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ARTICLE

Unmasking of latent synaptic connections in the cortex of the rat, elicited by facial nerve transection

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ABSTRACT Peripheral nerve injury elicits plastic changes in the cortex, resulting in reorganization of the somatotopic representation maps. These processes begin within minutes after nerve injury, and last for weeks. Although the mechanisms leading to these plastic changes are not known in a detail, a number of results suggest that the key element in the starting of these processes is a decrease in the function of the cortical GABAergic system, which allows the unmasking of pre-existing but normally silent synapses. The somatosensory and motor cortices of the rat brain are involved in strong and mutual interaction. This study of the early changes induced in this relationship by unilateral facial nerve transection (N7x) revealed that the disinhibition of associational and commissural connections caused by N7x allowed the appearance and enhancement of potentials in the motor cortices on both sides, evoked by right-side trigeminal stimulation, though these responses were strictly lateralized in normal animals. In response to the juxtacellular application of GABA and muscimol, reversal was observed in a small population of neurons (3 out of 84) tested with microelectrode recordings and pressure microinjections. These results suggest that a peripheral nerve injury (N7x) rapidly induces $GABA_A$ receptor-dependent disinhibition in the cortex.
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KEY WORDS

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Peripheral nerve injury (both afferents and efferents) causes time-predictable changes in the representation borders of the related cortical areas (Calford et al. 1996; Sanes and Donoghue 2000). However, little is known concerning the mechanisms inducing these changes. As regards the somatosensory cortex (SI), it is known that partial deafferentation is followed by a decrease in inhibition driven by the afferent inputs (Calford and Tweedale 1991). A decrease in GABA immunostaining in the cortex has also been reported (Alloway et al. 1989).

Similar changes have been observed in the motor cortex (MI) after de-efferentation. It is known that there is a reorganization of MI representations through associational connections within days and weeks after nerve injury (Donoghue et al. 1990; Sanes et al. 1990). It was recently established that these denervation-induced changes appear in two phases. The initial phase starts minutes after nerve injury, e.g. 4 minutes after unilateral transection of the facial nerve (N7x), intracortical microstimulation of the "de-efferented" MI area (vibrissa region) elicits vibrissal movements ipsilateral to the stimulation through the activation of pre-existing commissural connections (Toldi et al. 1996). Those branches of the facial nerve which are involved in vibrissal movements (and are cut) are known to be entirely efferent in rats (Semba and Egger 1986). During this short period of time, only the modification of pre-existing synaptic connections may occur, e.g. through the horizontal projections which traverse the representation borders (Huntley 1997). The mechanisms of this phenomenon remain to be elucidated. It is known that in the MI of the rat the somatosensory feedback of the forelimb participates in the maintenance of its representation borders (Sanes et al. 1992). Between the primary SI and MI, there is a strong functional interaction, described by both morphological and electrophysiological methods (Miyashita et al. 1994; Izraeli and Porter 1995; Farkas et al. 1999). These observations suggest a mutual interdependence between the somatosensory and motor systems of the rat. We considered it of interest to learn more about these modifying cortical connections after N7x.

We present evidence here that denervation-induced disinhibition allows the unmasking of pre-existing associational connections between the SI and MI in the contralateral hemisphere, and the unmasking of commissural connections between the MIs in both hemisphere. Evidence is additionally reported for a cellular mechanism possibly involved in disihibitional processes induced by peripheral nerve injury.

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Materials and Methods

Surgery

A total of 21 Sprague-Dawley rats (200-250 g) of either sex were involved in this study. The surgical procedures used followed the protocol for animal care approved by the Hungarian Health Committee (1998) and the international guidelines (European Communities Council Directives, 86/609/EEC). The animals were anaesthetized with an initial intraperitoneal dose of a Ketavet and Rompun mixture (10.0 mg/100 g, Cp-Pharma, and xylasine, 0.8 mg/100 g, Bayer AG, respectively). Maintenance doses were administered at hourly intervals. The right facial nerve (including its postauricular branch) was exposed and laid onto a special hook-shaped knife. The edges were retained, which allowed normal conduction. The nerve was transsected later, during the electrophysiological recordings. The MIs on both sides were exposed by craniotomy from about 2 mm to 5 mm anterior to the bregma, and from 0.5-5 mm lateral to the midline, according to the stereotaxic atlas of Paxinos and Watson (1982). The dura mater was removed and the cortical surface was covered with warm paraffin oil (Sigma, St. Louis, MO, USA) in order to prevent cooling and drying. The core temperature was maintained at 37ºC and the animals were kept at rest for at least 30 min after surgery.

