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MUSCARINIC CHOLINERGIC EFFECTS ON STIMULUS-EVOKED RESPONSES IN RAT PRIMARY SOMATOSENSORY CORTEX. AN ELECTROPHYSIOLOGICAL STUDY*

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Neuron terminals originating from the nucleus basalis magnocellularis (NBM) are the major source of the cortical cholinergic innervation, which is thought to play an essential role in higher brain functions. Electrophysiological studies have shown that activation of muscarinic cholinergic receptors caused a marked enhancement of sensory stimuli onto cortical neurons. Diminished cholinergic innervation of somatosensory cortical areas are manifested in decreased stimulus-evoked activity and impaired performance in a sensory discrimination task. We examined the effects of ACh and its muscarinic agonists on the response properties of layer IV-V barrel cortex neurons evoked by precisely controlled vibrissa deflections. The cholinergic pharmacons displayed their mostly facilitatory effects in latency-dependent manner: In most cases only one latency component of On and/or Off responses were changed.

Keywords: Cholinergic innervation — muscarinic receptors — somatosensory cortex — rat

Introduction

Several experimental and clinical evidence testify to the important role of cortical cholinergic innervation in maintaining normal cognitive functions. ACh either applied experimentally in the cortex /5/ or released from terminals of stimulated nucleus basalis magnocellularis (NBM) cells in sensory cortical areas /8/ increase the amplitude of responses evoked by sensory stimulation without affecting the background activity. The vibrissa-barrel pathway of the trigeminal system in the rat, firstly anatomically described by Woolsey and Van Der Loos /9/, offers a unique possibility to study the evoked responses in the primary somatosensory cortex induced by a

*Dedicated to Professor György Székely for his 70th birthday.

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controlled and adequate somatosensory stimulation (whisker deflection). In our study we examined the effects of ACh and its agonists on evoked unit activity of cells from identified barrels of the posteriomedial barrel subfield.

Material and Methods

A total of 36 Sprague-Dawley rats of both sexes were anesthetized with urethane (1.1 g/kg i.p.). After left side craniotomy microelectrode recordings were made at 0.3 mm intervals over the whole barrelfield to find the barrel related to the stimulated whisker. Mostly C1-3 vibrissae were deflected with a multiangular electromechanical stimulator. Stimulus waveforms were ramp-and-hold trapezoids. Details of surgical procedure, stimulation and the description of the recording equipment were published earlier /7/. Within the appropriate barrel the cells were excited quickly (6-10 ms) and powerfully (1-4 spike/stimulus onset and/or offset; On, Off response) by displacement of the related whisker. To the recording electrode filled with 2.5 M NaCl (5-10 MOhm) was adjusted a low impedance micropipette containing the drug. The distance between the electrode tips was not more than 50 μm. The following pharmacons were tested in 10^{-4}-10^{-3} M concentrations: acetylcholine, acetyl-L-carnitine, carbachol, scopolamine, atropine (the results of antagonist injection are not discussed in this paper). Before penetrations the optimal injection parameters solution were tested (25-40 pl/injection). In every second minutes from 4-6 min prior to the application to 16-20 min following the application peristimulus time histograms (PSTHs, bin width 5 ms) were produced. Activity levels of different latency components of On and Off responses taking from PSTHs figures were plotted versus time.

Results and Discussion

A total of 16 neurons were examined in detail from 81 isolated neurons in the somatosensory cortices of 36 animals. The results are presented in Table 1. ACh and its agonists increased the activity of at least one component of the On and/or Off responses in 65%. The effect of cholinergic drugs was mostly transient (4-6 min duration), however in some cases the effect was prolonged (up to 20 min or more), which is shown in our first example (Fig. 1). In this case an "unmasking" effect could be observed. After application of acetyl-L-carnitine, one latency component of the previously weak On response increased 6-8 times for 20 min compared to the control, while the first and third components remained unchanged. In most cases our observations were that the cholinergic pharmacons delivered "unmasking" effects on previously weak responses, and moreover, this mostly facilitatory

Table 1

ACh and its agonists induced changes of the components of On and Off responses evoked by vibrissa movements

On	Off	%
+	+	49
+	0	7
+	-	9
0	-	1
-	-	10
0	0	22
0	+ (§)	1
+ -	0 (*)	1
		100

+: one (in some cases more) of response components increased for longer duration than 4 min after drug injection

-: one (in some cases more) of the response components decreased for longer duration than 4 min after drug injection

0: there was no change (or shorter than 4 min) after drug injection

§: the exceptional case (presented in Fig. 2) when all of the components of an Off response increased after drug injection

*: the exceptional case when all of the components of an On response changed (increased and decreased) after drug injection

effect was manifested in changes of only one latency component of On and/or Off responses consisting of 2-4 components. In a few cases ACh or its agonists modified every component of the On and/or Off responses either in the same (Fig. 2 and §) or in different ways (in Table 1, *).

Although there are plenty of data proving cortical cholinergic effects in the cerebral cortex, their functional mechanisms in sensory processing are not well understood /6/. In rats the major means to explore the environment is the trigeminal system /2/. The major source of cholinergic innervation of the primary somatosensory cortex (SI) is the NBM /1/. Its cortical projecting neurons have restricted arborisation with little collateralisation giving the possibility of fine cholinergic tuning of small and restricted

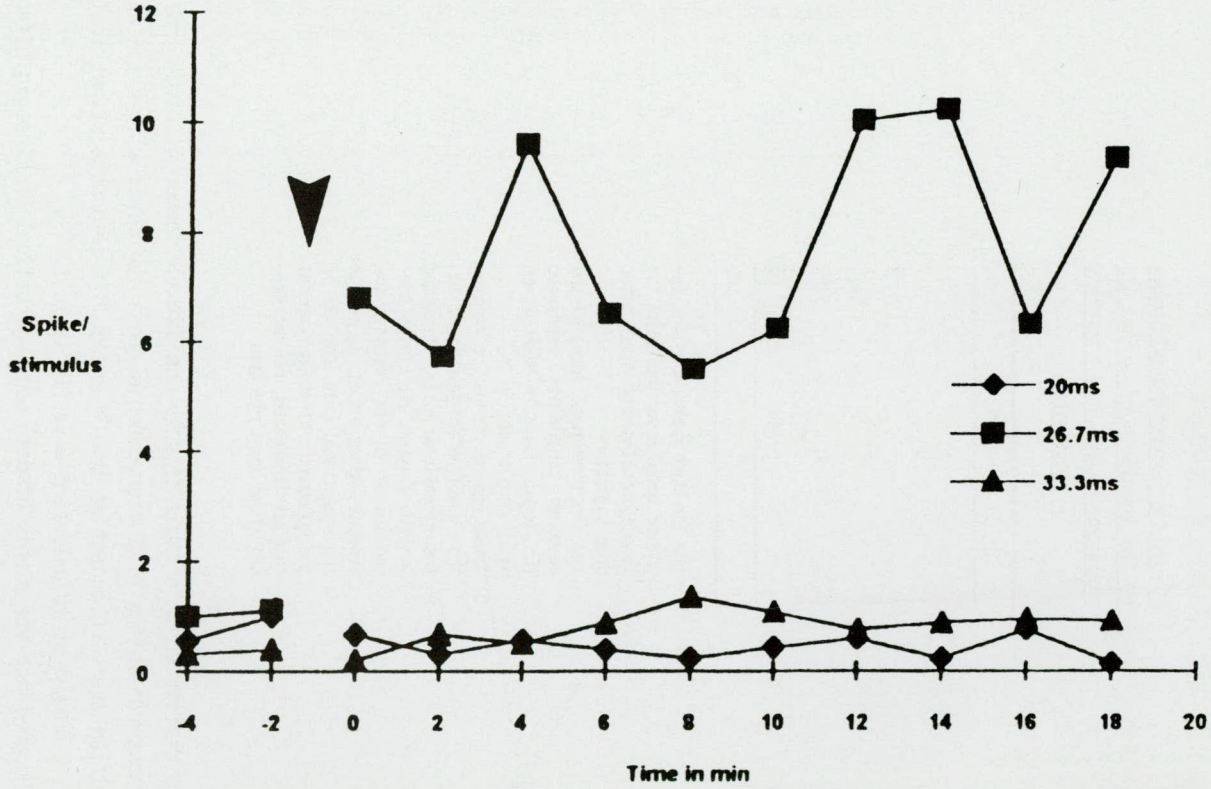

Fig. 1. Acetyl-L-carnitine increased the activity of the second component (with 26.7 ms latency) of the On response 6-8 times compared to the control level, while, the activity of the other two components have not changed at all

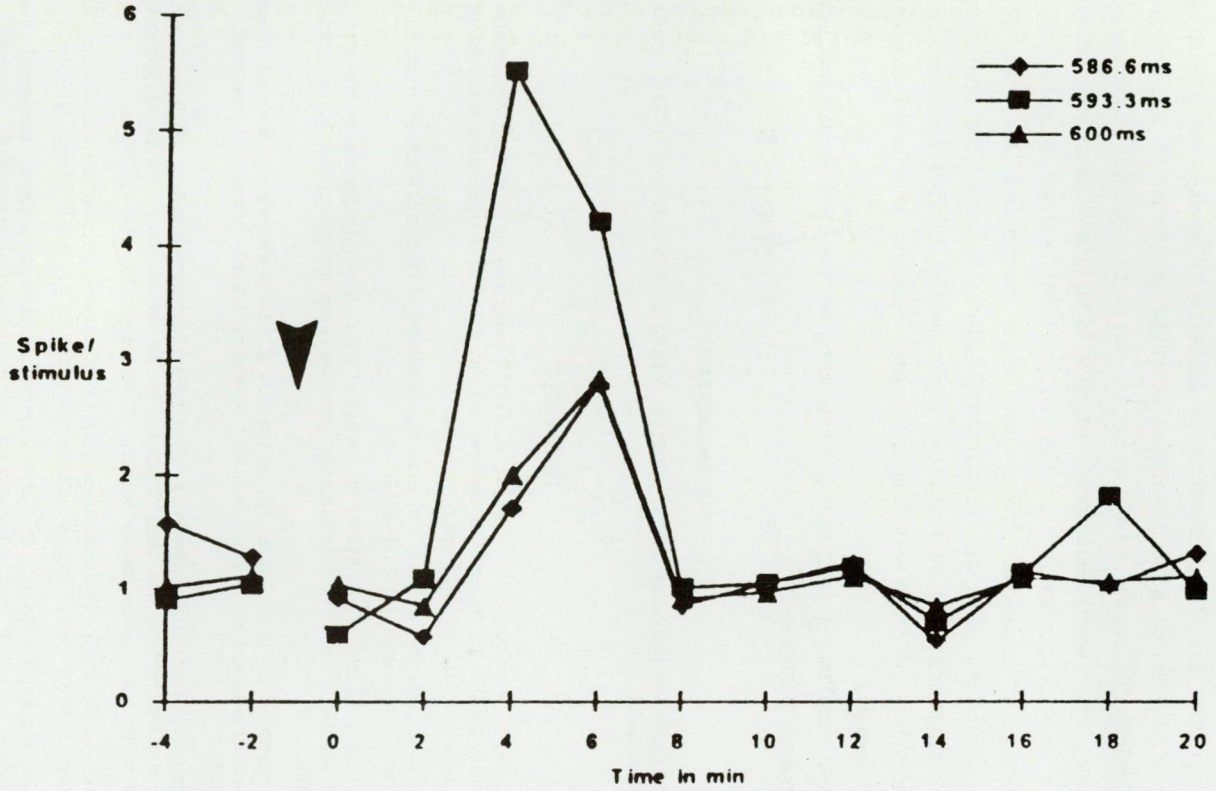

Fig. 2. Carbachol injection increased transiently the activity of all of the tree components of an Off response. This was the exceptional case when all the components of an Off response changed after drug injection

cortical areas of SI. This assumption is assisted by several NBM lesion studies /3, 4/. Decreased stimulus-evoked activity was found in the cholinergically lesioned SI cortex, while metabolic activity remained unchanged /4/. Furthermore, in a recent behavioural study a significant decreased performance in a sensory discrimination task of the NBM lesioned animals was observed because of disrupted sensory information processing /3/. These findings are consistent with our electrophysiological results. The cholinergic drugs developed (in our experiments) their mostly facilitatory action on evoked responses of layer IV-V barrel cortex neurons in a highly latency-dependent manner.

These results may help our understanding on the cholinergic modulation of the sensory processing.

Acknowledgement

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Stimulus-dependent muscarinic effects on evoked unit activity in the rat barrel cortex

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Abstract

The cerebral cortex receives a prominent cholinergic innervation, which is thought to play an important role in the regulation of its normal function. Electrophysiological studies have shown that activation of muscarinic cholinergic receptors results in a marked enhancement of excitatory stimuli onto cortical neurons. In the present study, we examined the effects of acetylcholine (ACh) and its muscarinic agonists (applied by pressure injection) on the response components of individual cortical neurons in layers IV and V of the rat somatosensory cortex in identified barrels (C2 and C3). It was found that the muscarinic agonists could modify the evoked unit activity (in most cases, they caused units to respond to previously minimally effective whisker stimuli), but the modulatory effect was highly dependent on the stimulus parameters, and in most cases, the effect was limited to only one component of 'on' or 'off' responses consisting of 2–4 spikes.

Keywords: Acetylcholine; Muscarinic; Somatosensory cortex; Barrel cortex; Whisker; Pressure injection; Extracellular unit activity

Although acetylcholine (ACh) is acknowledged to be a neurotransmitter in the cerebral cortex, its role in the cortical function is not well understood. The initial data indicated that ACh may be involved in sensory processing [3,5]. Indeed, the subsequent studies have shown that ACh can modify the cortical response to sensory stimulation [9,13]. The cholinergic effect on sensory evoked cortical responses has been studied in detail in the visual system. One group of researchers found that ACh increased the firing rate of cortical neurons only to the optimal visual stimulus [7,12]. Slightly different results were obtained by others, who found increased responses to both optimal and non-optimal visual stimuli [8,11]. Nevertheless, it was generally concluded that ACh improved the signal-to-noise ratio, since the evoked responses were usually larger without any increase in the background [11].

