulation at up to 20 different locations on the hindpaw. Based on PSTHs, response peak areas were analyzed and the activity was marked in a drawing of the hindpaw at the respective location by grey level coding. Locations that evoked no activity or low activity beyond a given threshold were not shown (cf. Fig. 3).

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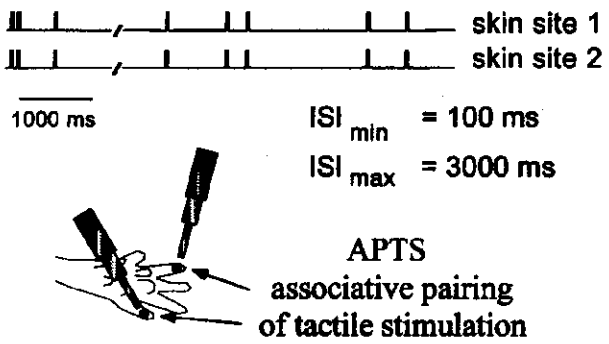

FIG. 1. Schematic illustration of the APTS (associative pairing of tactile stimulation) protocol. The centres of two non-overlapping receptive fields on two selected digits or on one digit and one pad were simultaneously stimulated for 6–15 h with a train of eight different interstimulus intervals between 100 and 3000 ms followed by a pause of 15 s.

To study the effects of APTS on the timing and amplitude of neuronal responses, PSTHs were recorded for tactile stimuli applied at the centre of a RF. Cumulative histograms were computed by summing the individual PSTH responses.

To induce plastic changes, the centres of two non-overlapping receptive fields on two selected digits or on one digit and one pad were simultaneously stimulated (APTS) for 6 or 15 h in 23 animals according to the following protocol. A train of eight different interstimulus intervals between 100 and 3000 ms were used randomly followed by a pause of 15 s (Fig. 1). After application of three trains there was an additional pause of 1 min to avoid adaptation and habituation.

We tested 35 right-handed human subjects before and after a 2 or 6 h APTS protocol in a two-alternative forced-choice tactile spatial discrimination task. The index finger of the right hand was tested, and the middle finger of the right hand or the index finger of the left hand served as controls. We used eight distances between 0.7 and 2.5 mm. Each session consisted of 10 randomized presentations of each distance. The pulse trains were recorded on tape and were played back via portable tape recorders (Walkman) which allowed unrestrained mobility of the subjects during the APTS period. The threshold was determined using a logistic fit function. To obtain stable and robust controls, each subject was tested on 5–6 successive days before the APTS was applied.

Results

Under normal conditions, maps of the hindpaw representation of somatosensory cortex (SI) in adult rats are characterized by small, low-threshold, cutaneous receptive fields (RFs), located on single digits, pads or parts of the heel, which define a fine-grained topographic representation. Application of 6–15 h of APTS on two digits or on one digit and one pad caused substantial expansions of the respective cortical skin representations, which was observed in all experiments, but which was variable in the spatial extent and overall shape (Fig. 2). In addition, emergence of new skin fields in cortical zones of former non-somatic responsiveness was observed, relocating the boundary of somatosensory cortex by up to 300–500 μm. As a consequence, the fine-grained topography of the hindpaw was replaced by a representation of multiple skin sites, dominated by the representations of the stimulation sites. Quantitatively, the size of that cortical area representing the skin fields on the stimulated digits or pads increased severalfold from an area of 0.06–0.15 mm^2 cortical surface under control conditions to 0.20–0.61 mm^2 after APTS.