Stimulation and electrophysiological recordings

The right vibrissa pad was stimulated with a bipolar needle electrode at threshold intensity (1 Hz frequency, 300 ms duration, 10 ms delay and 200-250 μA amplitude). The stimulation elicited clear movements of several vibrissae and evoked potentials in the vibrissal field of the contralateral MI. At the beginning of each experiment, cortical mapping of the evoked potentials was performed to find the appropriate point with evoked potentials with the highest amplitude in the MI contralateral to the stimulation (the punctum maximum, usually localized 2 mm lateral and rostral to the bregma). Continuous parallel recordings of evoked potentials were carried out from the punctum maximum of the contralateral MI and from its homotopic ipsilateral point. In another series of experiments, extracellular unit recordings were performed at the punctum maximum of the contralateral MI. The glass microelectrodes were filled with 2.5 M NaCl (impedance 15-20 MW). Action potentials with amplitudes at least three times higher than the noise were recorded and analysed. In this series of experiments, N7x was performed during the surgery. In some experiments, pyramidal tract stimulation was carried out for identification of the cells being recorded (Landry et al. 1984). For this stimulation, the ventral surface of the pyramidal tract was exposed, and a small flat bipolar electrode was gently placed on the surface of the pyramidal

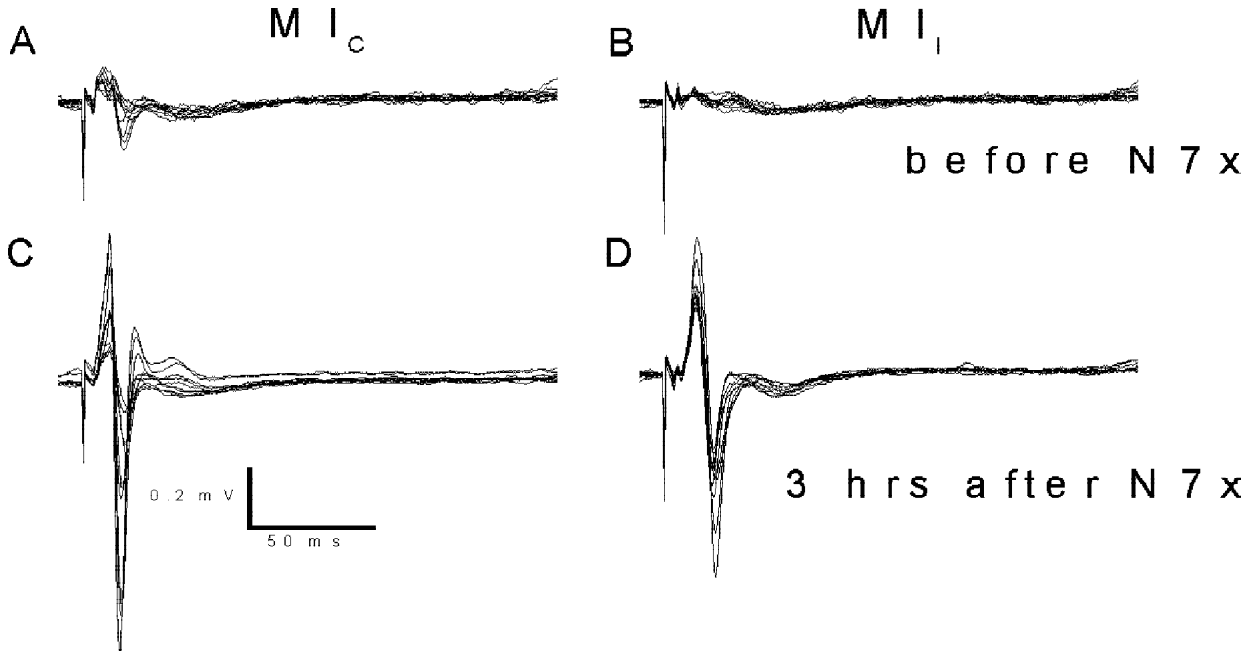

Figure 1. Evoked potentials of an N7x animal, recorded from the MIs on both sides: MI_c: primary motor cortex contralateral to both N7x and trigeminal stimulation. MI_i: primary motor cortex ipsilateral to both N7x and trigeminal stimulation. Each set of evoked potentials contains 10 averages cumulated from 60 sweeps. **A, B**: potentials recorded before N7x in MI_c and MI_i, respectively. **C, D:** potentials recorded 3 hours after N7x in MI_c and MI_i, respectively. Note that before N7x there were no evoked potentials in MI_i. Three hours after N7x, evoked potentials with large amplitude could be observed in the MIs in both hemispheres.

tract. Evoked potentials and unit responses were digitized and fed into a computer via an interface (Digidata 1200, pClamp604 software, Axon, Union City, CA, USA). Each registration contained 60 sweeps. Peristimulus time histograms (bin width 1.44 ms) were produced from unit responses.

Drug application

GABA or muscimol (10^{-3} M, dissolved in sterile saline) was applied juxtacellularly by pressure microinjection. For details of the electrode manufacturing, see Farkas et al. (1996). Briefly, two glass microelectrodes were glued together: one sharp electrode for recording and another one with a broken tip for drug application. Under a light microscope, the electrodes were adjusted so as to give a distance of ≤40 µm between their tips. At the optimal pressure, 25-40 pl of solution was applied.

Results