Unfortunately, the effects of ACh on the response properties of somatosensory cortical neurons have not been studied in the same detail as for the visual cortex. Additionally, in some investigations related to changes in cortical gross potentials responses were evoked by non-specific electrical forepaw stimulation [10,17], whereas in other studies, the cutaneous stimulation of the forepaw of cats [6,15,16] or of rats [1] served as somatosensory stimuli, but this incitement could not be accurate enough in all parameters. A unique possibility to study the cholinergic effect on a well-controlled evoked activity in the somatosensory cortex is offered by the vibrissa-barrel pathway [18] of the trigeminal system of the rat.

A total of 36 male and female Sprague–Dawley rats (body weight 200–350 g) were anesthetized with urethane (1.1 g/kg i.p.) and placed in a stereotaxic frame. A left side craniotomy was carried out and microelectrode recordings were made at 0.3 mm intervals over the whole barrelfield to find the barrel related to the stimulated whisker (in most cases one of the vibrissae C1–C3 was stimulated). The whisker was deflected individually by using an electromechanical stimulator. Stimulus waveforms were ramp-and-hold trapezoids that produced 1.2 mm whisker displacements of 300–600 ms duration at 10 mm from the base of the hair. The rise time of the stimulus was 30 ms.

Recording within the appropriate barrel, cells were excited quickly (6–10 ms) and powerfully (1–4 spikes per stimulus onset and/or offset) by deflection of the related

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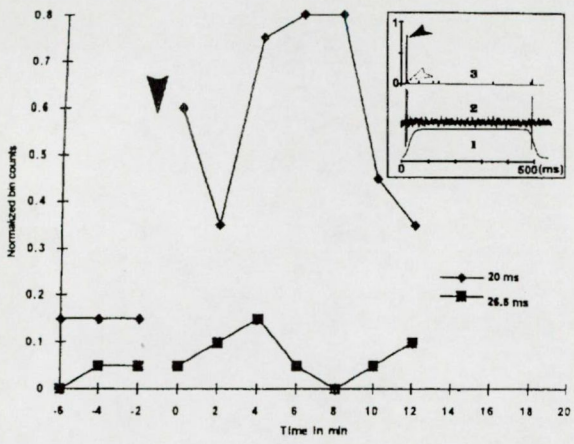

Fig. 1. Effect of ACh on the component with 20 ms latency of an 'on' response. The largest arrowhead indicates drug application. The response component with 26.6 ms latency did not change after drug application. The ordinate here and in Figs. 2–4 is normalized bin counts. Inset, 'on' and 'off' responses (2) to ramp-and-hold trapezoid whisker movement. The duration of whisker displacement was 500 ms (1). The solid arrowhead in the PSTH (3) points to the response component which increased after ACh injection, while the empty arrowhead points to the component (with 26.6 ms latency) which did not change after drug application. The ordinate of PSTH is normalized bin counts. PSTHs in all cases were based on 30 trials, with a bin width of 6.66 ms. The PSTH in inset shows the neuronal responses at 6 min following ACh application.

whisker. Details relating to stimulation and the angular sensitivity of the related cells have been published earlier [14]. To facilitate single-cell isolation, the recording electrode was constructed from a single glass micropipette filled with saline (2.5 M, 5–10 MΩ) and glued to the side of a low-impedance micropipette containing the drug. The distance between the electrode tips was 30–50 μm. The effects of ACh, acetyl-L-carnitine, carbachol, scopolamine and atropine were studied. The drugs were dissolved in sterile saline (0.9%) in 10^{-4}–10^{-3} M concentrations. Before penetration, the optimal pressure parameters (to eject 25–40 pl solution) were determined. It was confirmed several times that pure saline injection in a volume of 25–40 pl did not induce changes in neuronal activity. After unit responses to deflections of vibrissae stable for several minutes had been obtained in cortical layers IV and V, the drug was applied. Peristimulus time histograms (PSTHs; bin width 6.66 ms) were constructed at 2 min intervals, during 4–6 min before and 16–20 min after drug application.

A total of 81 neurons were isolated in the somatosensory cortices of 36 animals. The response latencies of all of these neurons to rectangular vibrissa movements were short (<10 ms), but in this study we used ramp-and-hold trapezoids for vibrissa stimulation (with 30 ms slope), which resulted in a 19–20 ms latency of the first action potential of the 'on' response consisting of 2–4 spikes (inset in Fig. 1). Mainly the neurons in barrels C2 and C3

Table 1

ACh and its agonists induced changes in components of 'on' and 'off' responses evoked by vibrissa movements in preferred angle

On	Off	Percent of cases
+	+	49
+	0	7
+	–	9
0	–	1
–	–	10
0	0	22
0	+[a]	1
+/–[b]	0	1
Total		100

+, one (in some cases at most two) of response components increased for longer duration than 4 min after drug injection; –, one (in some cases at most two) of the response components decreased for longer duration than 4 min after drug injection; 0, there was no change (or shorter than 4 min) after drug injection.

[a]The exceptional case (presented in Fig. 3) when all of the components of an 'off' response increased after drug injection.

[b]The exceptional case (presented in Fig. 4) when all of the components of an 'on' response changed (increased and decreased) after drug injection.

were investigated because the response properties (e.g. the preferred angle of their related vibrissa movement) of the neurons in these barrels are known [14].

At the very beginning of this study, it turned out that ACh and its muscarinic agonists influenced the 'on' and/or 'off' responses of some barrel cortical neurons in different ways (Table 1). However, the data which merely show the percentages of 'on' and/or 'off' responses increasing or decreasing after the application of ACh (or its agonists), do not offer sufficient information. This is specially true if it is taken into consideration that an increase or decrease in the 'on' or 'off' response after drug application is highly dependent on the stimulus parameters (e.g. on the angle of the related whisker movement). It was established earlier that the preferred stimulus, e.g. for the neurons in barrel C3, is the movement of the C3 whisker from its resting position in the caudal-dorsal direction [14]. If the C3 whisker was moved in a preferred

Table 2

Changes of 'on' and 'off' response components of three barrel cortex neurons after drug (ACh, cell 4; cabachol, cell 13; scopolamine, cell 16) application evoked by moving the related whiskers in preferred and reversed directions, respectively

Whisker movement	Cell 4		Cell 13		Cell 16	
	On	Off	On	Off	On	Off
In preferred direction	↑↓	↑	↑	↑	0	↓
In reversed direction	↓	↓	↑	↑	0	↑

↑, one of the response components increased after drug application; ↓, one of the response components decreased after drug application; 0, there was no change in the response after drug application.

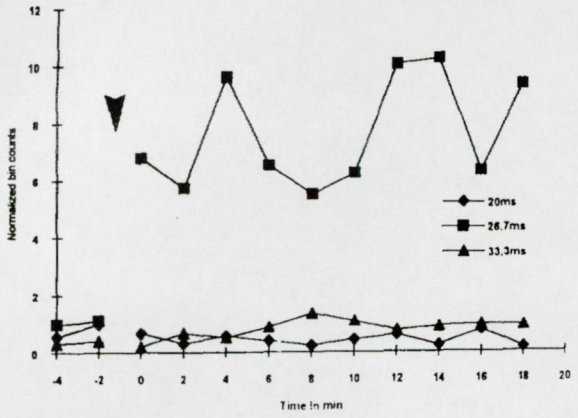

Fig. 2. Acetyl-L-carnitine increased the activity of the second component (with a 26.7 ms latency) of the 'on' response to 6–8 times the control level, while the activities of the other two components did not change at all. Arrowhead indicates the drug application.

direction, in more than 50% of the studied neuronal responses, it resulted in quite different changes in the 'on' and 'off' responses after drug application from those observed with stimulation in the reversed direction. Table 2 shows examples of this phenomenon. While both of the 'on' and 'off' responses of neuron 13 increased after drug application, independently of the direction of whisker movement, in the majority of neuronal responses, as shown in cells 4 and 16, the changes in response after drug application depended highly on the direction of whisker movement.

More detailed and precise information is offered by analyzing the changes in response components with different latencies, evoked by stimulation with optimal parameters. These studies showed that the drugs generally modify only one of the components within an 'on' or 'off' response. In our first example (this was the most predominant type), a very weak if any evoked response could be observed before ACh application (Fig. 1, see the ordinate). However, ACh injection resulted in an elevation of only the first of the two components of the 'on' response, while the level of activity of the component with 26.6 ms latency did not change (Fig. 1). In all those cases when ACh or its agonists increased a component of the 'on' and/or 'off' responses (altogether in 67%, see Table 1), this kind of effect of drugs could be observed. Another example is shown in Fig. 2 observed after acetyl-L-carnitine application. In that example, acetyl-L-carnitine injection induced an increase (to 6–8 times the control level) only in the second component of the 'on' response (with a 26.7 ms latency), while the first and third components remained unchanged (Fig. 2).

In most experiments, we observed that ACh and its muscarinic agonists induced units to respond stronger to previously minimally effective whisker stimuli (even stimulating with optimal parameters), and most frequently we found an increase in only one of the 2–4 components of the 'on' or 'off' responses (Figs. 1 and 2). Antagonists generally decreased only one component of evoked responses while they had hardly any effect on the background activity of the neurons (not shown here). There were two exceptional cases, when carbachol influenced all of the components of the 'on' or 'off' responses either in the same way (Fig. 3) or in different ways (Fig. 4).

The present study shows that: (1) ACh and its muscarinic agonists can modify the unit activity evoked in the barrel cortex of the rat, (2) the modulatory effects of these drugs are highly dependent on the stimulus parameters, and (3) in most cases, ACh and its muscarinic agonists modify only one component (having a precise latency) of an 'on' or 'off' response consisting of 2–4 spikes.

Observation (1) suggests that ACh and its muscarinic agonists exert a direct influence on the processing of so-

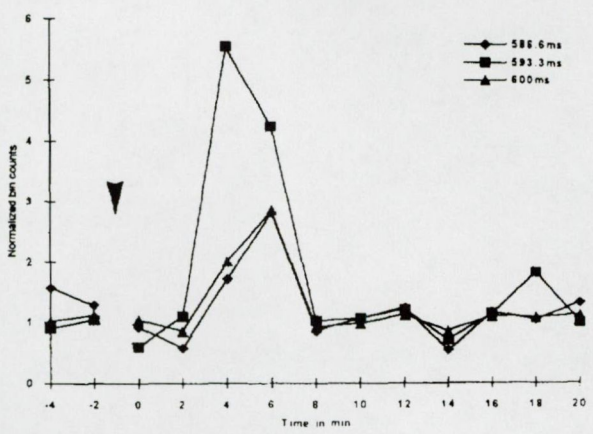

Fig. 3. Carbachol injection transiently increased the activities of all three components of an 'off' response. This was the exceptional case when all the components of an 'off' response changed after drug injection. Arrowhead indicates the drug application.

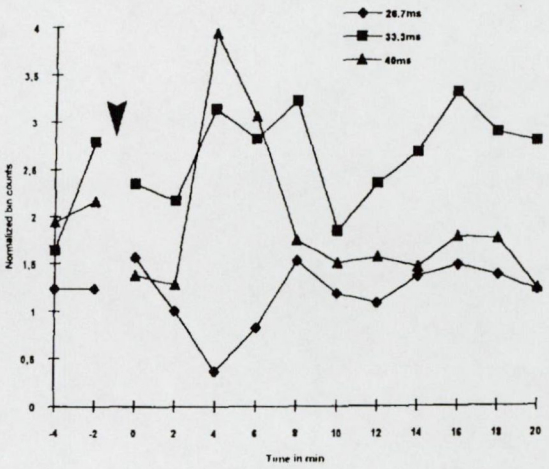

Fig. 4. The activity of the component with the shortest latency transiently decreased, while those of the two components with longer latencies increased after aspecific agonist carbachol injection. This was the exceptional case when all the components of an 'on' response changed after drug injection. Arrowhead indicates the drug application.

matosensory information in the barrel cortex of the primary somatosensory area of rat. This is in accordance with recent morphological and physiological observations which show that afferents from the nucleus basalis Meynert (NBM) to the somatosensory cortex are mostly cholinergic in the rat [2] and with the fact that NBM activation produces long lasting, cholinergically mediated alterations in the response properties of somatosensory cortical neurones [4].

Metherate et al. [6] found that ACh enhances only some response properties of a cell selectively rather than acting as a general excitant. Observations (2) and (3) are in agreement with this idea. The finding that muscarinic agonists and antagonists in most cases modify only one component of an 'on' or 'off' response (consisting of 2–4 spikes) suggests that ACh acts in only a very limited number of synapses of the intracortical network processing the actual somatosensory information within the barrel concerned.

All these observations indicate that ACh affects only specific components of the response circuitry which is not known in detail.

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Does the cortical representation of body parts follow both injury to the related sensory peripheral nerve and its regeneration?

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A study was made of the borderline between the physiological representations of the digits (D2, D3 and D4) and sinus whiskers in the rat primary somatosensory cortex after a contralateral infraorbital nerve crush. Following the injury, the physiological representation of the digits of the contralateral forepaw extended posterolaterally, occupying the anterolateral part of the whisker region (posteromedial barrel subfield). The extended physiological representation of the digits, though somewhat shrunken, remained after the reappearance of whisker-evoked responses, forming an overlapping area between the obligate digit and whisker representations. The findings emphasize the importance of afferent inputs in modulating cortical organization, but show that a reversible change in a sensory input (nerve damage) does not result in a perfectly reversible change in cortical representation.

Key words: Barrel field; Nerve crush; Sensory denervation; Somatosensory cortex; Somatotopic remapping; Trigeminal system

Introduction

The forepaw representation in the rat primary somatosensory cortex (SI) is organized topographically.[1–3] Waters and colleagues described the details of the organization of the forepaw representation and suggested that individual barrels within the forepaw barrel subfield (FBS) receive input from definite regions of the forepaw, forming a well-defined somatotopy.[3] A similar very precise structural and functional representation of the mystacial vibrissae also exists in the posteromedial barrel subfield (PMBSF) of the rat.[4,5] Despite the strict cortical representation of the body surface on the SI, a significant capacity to undergo functional changes in response to alterations of sensory input remains, even in the adult cortex.[6–9] Over the past few years, postontogenic functional plasticity has been observed in the somatosensory and motor cortices in adult animals of different species after circumscribed damage to peripheral receptor populations of the skin. In general, it was found that the input- or output-deprived cortical areas are occupied by the neighbouring sensory or motor representational fields immediately or days or weeks after denervation in both somatosensory and motor cortices.[7,8,10–12]

Much less is known about the central consequences of peripheral nerve regeneration following injury. A recent study indicated the existence of mechanisms in the developing brain that can create orderly cortical topography despite disordered sensory inputs.[13] Here we present electrophysiological evidence indicating that changes in the somatotopic map of the SI appear early after a nerve crush. However, we also demonstrate that entire reorganization in cortical topography does not take place after nerve regeneration in adult animals.