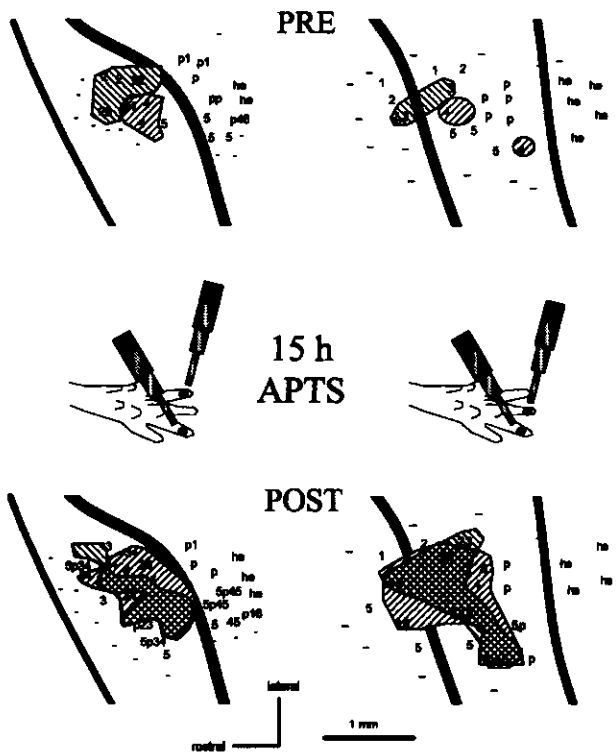

FIG. 2. Two examples (left and right columns) of cortical map reorganization after APTS. Control (pre) maps of the hindpaw representations defined in rat primary somatosensory cortex (top) based on reconstructions of highly enlarged brain photos. Black lines indicate blood vessels, penetration sites are marked. Scalebar = 1 mm. Numbers indicate digits 1 to 5, p pads, he heel. Bars indicate locations where cells could not be driven by low threshold cutaneous inputs. APTS was applied for 15 h to digits 2 and 4 (left) and on digits 3 and 4 (right). After APTS (bottom), the border region and the central hindpaw representation was remapped. In the experiments shown, new skin representations containing the stimulated skin fields emerged up to 400 μm beyond the control boundaries, while recording sites further rostrally maintained their unresponsiveness to tactile stimulation. Cortical areas representing the stimulated skin fields are highlighted by different hatching. Note emergence of substantial overlap of the cortical representation of the simultaneous stimulated skin fields.

Reorganizational changes of RFs were studied quantitatively by means of a modified response plane technique before and after 6–15 h of APTS (Fig. 3). Response planes were characterized by focal zones of activity surrounded by larger zones of less activity. After APTS, receptive fields showed normal low-threshold cutaneous characteristics. However, RFs were increased in size by integration of the stimulated skin sites. On the average, RF size increased from 45.4 ± 4.6 mm^2 (mean ± s.e.) skin surface under control conditions to 79.9 ± 8.22 mm^2 after APTS ($n = 94$, $p < 0.0007$, t-test). Enlarged RFs were predominantly found close to the stimulation sites but also up to 500 μm away from them, revealing a distance-dependent, directed enlargement towards the stimulated skin sites with the tendency to include them. Before APTS, there was a small degree of overlap between individual RFs with the RFs of the stimulated skin sites of 19.9 ± 3.11%. After APTS, the overlap increased to 34 ± 4.18% ($n = 94$, $p <$ 0.0007).

To verify that the effects described were indeed generated by the pairing induced coactivation patterns, we tested in three animals the effects of a so-called single point tactile stimulation (SPTS) consisting of a pattern of tactile stimulation with identical temporal characteristics to APTS for 8 or 15 h, which was, however, applied to only one single selected skin site on one digit or on one pad. We were not able to find changes of RF sizes within the accuracy of the method.

Neural responses to tactile stimulation were shown to differ with respect to their pharmacological properties. Late response components were shown to be NMDA receptor dependent, while early response episodes were NMDA independent.[18,19] We therefore analysed the response properties of SI neurones to computer-controlled tactile stimulation to determine whether the latency, duration and amplitude of the responses were altered following APTS. Peak response latencies and response amplitudes remained unchanged. In contrast, in all recordings after APTS the response episodes in the range between 25 and about 100 ms were enhanced (Fig. 4) indicating that APTS-induced plastic reorganization affects the amplitude of late, presumably NMDA receptor-mediated response components.

To determine the time course of the described effects, we compared the cortical maps and receptive fields before APTS, after APTS for 6 or 15 h, and then 12 h after the APTS interval in which no stimuli were delivered. The RF sizes returned to control conditions (Fig. 3), indicating reversibility of the