In the control animals, the electrical vibrissa pad stimulation elicited evoked potentials in the contralateral MI, but not in the ispilateral MI (Fig. 1 A and B). The parameters of the evoked potentials (shape, latency and amplitude) were identical to those published earlier (Toldi et al. 1999). Interestingly, the evoked responses in the contralateral MI were rapidly modified after N7x. A few hours after N7x, the amplitude of these potentials was highly enhanced and the latencies of all components had shortened (Fig. 1 C). More dramatic changes were observed in the ipsilateral MI. A few minutes after N7x, evoked potentials could be elicited in the MI ipsilateral to the stimulation, and their amplitude increased and remained high until the end of the experiments (Fig. 1 D).

A total of 84 neurons were isolated in the punctum maximum area of the vibrissal field in the contralateral MI after N7x. The response pattern and laminar distribution of the recorded neurons with responses to peripheral stimulation seemed to be identical to those described earlier in normal rats (Farkas et al. 1999). As expected, the application of GABA or muscimol inhibited most of these neurons (not shown here). However, a small percentage of the neurons (3 of the 84) displayed a reversal in response to the juxtacellular application of GABA or muscimol. Figure 2 shows an example. This pyramidal cell, recorded at a depth of 1,100 µm, responded to both pyramidal tract and peripheral vibrissa pad stimulation (Fig. 2 A, B). The application of GABA elevated the responses for 6 minutes (from 41 to 59 spikes/bin width, peak value taken from the first activation period: Fig. 2 C, D and E). The neuron did not display any signs of injury at any time during the registration period.

Discussion

Stimulation of the infraorbital nerve elicits well-characterized responses in the primary somatosensory (barrel) cortex of the rat (Welker 1976). The MI receives a somatotopically organized input from the SI (Miyashita et al. 1994; Izraeli and Porter 1995), and more than one-third of the MI units are also driven by peripheral somatosensory stimulation (Farkas et al. 1999). In normal rats, these evoked responses are strictly lateralized: they can be seen only in the MI contralateral to the stimulation, even during a prolonged period of stimulation (Farkas et al. 1999). After N7x, however, in 80% of the cases, the evoked responses were facilitated in the MI contralateral to the stimulation, and responses also appeared in the ipsilateral MI (Fig. 1). A few minutes after N7x, evoked responses could be observed and gradually increased in amplitude in the ipsilateral MI. In the rat, the MIs in both hemispheres are strongly interconnected by commissural fibres (Z borszky and Wolff 1982). The application of picrotoxin to the SI contralateral to the stimulation in normal rat elicited changes in the MIs in both hemispheres that were similar to the changes produced by N7x: the evoked potentials in the MIs on both sides were facilitated (Toldi et al. 1996, 1999), and some hours after picrotoxin application, large, type-II spike-waves (Coenen et al. 1995) could be detected. All these observations suggest that $GABA_A$ receptor-dependent changes take place in the SI and MI after N7x, which allow the unmasking of associative and commissural connections resulting in the appearance and facilitation of evoked potentials in the MIs in both hemispheres.