Materials and Methods

Animal preparation: In addition to intact adult Sprague–Dawley rats ($n = 2$, age > 70 days), litter mates ($n = 9$) in which the right infraorbital nerve was damaged by a crush where it exits the infraorbital foramen were studied. The rats were anaesthetized with Ketavet and Rompun (50 mg kg^{-1}, 4 mg kg^{-1}, i.p., respectively). The crush was performed by compressing the nerve with straight tweezers for 10 s. Cortical mapping was carried out immediately or 3, 7, 8, 10, 11, 12, 13 or 18 days after the nerve crush.

The hair over the skull was shaved. The head was secured in a stereotaxic frame (Kopf Instruments) throughout surgery, stimulation and mapping. Craniotomy was performed over the left hemisphere, extending from 2 mm anterior to 4 mm posterior to the bregma, and from 2.5 mm to 7 mm laterally of the midline. The dura mater was removed and the body temperature was maintained at 37.5°C with an automatic heating system.

Physiological stimulation and mapping procedure: The digits (D2, D3 and D4) of the right forepaw and the right side whiskers were stimulated with a fine

brush moved by an electromechanical stimulator (1 Hz, duration 500 ms).[14,15] Such stimulation resulted in evoked (on/off) responses in the corresponding cortical representation areas of these body parts. The mapping procedures were similar to those described by Li *et al.*[16] and were focused on to defining the border between the representation areas of the digits and whiskers. In order to accomplish this, we made closely spaced recordings of evoked responses from the cortical surface, performed electrode penetrations into the SI and recorded unit activities at a depth of 600–800 μm, since this depth has been shown to contain the barrel-associated neurons within the FBS[3] and the PMBSF.[4,5] Glass micropipettes (2–3 μm diameter) filled with 2.5 M NaCl (impedance 10–15 MΩ) were used to record unit and multiunit responses. Electrode penetrations were spaced at approximately 300–500 μm. Cortical maps were constructed on schemes (see Fig. 1) by marking the anteroposterior (p) and mediolateral (l) positions of recording points. All maps of recordings completely encompassed the parts of the SI which are the representation areas of the digits (D) and whiskers (W). Here we focus on the borderline between them, which was drawn midway between the distinct penetration sites associated with whiskers only and those exclusively related to digits (Fig. 1A). However, to define the boundary between the pure zones was possible only in control animals. After the infraorbital nerve damage, the cortical representation of the digits invaded the anterior part of the PMBSF, resulting in the appearance of an overlapping zone of D and W regions following the nerve regeneration (for details see below). In these animals, the posterolateral borderline of the representation of the digits was defined.

All procedures were carried out in strict accordance with the NIH Guide for the Care and Use of Laboratory Animals (Revised 1987).

Results

Digit representation in the rat SI is somatotopically organized: digits D2–D5 are organized into bands running in a medial to lateral direction.[2,3] Here we define not the detailed representation of the digits, but the posterior ends of the bands corresponding to the D2–D4 digits. This is the borderline between the somatosensory areas of the digits (D) and whiskers (W) (see control in Fig. 1A). The responses evoked by vibrissa stimulation disappeared immediately from the barrel cortex in animals whose infraorbital nerve was crushed. As the most striking effect of the infraorbital nerve damage, stimulation of the contralateral forepaw digits (D2, D3 and D4), which normally evoked cortical responses exclusively in their own cortical representation areas, also resulted in responses in the anterior part of the vibrissa-associated PMBSF. In other words, the border of the region of the digits shifted posteriorly. This phenomenon was observed minutes after the crush and was the most pronounced on day 3 post-crush (Fig. 1B). We tested the extension of the cortical representation of the digits and whiskers systematically for 18 days following the nerve crush. In spite of the reappearance of whisker-evoked responses 7–10 days after the nerve crush, an expanded digit representation could still be observed (Fig. 1C–E). A tendency to withdrawal might exist, but we did not find this tendency to be significant and never to be complete. The expansion of the representation of the digits resulted in an overlapping zone between the pure digit and the whisker cortical areas after recovery from the nerve injury. In this overlapping area, several recording sites were encountered where responses to both digit and whisker stimulation could be evoked (Fig. 1E).

Similarly, overlapping areas were created in experiments when picrotoxin was applied to the barrel cortex. Covering the cortical surface with a piece of paper soaked in a 10^{-4} M solution of picrotoxin resulted in changes which resembled those observed after an infraorbital nerve crush followed by regeneration; cortical responses to both whisker and digit stimulation could be evoked. In addition, silent cortical points were found in the overlapping areas. At these points, responses could not be evoked by either digit or whisker stimulation.

Discussion

Temporal infraorbital nerve damage (crush) not only resulted in the transient disappearance of the contralateral whisker-evoked responses from the barrel cortex, but also produced an expansion of the neighbouring representation area of the digits of the contralateral forepaw. A similar expansion of the somatotopic representation of the remaining digits was reported after partial digit amputation in flying foxes.[8] Denervation-induced plastic changes in the neocortex have been demonstrated not only in the somatosensory, but also in the motor cortex. Neurophysiological studies show that somatotopic representation maps in the primary motor cortex (MI) are also modifiable as a consequence of a peripheral nerve (facial) lesion during development[17] and also in adult animals.[10,12] In support of the electrophysiological results, morphological evidence suggests that synaptic reorganization in the facial nucleus begins within 1 h after facial nerve transection.[18,19] Our observations are in accordance with the findings of Calford and co-workers, who reported a similar type of immediate change in cortico-cortical

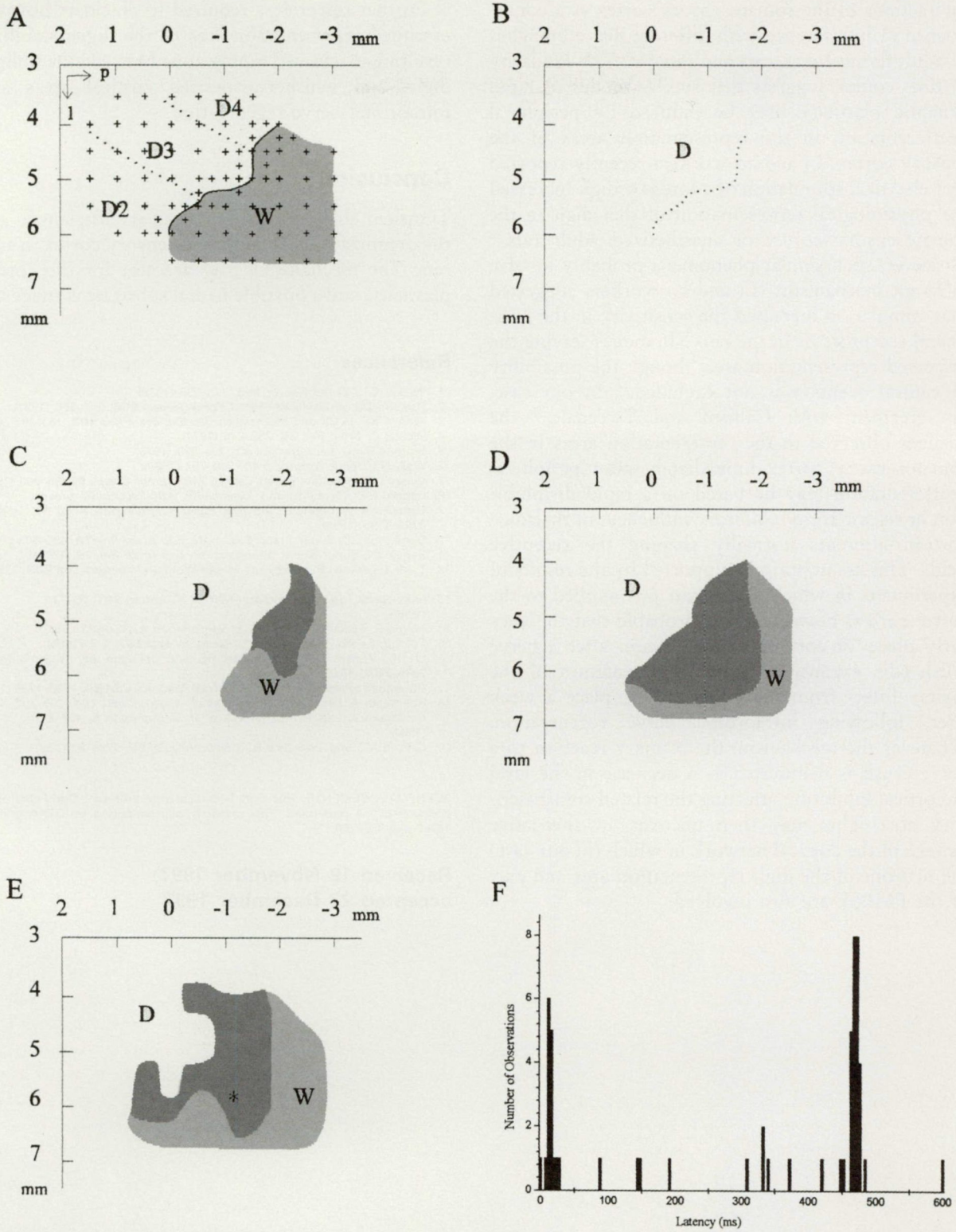

FIG. 1. The border between the physiological representation of digits 2, 3 and 4 (D2, D3, D4) and whiskers (W) in the left primary somatosensory cortex (SI) of adult rats. These maps contain only parts of the digit and whisker representations, focusing on the borderline between them. Zero on the horizontal scale represents the bregma. (**A**) The common border of the two areas, marked with a thick line, is clearly identifiable in control animals. Crosses sign individual recording sites tested throughout the experiments. (**B**) Three days after a right infraorbital nerve crush, the somatotopic representation of the digits is expanded posterolaterally, while whisker stimulation does not evoke a cortical response. The dotted line denotes the original borderline. (**C–E**) Following infraorbital nerve regeneration, whisker-related activation gradually reoccupies parts of the corresponding cortical area, while the representation of the digits remains expanded, resulting in a bimodal area marked with the darkest shading as was found in animals tested 8, 11 and 18 days after crush, respectively. (**F**) Post-stimulus time histogram of unit activity recorded in the bimodal overlapping area during forelimb stimulation, averaging 30 episodes of on/off stimuli. The recording site, originally a whisker-related point, is indicated by an asterisk in (E).

interactions of the somatosensory cortex as a consequence of interference with different nerve branches of adult flying-foxes, cats and rats.[7,8,20] The similarity in time course suggests that similar modes of rapid synaptic plasticity may be induced by peripheral deafferentation in the representation areas of the sensory cortex. Li and co-workers recently reported that electrical stimulation of a forepaw digit increased the physiological representation of that digit in the somatosensory cortex of anasthetized adult rats.[16] However, such similar phenomena probably involve different mechanisms. Li and co-workers suggested that stimulation increased the sensitivity in the peripheral receptors or in the nerve branches serving the increased representation area, though the possibility of central events was not excluded.[16] In our case, in agreement with Calford and Tweedale,[20] the changes observed in the representation areas in the somatosensory cortex immediately after peripheral deafferentation may be based on a rapid disinhibition or release from inhibitory influences of thalamocortical afferents normally shaping the receptive fields. This assumption is supported by the results of experiments in which picrotoxin was applied to the barrel cortex. However, it is probable that the very early phase of cortical reorganization after a nerve crush (the expansion of the representation of the digits) differs from the events taking place a week later, following infraorbital nerve regeneration. Whatever the mechanism, the primary reaction to a nerve crush is manifested as a decrease in the level of cortical inhibition affecting the related somatosensory area. This may then uncover an alternative pattern of the cortical network in which (in our case) the neurons of the digit representation area and part of the PMBSF are also involved.

Further research is required to elucidate how this extended representation area of the digits stabilizes, resulting in an overlapping zone between the obligate digit- and whisker-associated cortical areas after infraorbital nerve regeneration.

Conclusion

Transient elimination of an input is sufficient to alter the organization of the somatosensory cortex in adult rats. The mechanisms may account for the cortical plasticity, and a possible neural substrate is suggested.

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RESEARCH ARTICLE

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Comparative study of the neuronal plasticity along the neuraxis of the vibrissal sensory system of adult rat following unilateral infraorbital nerve damage and subsequent regeneration

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Abstract The aim of the present study was to examine the physiological consequences of a unilateral infraorbital nerve lesion and its regeneration at different levels of the somatosensory neuraxis. In animals whose right infraorbital nerve had been crushed, a large unresponsive area was found in the main brainstem trigeminal nucleus (Pr5). Responses evoked by ipsilateral vibrissal deflection in the middle of Pr5 reappeared only on days 22–35 after the nerve had been transected, whereas recovery from the nerve crush took only 7–9 days. However, no sign of short-term neuronal plasticity was observed in Pr5 after peripheral nerve injury. An enlargement of the receptive fields in two-thirds of the units and a lengthening in the delay of the evoked responses were observed as long-term plastic changes in Pr5 neurons after peripheral-nerve regeneration. In the ventral posteromedial nucleus of the thalamus (VPM) of partly denervated animals, however, only minutes or hours after the nerve crush, certain units were found to respond in some cases not only to the vibrissae, but also to mechanical stimulation of the face over the eye (two units), the nose (one unit), and the midline (one unit). Apart from the experiments involving incomplete denervation, the vibrissal representation areas of the VPM were unresponsive to stimulation of both the vibrissae and other parts of the face until nerve regeneration had occurred. In the somatosensory cortex, an infraorbital nerve crush immediately resulted in a large cortical area being unresponsive to vibrissal deflection. It was noteworthy, however, that shortly after the nerve crush, this large unresponsive whisker representation cortical area was invaded from the rostromedial direction by responses evoked by stimulation of the forepaw digits. In spite of the reappearance of vibrissa-evoked responses 7–10 days after the nerve crush, an expanded digital representation could still be observed 3 weeks after the nerve crush, resulting in an overlapping area of digital and vibrissal representations. The withdrawal of the expanded representation of forepaw digits was completed by 60 days after the nerve crush. The results obtained in Pr5, the VPM, and the cortex strongly suggest that the higher the station in the neuraxis, the greater the degree of plasticity after infraorbital nerve injury.