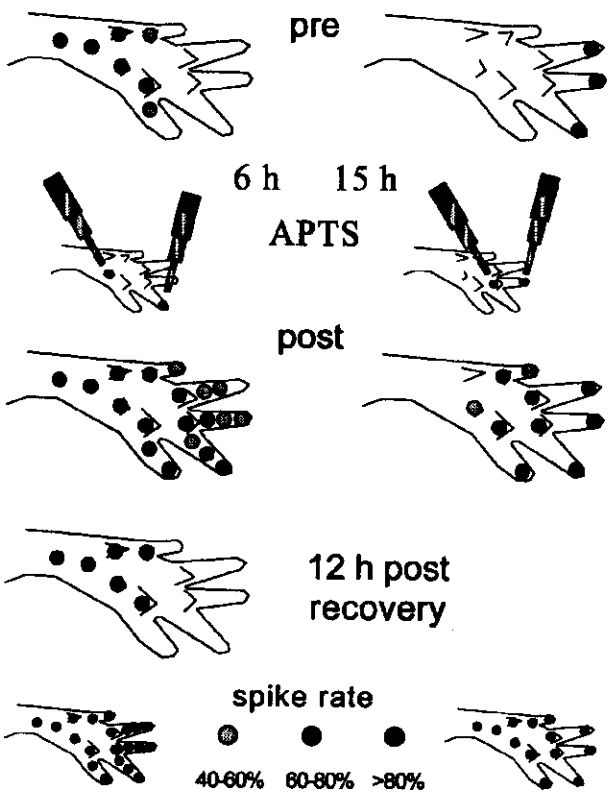

FIG. 3. Response planes of two cortical receptive fields (left and right columns) before and after 6 h (left) and 15 h (right) APTS. Under control conditions, the receptive fields are located over the pad region (left) and over digits 3 and 4 (right). APTS was applied for 6 h to digit 4 and pad 5 (left) and on digit 3 and pad 3 (right). After APTS, receptive fields are enlarged into skin field areas that were stimulated by the APTS protocol. Recovery was observed 12 h after terminating APTS, as illustrated for the receptive field shown in the left column. At the bottom, the locations on the skin used for constructing the response planes are shown together with the grey coding scheme expressed as relative spike rate (peak area in PSTH). Locations that evoked no activity or activity below 40% of maximal spike rates were not shown.

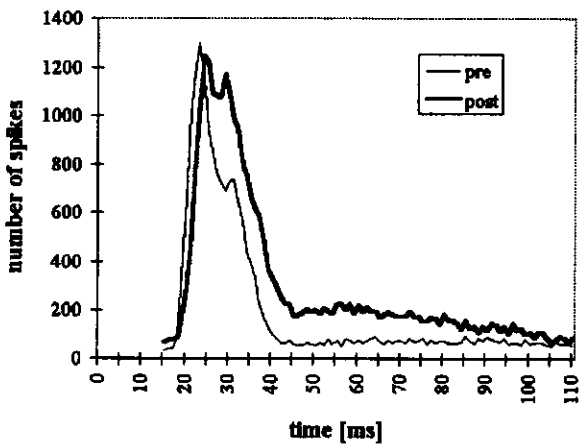

FIG. 4. Cumulative PSTHs to illustrate the effects of APTS on neural response properties. Single PSTHs were recorded following tactile stimulation applied to each RF centre. Binwidth is 1 ms. Sum of 220 recordings. While peak latency and peak amplitude remained unaffected by APTS, the response episodes in the range between 25 and about 100 ms were enhanced, suggesting the involvement of late, presumably NMDA-receptor mediated response components.

effects. Possible non-specific effects of the procedure were ruled out in four sham stimulation control experiments, in which the entire protocol was followed with the exception that no APTS stimulation was applied.

In order to explore the potential perceptual consequences of APTS-induced short-term plastic processes, we studied tactile spatial two-point discrimination performance in human subjects. After 2 or 6 h of an APTS protocol analogous to the above described electrophysiological experiments, we found a significant improvement in the spatial discrimination performance as indicated by decrease in discrimination thresholds from 1.37 ± 0.21 mm to 1.12 ± 0.22 mm after APTS ($n = 35$, $p = 0.000064$, 2-tailed t-test). Thresholds returned to normal 12 h after terminating APTS (1.37 ± 0.25 mm), indicating a reversibility of the changes in discrimination threshold similar to that seen in the electrophysiological experiments (Fig. 5). Inspection of the thresholds of the non-stimulated control fingers revealed no changes (1.43 ± 0.34 mm compared with 1.42 ± 0.35 mm after APTS; $n = 35$, $p = 0.9518$).

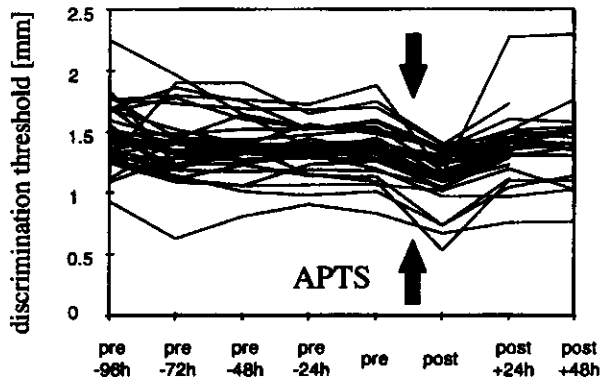

FIG. 5. Tactile two-point discrimination thresholds of the index finger of the right hand as measured in a two-alternative forced-choice discrimination experiment in 35 right-handed human subjects. Thresholds were measured 5 days before and immediately after APTS (arrows) and on two subsequent days. In all subjects thresholds were reduced immediately after APTS but returned to control values one day after APTS, revealing a similar time course of reversibility as described for the electrophysiological reorganizational changes in rats.