It is well known that GABA is the major inhibitory transmitter in the central nervous system of adult mammals (Macdonald and Olsen 1994). The opening of Cl^- channels in the cortical neurons of adult animals allows Cl^- to enter the cell because of the concentration gradient (inside 7 mM, outside 130 mM; Zigmond et al. 1999), which causes hyperpolarization of the cell membrane (Sivilotti and Nistri 1991). Under certain circumstances, however, GABA may act as an excitatory transmitter, e.g. after stimulation with high frequency, after axotomy, in heat stress or in a hypo-osmotic situation (van den Pol et al. 1996; Kaila et al. 1997; Taira et al. 1997). In these cases, an increased intracellular Cl^- concentration was found, which resulted in a Cl^- efflux (instead of an influx) during $GABA_A$ receptor activation (Ben-Ari et al. 1997; Kaila et al. 1997). In slice preparations containing a small ischaemic lesion, Neumann-Haefelin et al. (1995) showed that the neurons recorded closely to the infarct had a less negative membrane potential, and that there was a weaker GABA-mediated synaptic inhibition. From other studies, it is known that only a small decrease (10%) in GABAergic inhibition can lead to epileptic seizures through the horizontal spreading of cortical activation (Chagnac

Amitai and Connors 1989). Our own results demonstrate that in a few cases GABA or its agonist can serve as an excitatory transmitter (Fig. 2), possibly through a reversed Cl^- gradient. The presumed reversed Cl^-gradient of some neurons after N7x has been supported by a new histochemical technique. A transient Cl^- accumulation after N7x has been revealed in subpopulations of cortical neurons in the MIs and SIs in both hemispheres (Toldi et al. 2000). For technical reasons, at present we cannot prove that the Cl^--rich cells are identical with those of neurons which display a reversal in response to GABA, and we cannot prove that the Cl^- accumulation is the reason for the reversal effect of GABA. Disinhibition of a pyramidal cell (e.g. by inhibition of a GABA interneuron) cannot be excluded, which may also display a reversal in GABA effect in extracellular recording.

The clinical relevance of this type of studies is obvious. The injury of a peripheral nerve or an injury within the CNS (e.g. an ischaemic infarct) may cause serious malfunctions of the nervous system (phantom pain or synkinesia), which could be repaired at least partly during the recovery (Merzenich and Jenkins 1993; Heiss and Graf 1994; Johansson and Grabowski 1994; Nudo and Friel 1999). We hypothesize that the decrease in the GABAergic inhibition favours recovery by facilitating plastic changes, but in parallel with this the risk of seizures becomes greater.

Conclusions

After a facial nerve cut, there is a possibility for the appearance and enhancement of potentials in the MIs in both