Key words Neuronal plasticity · Trigeminal system · Somatosensory cortex · Nerve injury · Nerve regeneration

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Introduction

At one time, neuroscientists believed that the sensory systems in adults were stable, in contrast to the extensive and pervasive plasticity characterizing the development of the nervous system. Plastic changes in the primary somatosensory cortex (SI) were first detected in nerve-injury studies, where remapping of the remaining active inputs was observed (Rasmusson 1982; Kaas et al. 1984). In the past decade, numerous studies have demonstrated that the adult nervous system in fact possesses a pronounced plasticity (Calford and Tweedale 1988; Calford et al. 1996).

Lesions in the rat trigeminal system have become an important model for the study of central nervous system (CNS) pattern generation and plasticity (Klein et al. 1988; Kossut 1992; Hoeflinger et al. 1995; Jacquin et al. 1995). While denervation of the trigeminal system and its neuroplastic effects have been studied extensively (Chiaia et al. 1995; Lane et al. 1995), much less has been done to facilitate an understanding of its regeneration and of the effectiveness and quality of nerve repair. It is well demonstrated, for example, that an infraorbital nerve (ION) transection results in a rearrangement of the neuronal responses in the trigeminal system (Waite 1984). However, little is known about what happens con-

cerning the rearranged afferent neuronal activity or the plasticity induced by nerve injury following nerve regeneration. What are the consequences of peripheral-nerve regeneration regarding the functioning of related structures in response to the plastic changes induced in the CNS by peripheral nerve damage? To what extent does the rearrangement correspond to the normal innervation pattern? Relatively few studies have dealt with such questions (Arvidsson and Johansson 1988; Wall 1988; Melzer and Smith 1995, 1998; Florence et al. 1996).

In one of the most important early studies on neuronal plasticity induced by denervation, permanent transection of the sensory nerve to the vibrissae, the ION, was carried out, with regeneration being prevented. It was found that, in animals denervated as adults, no evidence of plasticity could be detected in the trigeminal nuclei and only a very limited reaction was apparent in the cortex (Waite 1984). Other results demonstrated that the receptive-field (RF) properties of the neurons of the ventral posteromedial nucleus of the thalamus (VPM) did not change immediately after the destruction of their input: only a gradual functional reorganization of the VPM was found (Rhoades et al. 1987). However, during the past decade, evidence has accumulated showing that partial sensory deafferentation generally results in responses to other stimuli, usually those engaging the sensory epithelium adjacent to the site of the lesion. Novel responses, often expanded RFs, are frequently observed immediately after denervation in the SI (Calford and Tweedale 1988; Byrne and Calford 1991; Calford et al. 1996), at a subcortical level (Nicolelis et al. 1993), or in both (Faggin et al. 1997).

Such controversies in the literature initiated the present comparative study on denervation-induced plasticity at three levels of the trigeminal neuraxis. Furthermore, it seemed to be interesting to examine the consequences of peripheral nerve regeneration on the functioning of the related structures or on the plastic changes induced in the CNS by the peripheral nerve damage.

The aim of the present study was to apply electrophysiological methods in the examination of both the short- and long-term consequences of a unilateral ION lesion and its regeneration at different levels of the related structures. The analyses were carried out at three levels of the trigeminal sensory system: in the brainstem trigeminal principal sensory nucleus (Pr5), in the VPM, and in the barrel cortex, following transection or crush of the right ION. The electrophysiological studies were carried out at different postoperative intervals: minutes or hours after denervation (P0) up to a 60-day delay (P60). In this study, we attempted to identify: (1) both the immediate and the long-term changes in the RFs of units in the above structures after denervation and during the subsequent regeneration, (2) the time needed for functional recovery of the related structures (the reappearance of an evoked response) after ION transection or crush, (3) the changes in some of the electrophysiological response parameters (e.g., latency) during ION regeneration. The main goal of the study was furthermore: (4) to establish whether the plastic changes induced by ION injury persist after functional recovery of the ION or whether they disappear as recovery is completed. Finally, an investigation was made of: (5) the abilities of structures at different levels of the trigeminal neuraxis to display neuronal plasticity.

Materials and methods

The surgical procedures used in this study followed the protocol for animal care approved by both the Hungarian Health Committee and international guidelines. Studies were made on intact Sprague-Dawley rats aged 60–100 days. A total of 98 animals were anesthetized with an i.p. injection of a mixture of Ketavet and Rompun (10.0 mg/100 g and 0.8 mg/100 g, respectively) and the right ION was exposed where it exits the infraorbital fissure. In 56 rats, the ION was crushed with fine tweezers (compressed for 10 s); in 18 littermates, the ION was transected. After denervation, the wound was closed and the animals were subjected to electrophysiological study (P0) or allowed to survive for at least 3 (P3) or at most 60 days (P60) before electrophysiological data were collected. Sham-operated littermates (n=24) served as controls. The groups and subgroups of this study are detailed in Table 1. Previously described (Toldi et al. 1994) extracellular recording, electromechanical vibrissa stimulation, and RF mapping procedures were employed to assess the responses of cells in the Pr5, the VPM, and the barrel cortex. Briefly, the deafferented or normal rats were anesthetized as described above. They were secured in a stereotaxic headholder (David Kopf Instruments) that provided access to Pr5 and VPM, or the barrel cortex was exposed. After surgery, the animals were kept at rest for 1 h. The core temperature was maintained at 37°C. Individual whiskers were deflected by using a multiangle electromechanical stimulator (Toldi et al. 1990). Before vibrissal stimulation, all of the vibrissae on the right side were cut to a length of 15 mm. The arm of the stimulator was attached to a vibrissa 10 mm from the base of the hair. The stimulus waveforms were ramp-and-hold trapezoids that produced 1.2 mm vibrissal displacements of 500 ms duration. The slope was 20 ms. In this work, the latencies were calculated by subtracting the slope from the measured latency values. After the correct recording site had been found (each vibrissa bend evoked vigorous "on-off" activities, with 1 or 2 spikes as an "on" response), the RF studies were performed. The vibrissae were stimulated one by one in rows A-E, and this was followed by mechanical stimulation of the fur on the face above the eye, the dorsal nose, the midline, or the skin of the forepaw digits, and in some cases electrical stimulation of the inside of the mouth. Not all these sites were stimulated in each experiment. Mechanical stimuli were delivered 20 times in a rostral-caudal direction (Toldi et al. 1994). Recording sites were encountered with the aid of a stereotaxic atlas (Paxinos and Watson 1982). Recording sites in Pr5, the VPM, and the cortex were controlled histologically. The animals were deeply anesthetized with an overdose of the xylazine/ketamine mixture and were transcardially perfused with 50 ml of physiological salt solution, followed by 300 ml of a 10% formalin fixative (4% formaldehyde in phosphate buffer). Parallel series of 10-μm coronal sections of the paraffin-embedded thalamus and cortex were made, and sagittal sections of the 50-μm thick brainstem were cut with a vibratome. Sections were stained with cresyl-echteviolet (Nissle-stained). Unit discharges were recorded extracellularly either with glass micropipettes filled with 2.5 M NaCl (impedance 15–20 MΩ), or with extrasharp tungsten electrodes (12 MΩ, 8°, A-M Systems). Electrodes were advanced in 3- to 5-μm steps by means of a Narishige hydraulic micromanipulator. The signals were fed into a differential amplifier with 50 Hz lower and 5 kHz upper frequency limits and were visualized on a Tektronix storage oscilloscope. The amplified unit responses were fed into a computer system via an interface (Digidata 1200, pClamp 6.0.4. software, Axon Instruments) and stored for further processing. Peri-

Table 1 The experimental groups in this study were as follows: a total of 98 animals were subjected to experiments in three main groups: (1) *Controls* (sham-operated), (2) *Crush* [infraorbital nerve (*ION*) completely or incompletely crushed], and (3) *Cut* (ION transected). Electrophysiological recordings were made in three structures: in the principal sensory trigeminal nucleus (*Pr5*), in the ventral posteromedial nucleus of the thalamus (*VPM*), or in the primary somatosensory cortex (*SI*, barrel-cortex). Electrophysiological studies started just after nerve injury (*P0*) or 3–60 days later (*P3–P60*)

No. in main groups	Controls (sham op.)	Crush	Cut	Total
	24	56	18	98
Recording sites				
Pr5	6	14 5 (P0) 1 (P6) 8 (P7–P60)	18 4 (P0) 4 (P7-P9) 10 (P15- P35)	38
VPM	12	26 20 complete ION crush: 3 (P0) 6 (P3-P6) 4 (P7-P10) 7 (P30–P50) 6 incomplete ION crush: 3 (P0) 2 (P23) 1 (P27)	–	38
SI	6	16 6 (P0) 4 (P3-P18) 6 (P60)	–	22

stimulus time histograms (PSTHs, bin width: 1.4 ms) were produced from registrations, each containing 50 trials.

Results

Response properties in principal sensory trigeminal nucleus

In order to assess the plastic changes induced along the neuraxis by ION injury and the following regeneration, recordings were made at the first synaptic station of the pathway in Pr5 of the trigeminal nuclear complex both in intact and in ION-injured animals (transected or crushed on the right side) and, later, during recovery.

Control animals

Thirty-eight cells in Pr5 were recorded in six control rats (Figs. 1 and 2, control column). The majority of the cells (82%) were not spontaneously active and most (68%) were adapting rapidly (Fig. 2). The response latencies of the cells in Pr5 were as follows: to 0° (deflection of vibrissae in the caudal direction): 3.28±1.17 (SD) ms; and to 180° (deflection of vibrissae in the rostral direction): 3.28±1.02 ms (Table 2). In most of the animals, 82% of the cells had RFs localized to only one vibrissa.

Infraorbital nerve-injured animals

In 18 animals, the right ION was transected; in 14 rats, it was crushed. Both types of injury resulted in a large unresponsive area in Pr5: ipsilateral vibrissal stimulation evoked no response in Pr5. Responses could be evoked only by stimulation of the lower jaw (dorsal part of Pr5) and, in a very limited ventral part of Pr5, by stimulation of the nose and cheek (Fig. 1A). However, responses were never found in the middle of Pr5 (in the vibrissal representation area) following stimulation of either vibrissae or these body parts. A difference between transected and crushed preparations was observed only in the duration of recovery. After ION transection, responses evoked in the middle of Pr5 by ipsilateral vibrissal deflection reappeared only on days P22–35, while the recovery from the ION crush took only 7–9 days. In consequence of the shorter recovery time and the lower degree of individual variation, mainly the crush injury was applied in further experiments. The response latencies were much longer in the ION-crushed or -transected and recovered animals (to 0°: 6.19±2.2 ms; and to 180°: 6.13±2.39 ms) than in the controls (Fig. 2 and Table 2).

The response properties (RF and latency) in Pr5 of denervated and recovered animals were followed up to P60, but only slight differences were observed in these parameters. We never found changes in RFs of Pr5 neurons shortly after ION cut or crush. A somewhat increased number of Pr neurons (11 of 37) responded to stimulation of more than one whisker (29.7%, in contrast to the 18% observed in the controls) after ION recovery. The response latencies became somewhat shorter with increasing postoperative time (the shortest latency found in a denervated and regenerating animal was 4.1 ms), but, because of the high standard deviations of the means at each examined postoperative time, the differences in latency decrease during recovery did not prove significant.

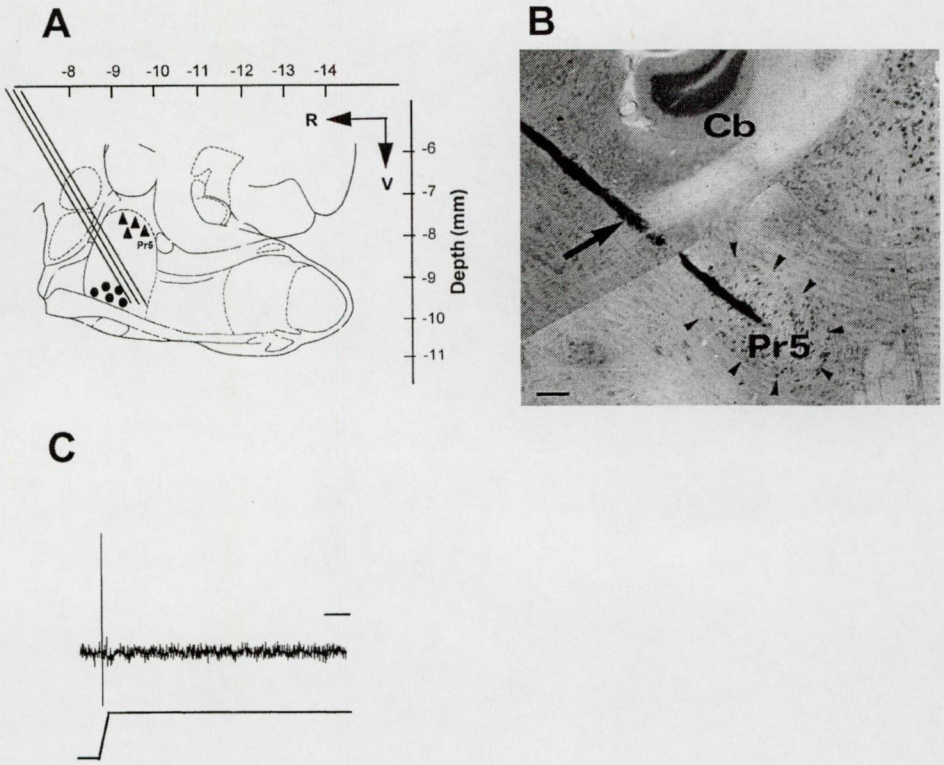

Fig. 1A–C Somatosensorily evoked responses in the principal sensory trigeminal nucleus (*Pr5*). **A** The Pr5 in stereotaxic coordinates according to the atlas of Paxinos and Watson (1982). Electrode tracks at the angle used in the experiments are shown. Penetrations were made in a matrix; 14–16 penetrations were obtained in an animal. *Triangles:* Dorsal part of Pr5 where the lower jaw-evoked responses are localized, *Circles:* cheek and nose representation in Pr5. Vibrissa-evoked responses localized in the central part of Pr5. *R* Rostral, *V* ventral. **B** Histological verification of the position of a recording electrode. The border of Pr5 is labeled with *arrowheads*. The *large arrow* points to the electrode track. *Cb* A small ventral part of the cerebellum. Calibration: 500 µm. **C** Response of a Pr5 neuron evoked by ipsilateral vibrissal deflection. Calibration: 20 ms