Discussion

In order to study systematically the effects of variation of the input statistics to induce plastic reorganizational changes in an intact animal, we attempted to modify synaptic efficiency by a stimulation protocol of associative pairing of tactile stimuli (APTS). This protocol generated temporal coactivation patterns which in turn induced significant reorganizational processes at the cortical level, including enlargement of receptive fields and cortical representational maps. In addition, late, presumably NMDA receptor-mediated response components were enhanced in amplitude, suggesting an involvement of glutamatergic synapses. Our results are consistent with the notion of correlational learning rules involved in this type of cortical plasticity.

The specific patterns of pseudorandomly distributed stimuli in time used in our APTS protocol were chosen, in addition to preventing adaptation and habituation, to by-pass the frequency-dependence of stimulation characteristic for LTP and LTD.[20] In spite of many successful attempts to characterize cortical plasticity separately *in vitro* and *in vivo*, there are still many open questions of how *in vivo* plasticity in terms of receptive field changes can be related to different forms of LTP and LTD. In a parallel study of cortical plasticity induced by intracortical microstimulation (ICMS) we could demonstrate that this type of plasticity that leads to similar RF enlargement[10,15] as described for APTS, was most probably mediated by LTD as indicated by *in vitro* experiments utilizing identical modes of induction.[21] These results demonstrate a non-trivial relationship between *in vitro* and *in vivo* forms of plasticity. Further studies are needed to unravel the underlying mechanisms involved in mediating the effects of ATPS.

Our provision of temporally coherent inputs by simultaneous paired tactile stimulation at two skin sites can be used to study *in vivo* implications and constraints of input statistic-based cortical plasticity. In this first experimental series, we compared the effects of associative pairing generating co-activation with a protocol of tactile stimulation applied to only one single point (SPTS) which revealed no reorganizational changes for SPTS, indicating that co-activation is indeed a crucial parameter for induction of plasticity. It is conceivable that the sensitivity of temporal separations and the existence of local predictive learning rules[22] can be tested by introducing either temporal delays or anticorrelated temporal patterns of stimulation between the two locations. Our approach can be further extended to study a possible distance- and ISI-dependency (interstimulus interval) of APTS by systematic variation of the spatial separation distance between the two stimuli.

Remarkably, the APTS protocol was similarly effective at the perceptual level by enhancing the spatial discrimination performance. This suggests that fast plastic processes based on variation of the input statistics can have perceptual consequences, supporting the significance of cortical reorganizational processes without involving specific types of reinforcements. At first sight, the enhancement of the discrimination performance might appear sur-

prising in view of the reported receptive field enlargement. However, perceptual thresholds are usually lower than corresponding single neurone properties. Hyperacuity, for example, can not be explained based on concepts of receptive field sizes of single cells. When interpreting our results one has to bear in mind that the changes of cortical response properties included an enlargement of RF size and a substantial reorganization of the representational maps accompanied by an increase of RF overlap, which increases the number of neurones activated by the stimulation of a selected skin field. Concomitantly, response durations became longer, increasing the time over which the neurones are active. It appears reasonable that all changes together enable higher cortical levels to perform a faster and more elaborate decoding and processing of information. The implications of increased RF size and overlap are formalized in the coarse coding principle[23–25] which was established in order to explain the frequently observed broad tuning properties or large RF sizes which nonetheless allow a fine discrimination performance at a behavioural level by populations of neurones. We could demonstrate that a theoretical analysis of our electrophysiological APTS data in the framework of the coarse coding predicts a 30–40% increase of spatial resolution (Eurich *et al*, unpublished data) which matches the range of improvement observed in our APTS psychophysical experiments in humans.

Generally, the short time-scale of APTS-induced reorganization at the cortical and psychophysical level and the aspect of reversibility support the hypotheses of fast modulations of synaptic efficiency in existing, but dynamically maintained networks. Such a system may represent the neural basis for lifelong adaptive sensory and perceptual capacities.

Conclusion

We studied cortical plastic reorganization induced by an associative pairing of 'natural' (i.e. tactile) stimulation applied to two separate skin locations (two non-overlapping RFs) of the hindpaw of adult rats. This protocol was chosen to investigate in the intact brain the constraints of cortical reorganization elicited by coactivation patterns following as closely as possible the idea of a Hebbian pairing protocol. This protocol allows us to vary systematically the type of input statistics which are hypothesized to play a crucial role in the induction of cortical plasticity. Here we report the existence of fast (6–15 h) and reversible reorganizational changes of RFs, representational maps and response characteristics of somatosensory cortical neurones. No changes could be induced when only one single skin site was stimulated strongly, supporting the importance of co-activation.

In order to clarify how far this type of plastic reorganization is relevant at the perceptual level, we studied in humans the impact of an analogous APTS protocol by measuring spatial discrimination performance. Our APTS protocol was similarly effective at the perceptual level by enhancing the spatial discrimination performance. This suggests that fast plastic processes based on variation of the input statistics can have perceptual consequences, supporting the significance of cortical reorganizational processes without involving specific types of reinforcements.

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