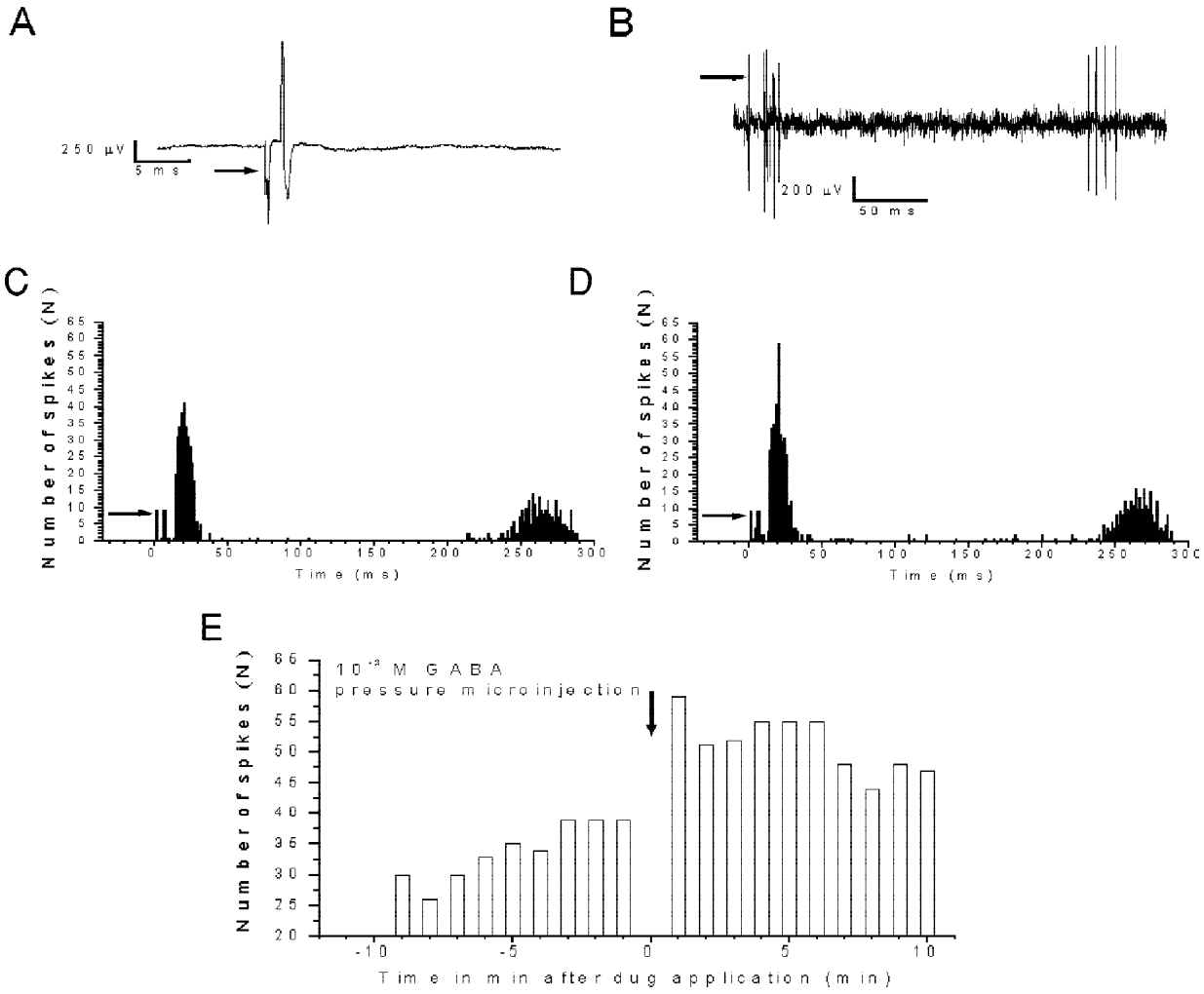

Figure 2. Responses of an identified pyramidal neuron to 10^{-3} M GABA pressure microinjection. **A**: The pyramidal tract stimulation elicited an antidrom response with short latency (2-3 ms). **B**: After identification of a pyramidal cell, its somatosensory input was tested with contralateral vibrissa pad stimulation. Poststimulus time histograms of a neuron show the evoked unit activity 1 min before **(C)** and after **(D)** drug application. The motor cortical neurons displayed the typical response pattern described previously (Farkas et al. 1999). **E**: The change in evoked unit activity elicited by GABA microinjection. This graph shows the time course of the change in spike activity of the neuron presented in **C** and **D**. Horizontal arrows point to the stimulation artefacts (in **A**, **B**, **C** and **D**), while the vertical arrow in **E** indicates the drug application.

hemispheres, evoked by unilateral trigeminal stimulation. The bases of the new activity pattern are the cortical associational and commissural connections, which are partly disinhibited after peripheral nerve injury. Our observations at a cellular level suggest that the reason for this phenomenon might be a $GABA_A$ receptor-dependent decrease in inhibition, caused by the transient Cl^- accumulation of some pyramidal cells in the de-efferented MI.

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