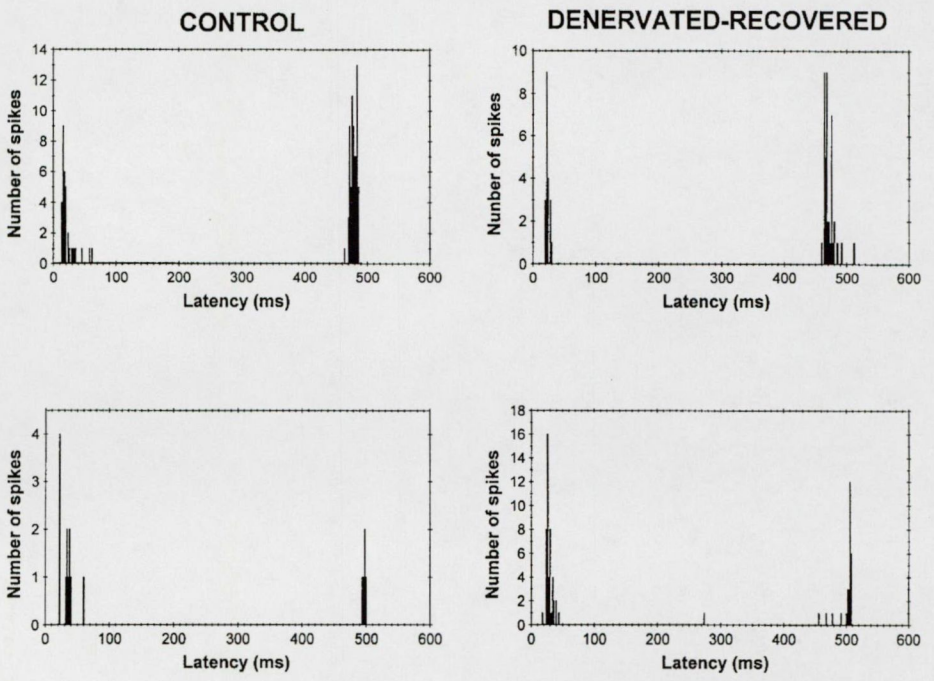

Fig. 2 Peristimulus time histograms (PSTHs) of evoked activities of principal sensory trigeminal nucleus (Pr5) neurons observed in controls (1st column) and in denervated-recovered animals (2nd column). The number of evoked spikes in the PSTHs in the Pr5 neurons was not high because of the rapid adaptation

Response properties in ventral posteromedial thalamic nucleus

Recordings were made on 64 vibrissa-responsive neurons in the VPM of 12 control animals and on 72 vibrissa-evoked unit activities from 26 rats during their recovery from the contralateral ION crush.

Control animals

Evoked activities both in controls and in ION-crushed and recovered animals were mapped systematically in the VPM. The distances between electrode tracks were 150–200 µm (Fig. 3A,B). In intact rats, the majority (~78%) of the isolated VPM cells were activated by only one vibrissa. Only 15 cells (~23%) were sensitive to

Table 2 Mean latencies (±SD) of responses evoked in the principal sensory trigeminal nucleus (*Pr5*), the ventral posteromedial nucleus of the thalamus (*VPM*), and the primary somatosensory cortex (*SI*) by mechanical vibrissa deflection in the caudal (0°) and the rostral (180°) direction. Differences between mean latencies of controls and of animals with the infraorbital nerve (*ION*) regenerated are all significant at levels of 0.03 (Pr5) and 0.01 (VPM, SI)

Direction	Controls		Denervated-recovered	
	0°	180°	0°	180°
Structure				
Pr5	3.28±1.17	3.28±1.02	6.19±2.21	6.13±2.39
VPM	7.30±1.31	8.34±3.06	Complete ION crush: 21.75±10.84 Incomplete ION crush: 14.51±11.30	Complete ION crush: 18.84±7.11 Incomplete ION crush: 15.10±10.10
SI	16.31±12.35	–	36.31±23.09	–

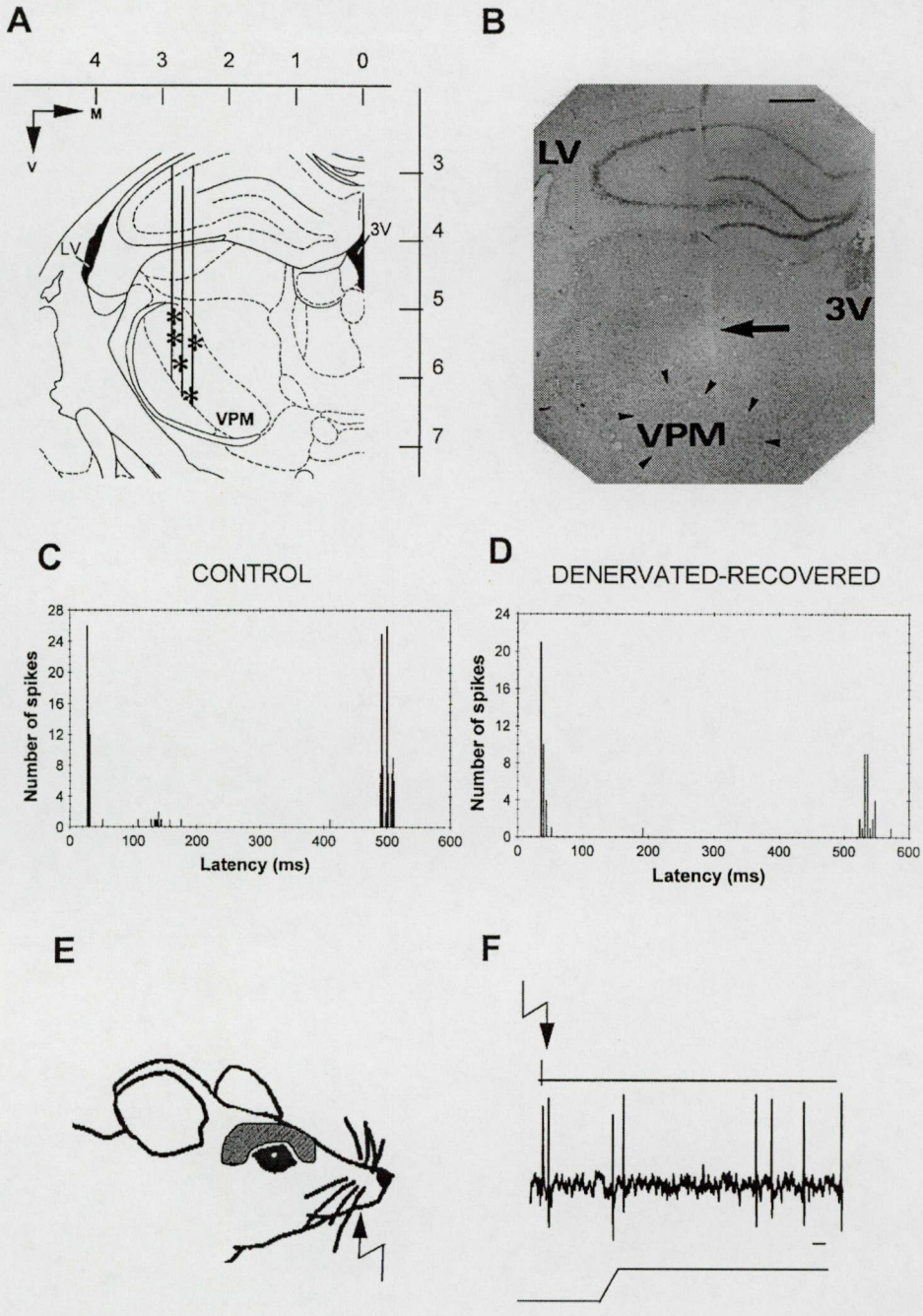

Fig. 3A–F Stimulation sites on the face and evoked responses in the ventral posteromedial nucleus of the thalamus (*VPM*) of the rat. **A** The VPM in stereotaxic coordinates according to the atlas of Paxinos and Watson (1982). *Parallel lines* show the electrode tracks used in the experiments (16–18 penetrations per animals were obtained in a matrix). *LV* Lateral ventricle, *3V* third ventricle. **B** Histological verification of the position of a recording electrode. The border of the VPM is labeled with *arrowheads*. **C** "On-off" neuronal responses evoked by contralateral vibrissal deflection in the VPM of a control. **D** "On-off" responses in the VPM of a denervated-regenerated animal (see the longer latencies). **E** In partly denervated animals, the vibrissa pad was stimulated electrically (*arrow*), while other parts of the face (e.g., over the eye) were stimulated mechanically (*shaded*). **F** In partly denervated animals, both electrical vibrissa pad stimulation (*arrow*) and mechanical stimulation of the common fur (e.g., over the eye, *half-trapezoid*) evoked responses in the vibrissal representational areas of the VPM shortly after the injury. Calibration: 20 ms

more than one vibrissa (up to 3), but never to stimulation of other parts of the face (e.g., the nose, lower jaw, inside of the mouth, etc.). These vibrissa-driven cells were localized in the medial part of the VPM (Fig. 3B). The latencies of the vibrissa-evoked responses observed within this medial part of the VPM were as follows: to 0°: 7.30±1.31 ms; and to 180°: 8.34±3.06 ms (Table 2). Most of the cells adapted rapidly.

Rats whose contralateral ION was crushed and allowed to recover

In 3 of 20 rats, immediately after the unilateral complete ION crush (P0), the VPM responses to vibrissal stimulation were primarily studied (see Table 1 to follow the experiments). However, in the medial part of the VPM, responses evoked by stimulation of either a vibrissa or other parts of the face were not observed. This part of the VPM was totally unresponsive. The result was the same in those 6 experiments in which a longer postoperative time was allowed (from P3 to P6). Recovery from the crush could be detected on days 7–9. From this time on, an increasing number of units in the VPM responded to vibrissal stimulation (Fig. 3D). The latency of the responses, however, varied considerably. The latency to vibrissal deflection in the caudal direction (0°) was found to be 21.75±10.84 ms, while in the rostral direction (180°) it was 18.84±7.11 ms (Table 2). It is worthy of mention that, in the VPM of most of the denervated and recovering animals, the units usually demonstrated a relatively high spontaneous activity as compared with that found in the controls. During the recovery, the RF of the units became somewhat larger. Some units could be activated by the stimulation of five vibrissae, while others responded to a smaller number, most of them to only one.

In seven further animals, after ION crush, apart from the slight (but highly varying) increase in the RFs and the significant increase in the response latencies relative to the controls, the units in the VPM did not display definite plastic changes between days P30 and P50.

In six other animals, an *incomplete ION crush* was performed. With sharp forceps, only about 60–80% of the ION was crushed. Unfortunately, we did not identify the vibrissae corresponding to the ION that remained intact. In 3 of the 6 incompletely denervated animals, altogether 11 vibrissa-evoked unit responses could be observed in the medial part of the VPM on P0. Among them, only minutes or some hours after the ION crush (P0), four units were found to respond not only to the vibrissae, but also to mechanical stimulation of the face over the eye (2 units), of the nose (1 unit), and of the midline (1 unit) (Fig. 3E and F). The other three of the six incompletely denervated animals were used for electrophysiological study on day P23 (2 animals) or P27 (1 animal). The results of these experiments, however, did not differ from those observed in completely denervated and regenerating animals, except for the shorter mean latencies (to 0°: 14.51±11.3 ms; and to 180°: 15.10±10.1 ms), which was probably due to the incomplete denervation. In contrast to the experiments on P0, in the P23 and P27 incompletely denervated animals, we could not evoke any response in the vibrissal region of the VPM by stimulation of other parts of the face, with the exception of the reappearance of the vibrissa-evoked responses.

In summary, apart from the experiments involving incomplete denervation (P0), the vibrissal representation areas of the VPM were unresponsive to stimulation of the vibrissae or other parts of the face before ION regeneration had occurred.

ION crush-induced plastic changes in the primary somatosensory cortex

In six control animals, we mapped the responses evoked in the primary somatosensory cortex by stimulation of the vibrissae and the body parts mentioned above (Figs. 4A, controls, and 5A). The sites of the responses in the facial region of the SI were the same as described earlier (Welker 1971; Waite 1984; Tracey and Waite 1995). The responses evoked in the barrel cortex by vibrissal deflections were also similar to those already described: the cells within a barrel responded best to one vibrissa, the principal vibrissa (Fig. 4A, control column), but could commonly be driven less effectively and with a longer latency by several surrounding vibrissae (Armstrong-James and Fox 1987; Welker et al. 1993; Toldi et al. 1994).

ION crush (in 16 animals), however, caused deafferentation of all the mystacial vibrissae and immediately resulted in a large cortical area unresponsive to vibrissal deflection. It was interesting to learn that this large unresponsive vibrissal representation cortical area was invaded from the rostromedial direction by the responses evoked by the forepaw digits, shortly after the ION crush (P0 and P3, see Fig. 5B). We attempted to stimulate areas from many other body parts (e.g., the fur on the face above the eye, the dorsal nose, the midline, the inside of the mouth, the forearm, or the ear), but, interestingly, only the representation of the digits on the contralateral forelimb revealed invasion into the barrel cortex.

Digital representation in the rat SI is somatotopically organized; digits D2–D5 are organized into bands running in a medial to lateral direction (Dawson and Killackey 1987; Waters et al. 1995). Here, we defined not the detailed representation of the digits, but the posterior ends of the bands corresponding to digits D2–D4. This is the borderline between the somatosensory areas of the digits and vibrissae (Fig. 5A). The responses evoked by vibrissal stimulation disappeared from the barrel cortex immediately after ION crush. As the most striking effect of ION damage, stimulation of the contralateral forepaw digits (D2, D3, and D4), which normally evoked cortical responses exclusively in their own cortical representa-

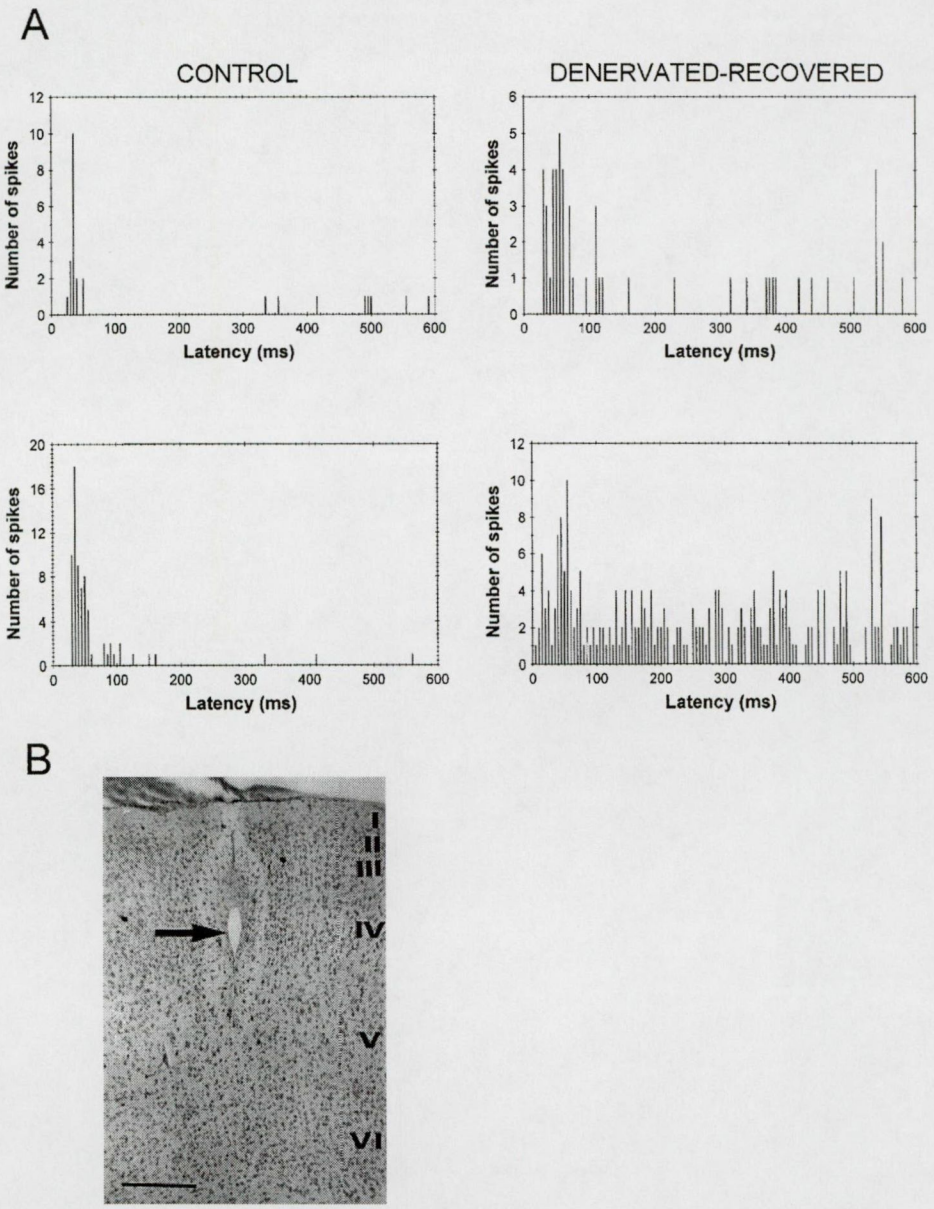

Fig. 4A–B Peristimulus time histograms (PSTHs) of whisker-evoked responses in controls and in infraorbital-nerve (ION) denervated-recovered animals. **A** Whisker deflection evoked "on" responses with short latency in the posteromedial barrel subfield (PMBSF) of control rats. In rats in which the ION was crushed and allowed to recover, the weakly evoked responses had long latencies. **B** Unit recordings were made in layer IV of the PMBSF. The *arrow* points to the lesion made by the tip of the recording electrode. Per animal, 50–70 penetrations were obtained in a matrix in the primary somatosensory cortex. Calibration: 500 μm

tion areas, also resulted in responses in the anterior part of the vibrissa-associated posteromedial barrel subfield (PMBSF). In other words, the border of the region of the digits shifted posteriorly. This phenomenon was already observed only minutes after the crush (P0) and was most pronounced on day 3 (P3) (Fig. 5B). We tested the extension of the cortical representation of the digits and vibrissae systematically for 60 days following the ION crush. Though the vibrissa-evoked responses reappeared 7–10 days after the ION crush, an expanded digit representation could still be seen 18 days after nerve damage. The expansion of the digital representations and the reappeared whisker-evoked responses resulted in an overlapping zone between the pure digital and the vibrissal cortical areas during recovery from the ION injury (Fig. 5C). In this overlapping area, several recording sites were encountered where responses to both digital and vibrissal stimulation could be evoked, but the representations were discontinuous. The spontaneous cortical activity in the PMBSF of the denervated-recovered animals was relatively high, but the evoked responses, mainly during the early recovery days, were weak, with long latencies (Fig. 4A, denervated-recovered). Following the changes in the cortical representations of the digits and whiskers up to 60 days after ION damage, it was found that the rearrangement in the cortical map is nearly completed by that time. The representational maps of the digits and vibrissae in the SI were found to be similar to those for the controls, except for some recording sites (Fig. 5D).

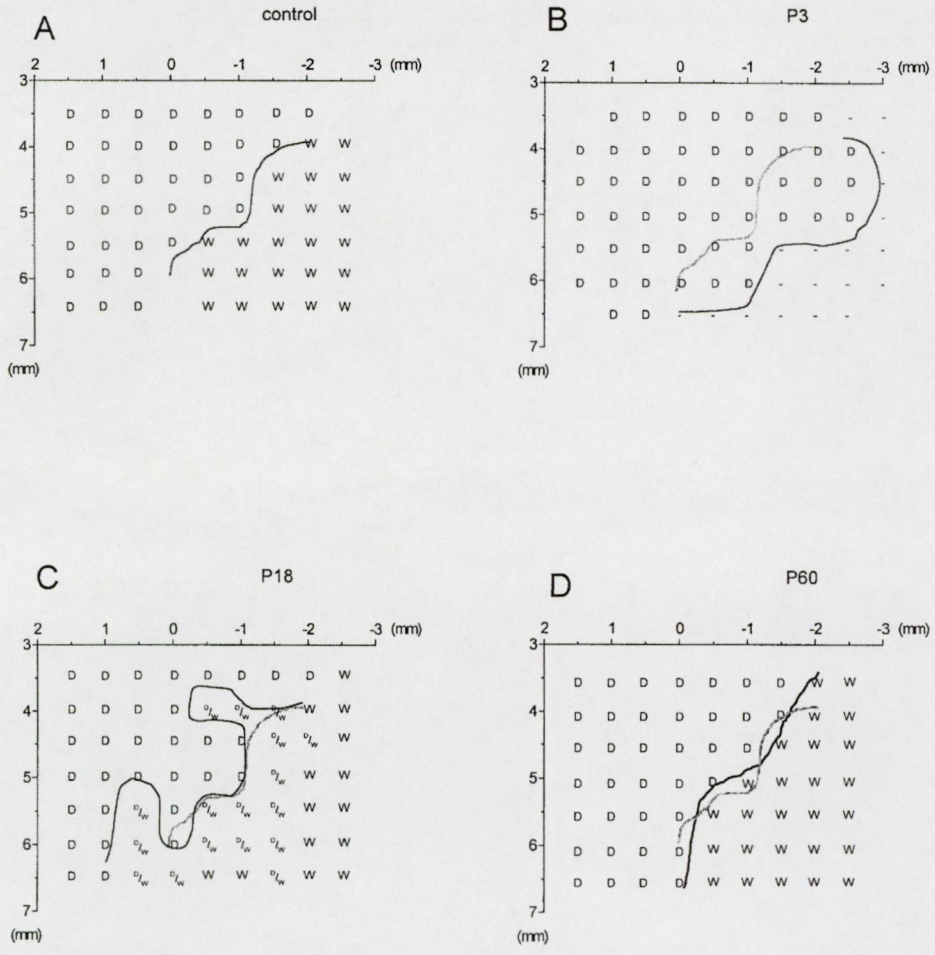

Fig. 5A–D Reorganization in cortical maps of digit and whisker representations induced by injury and subsequent regeneration of the contralateral infraorbital nerve (ION). **A** The posterolateral border of the physiological representation of digits 2, 3, and 4 in a control. **B** Contralateral ION crush resulted in a large unresponsive area to whisker deflection in the posteromedial barrel subfield (PMBSF), but stimulation of the digits evoked responses in the enlarged representation. The border of the digital representation was shifted posteriorly. *Grey line:* The original border between the digits and whisker regions, *black line* the posterior border of the enlarged digital representation. The shift in digital representation could be observed within minutes/hours after the ION crush. **C** The expanded digital representation and the reappeared whisker responses resulted in an overlapping zone between the pure digital and the vibrissal cortical areas ($^{D}/_{W}$). *Grey line* The original border between D and W areas, *black line* posterior border of the pure digital area. **D** 60 days after ION crush, the representational maps of the digits and vibrissae in the primary somatosensory cortex were found to be quite similar to those of the controls. *D* Digit representation, *W* whisker representation. - no evoked response. Zero on the horizontal scale represents the bregma

The latencies in PMBSF were only tested by deflection of vibrissae in the caudal direction (to 0°). The latency in the controls was found to be 16.31±12.35 ms and in the recovered animals to be 36.31±23.09 ms (Table 2). These latencies decreased during the following recovery time (showing a high variation from animal to animal), but did not reach the control level up to P60.

Discussion

This work involved a comparative study of the effects of the neuronal activity following unilateral ION transection or crush at three levels of the trigeminal system: Pr5, the VPM, and the facial area of the SI. In the control animals, the neuronal activities and the properties of the evoked neuronal responses in each structure were similar to those reported by various researchers during the past two decades. Most of the neurons in Pr5 had small RFs, limited to one vibrissa, as found by Jacquin et al. (1988). Units in Pr5 were not spontaneously active and most adapted rapidly. This observation was similar to that found by Waite (1984). Our estimate of the mean latency of evoked responses in Pr5 (3.28 ms) is somewhat longer than that reported by Waite (2.8 ms). The difference is probably explained by the ramp-and-hold trapezoidal stimulus, with a 20-ms slope used in our experiments. However, this difference in latency does not disturb the comparison between the latencies of the controls and the animals with regenerating ION in the course of our experiments. The topographic organization of the vibrissal representation found in our experiments is similar to that described by Waite (1984). The neurons in the VPM also exhibited small RFs. Most cells were activated by only a

single vibrissa, similar to that described by others (Rhoades et al. 1987; Ito 1988; Armstrong-James and Callahan 1991; Chiaia et al. 1991). The cortical map of the SI in the control animals was similar to that described by Welker (1971). These findings, together with our own earlier ones (Toldi et al. 1994) regarding the RF characteristics, and the adaptation and direction sensitivity properties of the barrel cortex neurons, are in close agreement with those of previous investigations (Welker 1976; Kossut 1992; Welker et al. 1993).

While the results we obtained on the controls can be compared with those from a large number of other experiments, there have been relatively few studies that are actually analogous to our own. The two special features of our study were that: (1) we allowed the injured ION to recover, and (2) a complex study was carried out to compare the plasticity capacities of the main three structures along the trigeminal neuraxis.

Concerning the five goals detailed in the Introduction, it can be stated that:

1. Slight changes were found in the RFs in only some of the Pr5 neurons as compared with the controls. Though the model is somewhat different, the results are similar to those reported by Waite (1984). In contrast, higher-order neurons have been shown to develop new RFs very shortly after a peripheral nerve crush. In animals with a partly denervated ION, some neurons in the vibrissal representation areas of the VPM exhibited an immediate shift in the RFs responding to stimulation of other parts of the face (Fig. 3). After recovery from the complete ION crush, in some animals RF enlargement was also detected in the VPM, but the most significant plasticity induced by peripheral nerve denervation was found in the SI (Figs. 4 and 5). Our results demonstrated that temporary ION damage (crush) not only resulted in a transient disappearance of the contralateral vibrissa-evoked responses from the barrel cortex, but also produced an expansion of the neighboring representation area of the digits of the contralateral forepaw (Fig. 5). A similar expansion of the somatotopic representation of the remaining digits was reported after partial digital amputation in flying foxes (Calford et al. 1996).
2. The time needed for the functional recovery of vibrissal afferents from the injury depended greatly on the type of denervation. Recovery from the ION transection required 22–35 days, while that from the ION crush took only 7–9 days. The most likely explanation of these findings appears to be the discontinuity of the ION after transection (Holstege 1991), while the physical continuity of the proximal and distal stumps of the damaged ION fibers persists after crushing. The physical continuity allows the regeneration to proceed more quickly, because the regenerating axons grow toward denervated targets within the endoneural tubes formerly occupied by intact axons and their myelin sheaths (Fu and Gordon 1997). Although the nerve injury caused by crushing of the ION with tweezers in our experiments was checked histologically in several cases and was always found to be perfect, it can not be excluded that, in some cases, a few fibers were spared, which helped the nerve recover. The changes in the fine structure of the related trigeminal centers following the peripheral nerve damage might also be an important factor.
3. The most pronounced change in all related structures was the increase in the latency of the evoked responses during recovery from the ION injury (Table 2). Waite and Cragg (1982) compared the latencies of evoked responses in adult animals subjected to ION crush or transection as newborns. Though their measured latency was somewhat shorter (probably because of the different stimulating technique and mainly because the ION injury was produced in newborn animals), the observations were similar: the ION-crushed animals suffered only minor modifications, while the ION-transected animals displayed a much longer recovery time.
4. The main purpose of the present study was to establish whether plastic changes induced by ION injury persist or disappear as recovery progresses. As we found the most significant neuronal plasticity in the SI, we limit the discussion of this point to the cortical observations. Our results demonstrated that temporary ION damage (crush) not only resulted in a transient disappearance of the contralateral vibrissa-evoked responses from the barrel cortex, but also produced an expansion of the neighboring representation area of the digits of the contralateral forepaw. Our observations are in accordance with the findings of Calford and colleagues, who reported a similar type of immediate change in the cortical representation of the somatosensory area as a consequence of interference with different nerve branches of adult flying-foxes, cats, and rats (Calford and Tweedale 1991; Calford et al. 1996; Clarey et al. 1996). The similarity in time course suggests that similar modes of rapid synaptic plasticity may be induced by peripheral deafferentation in the representation areas of the sensory cortex. By 7–10 days after the ION crush, the vibrissa-evoked responses had reappeared, but the expanded digit representation could still be observed 18 days later (Kóródi and Toldi 1998). However, following the induced changes in the cortical representational map up to 60 days, its reestablishment was found to be nearly complete by that time. The borderline between the representations of digits and whiskers was similar to that for controls (Fig. 5D). This result resembles that obtained in monkeys. Recordings from the hand representation of monkeys after nerve crush and regeneration revealed maps that were totally normal, and when the same monkey was recorded both before and after nerve crush and regeneration, the individual features of the maps were found to return (Wall et al. 1983). Our results in the rat, however, showed that the ION injury-induced extended representation area of the digits stabilized transiently for weeks, resulting in

an overlapping zone between the obligate digit- and vibrissa-associated cortical areas during ION regeneration.

5. The comparative study of neuronal plasticity at three levels of the trigeminal neuraxis demonstrated that, in the animals whose ION was transected or crushed as adults, short-term plasticity could not be observed and rather limited long-term plastic changes were detected in Pr5. In the thalamus, both short-term and long-term plastic changes took place, whereas an immediate significant reorganization in cortical representation was observed in the SI. With the exception of a very small number of cells recorded by Devor and Wall (1981a, 1981b), damage to peripheral nerves does not appear to produce an immediate shift in the RF characteristics of spinal neurons. Similarly, Waite (1984) did not observed an unmasking of new RFs in the trigeminal brainstem complex after acute transection of the ION. Higher-order neurons, however, have been shown to develop new RFs, in some cases at very short intervals (Nakahama et al. 1966) and, in other models, with a longer delay after peripheral nerve transection (Rhoades et al. 1987) in the VPM. This kind of plasticity of the human brain has very recently come to the center of interest of research (Davis at al. 1998; Kaas 1998; Ramachandran et al. 1998). Finally, a number of studies (reviewed in Calford et al. 1996) have shown that transection of a peripheral nerve can result in the unmasking of new RFs at very short intervals (hours or even minutes) in the SI. However, the elegant experiments of Faggin et al. (1997) disclosed that, following the onset of the reversible deactivation, immediate and simultaneous sensory reorganization could be observed at all levels of the somatosensory system. They concluded that the spatiotemporal attributes of cortical plasticity are paralleled by a subcortical reorganization.

Our technique is probably not that sensitive, but the results observed with this technique do indicate that either short- or long-delay reorganization in the brainstem after peripheral injury may be limited. Results obtained in the VPM and the cortex strongly suggest that the higher the stations in the neuraxis, the more plasticity that occurs. This suggestion fits in well with results hypothesized several years ago (Merzenich et al. 1988; Pons et al. 1988) and recently observed in a quite different model with a different method (intrathalamic and intracortical microstimulations; Dinse et al. 1997).

At present, the mechanisms of denervation-induced plasticity and reorganization in the somatosensory and motor systems are unknown, but are undergoing increasingly active investigation both by others (for reviews, see Kaneko et al. 1995) and by ourselves (Toldi et al. 1996a, 1996b; Farkas et al. 1998; Kóródi and Toldi 1998). Thalamic involvement in these processes is probable (Diamond et al. 1992a, 1992b; Weinberger 1995), but the main site of plasticity is indisputably the cortex. Accumulating evidence has led to the development of a hypothesis that both cholinergic and γ-aminobutyric acid-containing projections from the basal forebrain play important roles in the production of a state in the cortex that permits neuronal plasticity to occur (Dykes 1997). However, at present, it appears that new conceptions of the sensory cortical function are needed to incorporate the new data and to allow an understanding of these processes.

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A COMBINED ELECTROPHYSIOLOGICAL AND BEHAVIOURAL STUDY FOR THE ASSESSMENT OF ACTIVITY-DEPENDENT CHANGES IN MICE*

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Activity-dependent adaptive changes in the nervous system involve structural and functional changes in the cortical circuitry. In this work the cortical function was studied by repeated recording of the somatosensory and motor potentials evoked by whisker deflections after altered sensory-motor experience in adult mice. The latencies of motor and somatosensory evoked potentials were found to shorten, while their amplitudes decreased, after a behavioural challenge involving the vibrissal apparatus. Sensory deprivation achieved by whisker trimming resulted in a partial reversal of the changes observed after increased activity. The derived parameters imply that cortical information processing speeds up as a result of experience, while decreased activity has the opposite effect. The methods used throughout the experiment were minimally invasive, and thus proved to be sufficient for the long-term follow-up of cortical functions.

Keywords: Experience-dependent plasticity – evoked potential – barrel cortex – mice

INTRODUCTION

Life is a long history of sensory experience, in the course of which the nervous system proves capable of adapting to the ever-changing environment. A fundamental issue in neurobiology is the understanding of the morphological and physiological correlates of these adaptive changes in the nervous system. Controlled alterations of sensory input permit studies of the consequent structural and functional changes, i.e. studies of experience-dependent neural plasticity.

The barrel field region of the rodent primary somatosensory cortex is often used as a model of cortical plasticity. This area of the cerebral cortex contains discrete groups of neurons in layer IV, called barrels, which are related one-to-one to the large mystacial vibrissae on the contralateral face [27]. Investigations have long been performed of the question of whether there are any changes at the morphological, metabolic, electrophysiological or molecular levels following alterations in sensory experience caused, for example, by transection of the infraorbital nerve, the removal of

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whisker follicles, whisker plucking or trimming, or passive stimulation of the whiskers.

After neonatal whisker follicle removal, the corresponding barrels fail to develop [22], while partial elimination of the vibrissal input in young adult animals results in topographic reorganization of the barrel cortex [11]. A decreased number of GABA-ergic (GABA = gamma-aminobutyric acid) synaptic contacts in layer IV of the barrel field has been shown to be a consequence of whisker plucking from birth [16]. This has been proposed as the structural substrate for experience-dependent plasticity. Following partial vibrissectomy, a higher dendritic spine density was found in the barrel columns corresponding to the spared whiskers, accompanied by an enhanced elongation and branching of the axons [11]. Deprivation was also shown to decrease the dendritic spine motility in the corresponding barrels [14].

Histochemically, neonatal whisker plucking or follicle removal resulted in decreased GABA immunoreactivity in layer IV of the barrel field [10, 15], as well as a lower level of muscimol binding and severe cytoarchitectonic malformations [20]. Follicle removal in adults diminished the GAD (glutamic acid decarboxylase) immunoreactivity [23] and cytochrome oxidase staining [26], and transiently depressed muscimol binding [20]. Whisker trimming, a relatively mild intervention, also resulted in a decreased GAD immunoreactivity [1], diminished muscimol binding [7], decreased cytochrome oxidase staining [12] and lowered levels of energy-related enzymes [6] in the deprived rows of barrels. Besides an expansion of the functional representation, trimming all but one whisker caused a site-specific upregulation of CRE-mediated gene transcription in layer IV [3]. Experiments in which animals were subjected to increased passive whisker stimulation revealed an altered deoxyglucose uptake [25] and a transient increase in GAD immunoreactivity [24].

Cellular electrophysiological studies have proved that neonatal deprivation causes the deprived barrel neurons to have an increased receptive field size, spontaneous activity and evoked responses, and a decreased angular selectivity [19]. In adolescent rats, there is a rapid depression of the responses to the deprived sensory input and an upregulation of the responses of the spared barrels [8]. The most widely used procedure for the study of experience-dependent plasticity at the level of cellular physiology is the whisker pairing paradigm, where two adjacent whiskers are spared on the muzzle [2, 5]. Although the altered input causes the response to the central receptive field (the principal whisker) stimulation to increase, the response to the spared surround receptive field whisker increases much more intensely, while the responses evoked by the non-paired (trimmed) whiskers decrease. It has been assumed that a redistribution of activity takes place across the barrel columns [13]. This surround-whisker bias fails to develop under the cholinergic depletion of the cortex caused by lesion of the basal nucleus of Meynert [4]. Similarly, plastic changes evoked by whisker pairing are prevented by application of the NMDA receptor antagonist D-AP5 [18].

Very few studies have addressed plastic changes from the aspect of behaviour. However, the role of the barrel cortex was implied in tactile discrimination tasks carried out following cortical ablations [9]. The importance of active tactile exploration

in the development of the normal spatiotemporal patterning of the neuronal receptive fields has also been demonstrated [17].

In the above-mentioned studies, unidirectional changes were investigated, since repeated measurements on the same animal were not possible, in consequence of the nature of the approach.

In the present work, our purpose was to identify physiological correlates of cortical plasticity, however subtle, that arise as a result of mild, nearly natural alterations of sensory experience (an increased and a subsequent decreased use of the vibrissae) in adult mice, using the most gentle electrophysiological technique possible. Furthermore, to follow activity-dependent changes, their time course or reversibility, it was desirable to apply techniques that allow repeated measurements on the same animals. Therefore, we applied the minimally invasive epicranial evoked potential recording method [21], which proved to be sufficient for repeated use. As for the change of the sensory experience, we also applied natural, non-invasive interventions. Thus, the animals were first subjected to a behavioural challenge, the radial arm maze, which requires active use of the vibrissal system and also motor skills. Their whiskers were then trimmed to create a state of sensory deprivation in the same group of animals. The consequences of each alteration were examined by recording epicranial evoked potentials measured above the somatosensory and motor cortices of the contralateral hemisphere.

MATERIAL AND METHODS

A total of 13 12-week-old male CFLP mice weighing 32–36 g at the start of the experiment were used. Seven of them (RM group) ran the radial arm maze after the initial electrophysiological recording and suffered whisker trimming after the second one. Six animals served as age-matched controls (CTR).

All three consecutive electrophysiological recordings were carried out under Nembutal (pentobarbital, 60 mg/kg i.p.) anaesthesia. The body temperature was kept at 37 °C. Following incision of the scalp, the head was fixed in a stereotaxic frame and the two recording electrodes were placed on the exposed skull surface, one over the barrel field (co-ordinates 2.5 mm posterior and 3 mm lateral to the bregma), and the other over the primary motor cortex (1 mm anterior and 1.5 mm lateral to the bregma). Electroconductive paste was used to decrease resistance.

Mechanical stimulation of the vibrissae was carried out by means of an electrically controlled mechanical device. Deflections of the vibrissae could be made in any direction, with variable speed and waveform. In this case, the stimulus waveforms were ramp-and-hold trapezoids that produced a 3 mm dorso-ventral displacement of all whiskers at 10 mm from the base of the hair. One stimulus cycle consisted of a displacement of the whiskers from their resting position (stimulus onset) and the return to the initial position (stimulus offset). The stimulus duration was 500 ms, the slope lasted for 30 ms and the frequency of stimulation was 1 Hz.

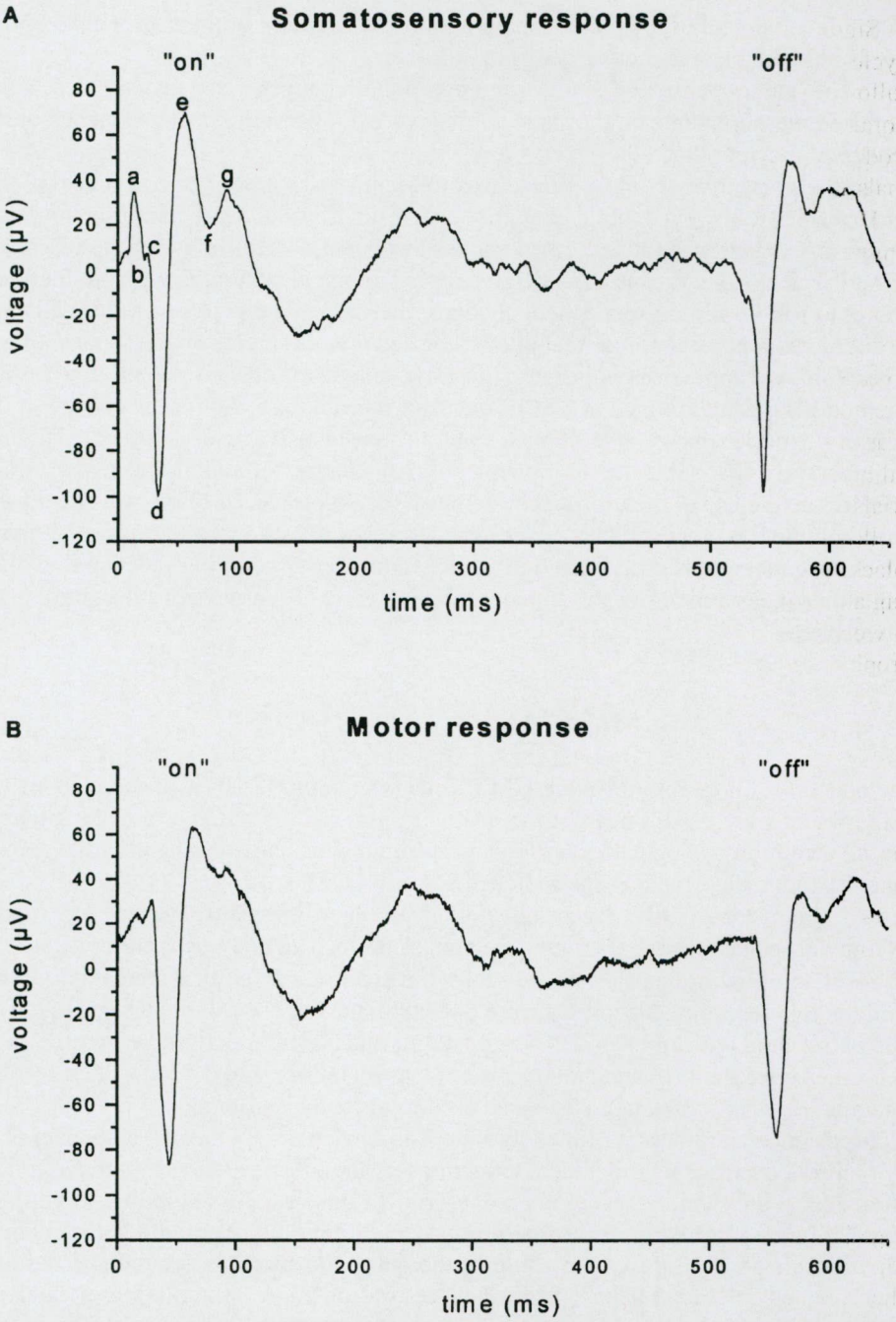

Fig. 1. Responses recorded over the somatosensory (A) and motor (B) cortices. Different components (a–g) are marked at the somatosensory "on" component

Signals were amplified (×5000) and filtered (5 Hz and 5 kHz), and 10 times 30 cycles were stored for averaging and analysis. *"On"* responses were defined as those following the stimulus onset within 120 ms, while *"off"* responses had the same temporal relation to the stimulus offset. At the end of the recording procedure, the electrode paste was washed off and the scalp was closed with surgical sutures. The animals were kept under observation until awakening.

During the 6-week interval between the first and second recordings, one group of animals ran in the radial arm maze. The set-up, made of Plexiglas, was elevated 50 cm above the ground; it had a central platform (d = 15 cm) and 8 identical arms (6×40×10 cm). Each of the 24 trials per animal lasted for 10 minutes. The 8 arms of the maze were situated 6 cm from the central platform, and therefore the animals had to cross a gap, but they could palpate the entrance of the arms by using their whiskers prior to entering. All the arms had a sandpaper doorstep and all were baited, but there was only one through which the animals could gain access to the food. This rewarded arm was distinguished from all the others by a differently textured doorstep. All trials were carried out in a dark, soundproofed room under infrared video control. Following this behavioural challenge, the second electrophysiological recording took place. The same group of animals then had their whiskers trimmed continuously during a 6-week period so as to prevent whisker growth above 10 mm. At the end of the 6 weeks, the whiskers were allowed to grow somewhat longer before the third electrophysiological recording was carried out.

RESULTS

In the course of the first electrophysiological recording, we identified 7 different peaks of the *"on"* and *"off"* responses, designated by the letters from *a* to *g*. Their latencies were characteristic, ranging from 13 ms (*a*) to as long as 87 ms (*g*) for the *"on"* response recorded by the electrode placed over the somatosensory cortex, while the peaks of the *"off"* response lay in the range 520–602 ms. The *"on"* responses that were led off from the motor cortex had latencies in the range 17–95 ms, while the *"off"* response peaks appeared at 525–615 ms. Representative examples of the waveforms are shown in Fig. 1.

The amplitudes of the somatosensory and the motor peaks were generally in the interval 10–200 μV, but only the highest peaks (*d*, *e* and *g*) exceeded 100 μV. At the first recording, no differences were observed between the two groups of animals as concerns the latencies and amplitudes.

As revealed by the second electrophysiological recording session, running in the radial maze, i.e. an increased use of the vibrissal sensory-motor system, resulted in significant changes in the mentioned physiological parameters. While the latency of the *"on"* response underwent no major alterations, that of the somatosensory *"off"* responses decreased in both groups. The change was more pronounced in the RM group (on average, there was a 9.7 ms shortening of the latencies in the RM group, while in the CTR group it was 7 ms), with significantly shorter latencies for 4

response components (*a, c, d* and *g*) in the RM group (514.7 ms vs. 518.7 ms, 522.1 ms vs. 528 ms, 530.4 ms vs. 537.8 ms, and 584.3 ms vs. 596.3 ms). The motor response latencies shortened significantly in both groups by the second recording, with an average of 11.4 ms in the CTR, where 4 peaks occurred earlier, while in the RM group 6 peaks had, on average, 9 ms shorter latencies than before the maze running. The latency difference between the *g* component of the *"on"* somatosensory and motor responses exhibited a significant decrease following an increased use of the vibrissal sensory-motor system, changing from 7.6 ms to 0.5 ms, in this way becoming significantly shorter than that of the CTR group (9.1 ms) at the second recording.

The amplitude of the responses was also significantly changed in the RM group after the radial maze running, for both the somatosensory (43 μV decrease on average) and motor (16 μV decrease on average) response components, whereas in the CTR group they did not display any alteration.

At the third recording, following sensory deprivation, the latencies of the response components were further shortened, but this tendency was much less pronounced than after the increased sensory-motor activity. Although the RM latencies were on average 0.9 ms shorter, several peaks occurred later than before deprivation. The CTR animals had 2.74 ms shorter latencies than before deprivation. The motor latencies after the decreased sensory activity were also shortened on average in the RM group, with a significant difference between the two groups for the *e* (55.9 ms vs. 66 ms) and *f* (72.9 ms vs. 90.9 ms) components of the *"on"* response, and for 3 components (*a, b* and *e*) of the *"off"* response (515.8 ms, 521.5 ms and 558.5 ms vs. 521.2 ms, 526.6 ms and 567.6 ms, respectively). Other peaks (*c, f* and *g*) appeared with longer latencies. The latency difference between the somatosensory and motor *g* peaks increased in the RM animals (to 4.2 ms) during deprivation, and thus there was no longer any difference as compared to the value measured before the increased activation, but it was still significantly shorter than that in the CTR group. This value gradually increased in the CTR animals throughout the three consecutive recordings. The width of the motor *"off"* complex (*a–g* distance) increased significantly in the deprived RM group, changing from 74.2 ms on average to 96.3 ms.

As for the amplitudes, the initial decrease observed after the increased sensory-motor activity did not continue, but the differences as compared to the initial values were still significant in the RM group. However, the amplitude of the earliest peak of the somatosensory response increased (from 22.9 μV to 37.7 μV) towards the initial value of 66.3 μV. The initially decreased motor amplitudes increased for the *a, b,* and *c* components of the *"on"* response during the period of deprivation in the RM group (23.4 μV, 11 μV and 28.1 μV vs. 8.9 μV, –3.4 μV and 9.1 μV, respectively), so that they were not significantly smaller than the values observed before the increased activity (21 μV, 8.4 μV and 21.7 μV). In the CTR group, significantly smaller motor amplitudes were obtained during the third recording for the early components (*a, b* and *c*) of its *"on"* response (9.2 mV vs. 26.7 mV, –1 mV vs. 15.5 mV, and 17.4 mV vs. 35.7 mV) as compared to the initial values obtained at the first recording. The *"off"* component of the motor response revealed similar, though not significant changes in amplitude.

DISCUSSION

In the present study, we set out to detect changes in cortical function evoked by alterations of sensory experience in adult mice. For this purpose, we first subjected the animals to a behavioural demand, where they had to use their whiskers actively, and thus the activity of the vibrissal sensory-motor system was increased. Subsequently, we trimmed all the whiskers of the animals, thereby creating a state of sensory deprivation. We followed the consequent physiological changes by recording the somatosensory and motor evoked potentials epicranially. We identified 7 components of all *"on"* and *"off"* responses, characterized by their latency and amplitude, and we analysed these *post hoc*. A main feature of the experiments was that the alteration of the sensory experience and the recording procedure were only minimally or not at all invasive, which allowed repeated measurements on the same animals.

The fact that ablation of the barrel cortex impairs the vibrissal roughness discrimination [9] implies that this region is an active site of experience-induced adaptive changes. Following an increased use of the sensory-motor apparatus of the whiskers, a pronounced shortening of the latencies of the somatosensory evoked potentials could be observed. This occurred in both groups, but resulted in a significantly quicker response in the RM group that ran in the radial maze, suggesting a faster cortical processing as a result of the increased use. In studies of experience-dependent plasticity, it should also be borne in mind that the recording procedure itself, which consists of continuous stimulation for about a half an hour, is equivalent to increased use. Thus, reproducibility of the responses should be evaluated with regard to the fact that animals subjected to subsequent recordings are naive only on the first occasion. This could be one reason for the latency shortening observed in both the RM and CTR groups at the second recording. Although the mechanism of the latency shift is unclear, explanations should be sought in the physiology of the ascending thalamocortical pathway and in that of the local and projection circuitry of the cortex since both early and late components of the evoked potential complex exhibited alterations. Functional reorganization of the cortical connections as a result of the altered sensory experience is suggested by Simons and Land [19], who found that the first peak in the post-stimulus time histogram of deprived barrel neurons occurs during the period when the activity in the non-deprived cells is most strongly inhibited. Studies addressing the role of different transmitter systems in activity-induced changes indicate that GABA, glutamic acid and acetylcholine play important roles in these processes [4, 18, 24]. These neurotransmitters may also have an impact on the function of the local cortical circuitry, thereby influencing the response latencies. It has been demonstrated by Welker et al. [25] via the monitoring of deoxyglucose uptake that increased use affects the metabolic characteristics of the cerebral cortex, which correlates with the alertness of the cortex to respond to ascending stimuli, and thus with the response latency. By measuring the latency differences between corresponding sensory and motor peaks, we have also found that the sensory-motor processing of the vibrissal system is accelerated after an increased use of the whiskers, which may reflect a more efficient response transformation as a result of experience.

After sensory deprivation, carried out by whisker trimming, there was still a shortening of the latencies on average, but some of the response peaks occurred with slightly longer latencies, suggesting that an opposite mechanism was taking place as compared with that during increased sensory activity. The motor latencies for the later response components were also found to lengthen in the RM group following sensory deprivation, while the early components appeared with shorter latencies, implying that peaks of different origins display different dynamics of change in response to alterations in experience. After deprivation, the cortical processing seemed to slow down, as suggested by the latency difference between the *g* peak of the motor *"on"* response and the same peak of the somatosensory response. The widening of the response complex reflects the same effect of sensory deprivation on the cortical processing. The decrease in cytochrome oxidase staining and the lower levels of energy-related enzymes associated with sensory deprivation [6, 12] may provide some explanation for the slower cortical activity observed here after whisker trimming.

The amplitudes also altered throughout the experiment. Following an increased use of the sensory-motor apparatus in the radial maze, both the somatosensory and the motor amplitudes were decreased significantly. A known consequence of a higher level of activity is an increased GAD immunoreactivity [24], which may control the receptive fields of the cortical neurons. This may have the benefit of sharpening the neural responses, making them more specific to a given stimulus, restricting the flow of excitation, which is reflected by smaller amplitudes, i.e. a decreased level of cortical synchronization.

The amplitudes after sensory deprivation revealed an opposite tendency. The increase in amplitude is in accordance with the finding that both the GABA immunoreactivity and the GAD immunoreactivity are lower in the barrel cortex following sensory deprivation [15, 23], while the number of GABAergic synapses is decreased [16], giving rise to a decreased level of inhibition and greater cortical synchronization, reflected by higher amplitudes in our study.

We have established that an increased and subsequently decreased sensory activity provoked somewhat opposite changes in such physiological parameters as the latency and amplitude of the somatosensory and motor evoked potentials. The approach used here proved sufficient for the detection of mild physiological alterations resulting from changes in sensory experience, and provides a useful protocol for the examination of dynamic alterations in neural function.

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DISSERTATION SUMMARY

Plasticity of the somatosensory cortex in adult rodents

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Life is a long history of sensory experience, in the course of which the nervous system proves capable of adapting to the ever-changing environment as well as to behavioural challenges and pathological alterations. This ability is termed plasticity. A fundamental issue in neurobiology is the understanding of the morphological and physiological correlates of these adaptive changes in the nervous system. Controlled alterations of sensory input permit studies of the consequent structural and functional changes, *i.e.* studies of experience-dependent neural plasticity.

In our experiments we have been studying alterations of functional parameters with electrophysiological methods on the so-called barrel field of rodents. This region of the cerebral cortex contains discrete groups of neurons in layer IV, called barrels, which are related one-to-one to the large mystacial vibrissae on the contralateral face. Anteromedially we can find the forepaw representation area adjacent to the barrel field, which is also topographically organized. Despite the strict cortical representation of the body surface on the primary somatosensory cortex (SI), a significant capacity to undergo functional changes in response to alterations in sensory input remains even in the adult cortex.

First, we investigated the consequences of infraorbital nerve injury on the organization of cortical representational maps in adult rats. The infraorbital nerve is a sensory branch of the trigeminal nerve that innervates the whisker follicles of the face, so its injury eliminates the input of the contralateral barrel cortex. Recording somatosensory evoked potentials and extracellular unit activity over the barrel field as well as on the adjacent forepaw representation area we present evidence indicating that changes in the somatotopic map of the SI appear early after nerve crush. We closely studied the borderline between the physiological representation of the sinus whiskers and the digits. Following the injury, the physiological representation of the digits of the contralateral forepaw extended posterolaterally, occupying a part of the whisker region. The extended physiological representation of the digits, though somewhat shrunken, remained after the reappearance of whisker-evoked responses, forming an overlapping area between the obligate digit and whisker representations. Thus, we demonstrate that entire reorganization in cortical topography does not take place after nerve regeneration in adult animals.

Our purpose was then to identify physiological correlates of cortical plasticity, however subtle, that arise as a result of milder, nearly natural alterations of sensory experience, leaving the nervous system intact. So we examined the changes of somatosensory evoked potentials after increased and a subsequent decreased use of the vibrissae in adult mice. Thus, the animals were first subjected to a behavioural challenge, the radial arm maze, which requires active use of the vibrissal system and also motor skills. Their whiskers were then trimmed to create a state of sensory deprivation. Furthermore, to follow activity-dependent changes, their time course or reversibility, it was desirable to apply a technique that allows repeated measurements on the same group of animals. Therefore, we applied the minimally invasive epicranial evoked potential recording method, which proved to be sufficient for repeated use. The consequences of each alteration were measured above the primary somatosensory and motor cortices of the contralateral hemisphere. The latencies of the evoked potentials were found to shorten, while their amplitudes decreased, after the behavioural challenge involving the vibrissal apparatus. Sensory deprivation achieved by whisker trimming resulted in a partial reversal of the changes observed after increased activity. Some derived parameters imply that cortical information processing speeds up as a result of experience, while decreased activity has the opposite effect.

Our results demonstrate that the nervous system is capable of adaptive changes, such as reorganization of cortical maps or change of the dynamics of the evoked potentials at adult age, and these functional alterations are detectable and traceable even by minimally invasive physiological methods through repeated measurements on the same group of animals.

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