THESES OF THE DISSERTATION

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# PLASTICITY OF THE PRIMARY SOMATOSENSORY CORTEX IN ADULT RODENTS

Szeged
2002

The "on" and "off" responses are differentially influenced by increased or decreased use of the system, that suggests different mechanisms in their generation.

Our pharmacological results suggest that the acetylcholine and its muscarinic agonists play a modulatory effect on the barrel cortex. The fact that same drug exerts different effects on the same neuron depending on the direction of the stimulus results that direction coding is a segregated function within barrels, i.e. different neuron populations are responsible for the processing of stimuli in different directions. The behaviour of "on" and "off" responses suggests the same. That cholinergis agents affect only one latency component of the evoked activity implies that these drugs act only on a small number of synapses in the barrel field.

The above results are hopefully heplpful in understanding better the plastic changes of the cerebral cortex, thus taking us closer to the understanding of higher cortical functions.

## OBJECTIVES



## INTRODUCTION

Throughout our life the nervous system is continuously adapting to the ever changing environment, to behavioural challenges. and pathological states. This capacity is termed *plasticity*. A fundamental issue in neurobiology is the morphological and physiological background of these adaptive changes. The sensory, motor and higher cognitive functions can be induced to adapt by different types of challenges. The brain itself can get damaged, or the peripheral nerves, elimination of receptors causes the sensory system to loose its inputs, while damage to the motor system of the body deprives the neural motor system of its efferents, but changes of the environment – its being enriched or less rich in stimuli – or challenges on the behavioural level, learning tasks can all induce adaptive mechanisms to switch on. In our present experiments we will make an attempt to detect some details of denervation, deprivation or increased use induced plastic changes on the primary somatosensory cortex of adult rodents. Thereafter we examine the effects of the plasticity associated cholinergic system on the evoked unit activity of neurons on the *barrel field*.

**1**. In the first set of experiments we were trying to find out how the primary somatosensory cortex of adult rodents reacts to the elimination of its input. We crushed the infraorbital nerve (ION) and followed the dynamics of cortical topographical reorganization., in other words, the representational plasticity the primary somatosensory cortex.

We measured how the spatial organization of the affected cortical area was affected, as well as the neighbouring zone and their spatial relations. We followed the changes in time until the crushed nerve functionally regenerated. We tried to answer the question if the elimination of the input is mirrored in the cortical organization of the affected input. If it is, then for how long the changes are detectable and if its extent changes during the time of regeneration. The experiments were carried out on rats, and following the crush of the infraorbital nerve, that sensorily innervates the whisker pad, we mapped the extent of the whisker and forelimb representation areas on the primary somatosensory cortex, with special interest on their representational boundary. We examined the presence or absence of the evoked responses upon whisker and forelimb stimulation. The measuring points were placed 300-500 μm from each other. The measurements were carried out simultaneously on the cortical surface and intracortically. The animals were examine immediately, 3, 7, 8, 10, 11, 12, 13, 18 and 60 days after the injury.

**2**. In our second experiment we tried to detect plastic changes induced by more delicate interventions. For this reason we subjected the vibrissal system to increased use, then by cutting the whiskers we inhibited its function preserving the nervous system intact. We examined



## DISCUSSION

Our experiment on representational plasticity is new in the sense that it employs natural stimulation, from two different body parts and follows the changes for up to two months. The observed changes are most likely the consequences of disinhibition caused by the elimination of the thalamo-cortical and callosal inputs that normally shapes receptive fields. There might be different mechanisms responsible for the changes occurring later. It is supposed to be a consequence of a new balance between excitatory and inhibitory influences on the cortex. The morphological substratum behind it is thought to be the synaptic reorganization, that is supported by numerous molecular and cellular results.

Preserving the nervous system intact, simply increased and decreased use of the vibrissal system can induce plastic changes on the cortical level in adult age. In our experimental paradigm it was possible to measure evoked potentials repeatedly on the same group of animals. During deprivation a reduced cytochrome-oxydase staining as well as a decreased level of energy related enzymes was found that might be partly responsible for the slowing of cortical processing. During development an experience-dependent change in glutamate receptor constellation was described. NMDA receptors are gradually replaced by AMPA receptors that are known to have faster dynamics, thus might be responsible for shortening of latencies.

**Cortical plasticity following increased use of the ION and deprivation**

We took an attempt to induce cortical plasticity in adults preserving the integrity of the nervous system. We found, that following increased use of the vibrissal apparatus causes a shortening of latencies of some components of the evoked responses. Based on these results we concluded the fastening of information processing in the cortex as a result of increased use. Cutting the whiskers induced an opposite effect, i.e. the latencies shortened to some extent. We noticed that the "on" and "off" responses did not change on the same way.

**Effects of cholinergic drugs on the evoked unit activity**

Based on the literature the role of acetylcholine in cortical plasticity is feasible. As the effect of this neuro.modulator is not fully understood we set out to measure its effects on evoked unit activity in the barrel cortex. We found that the acetylcholine usually effects only one latency component of the evoked activity, and this modulatory effect can either be excitatory or inhibitory. These effects seemed to be dependent on the stimulus parameters, especially its direction. The "on" and "off" response complexes changed in different ways under the influence of the same drug.

the changes of the somatosensory cortical evoked potentials. We attempted to find out if the consequences of these physiological or nearly physiological interventions are detectable by minimally invasive electrophysiological techniques, and if they are, then what is their nature. To accomplish this we recorded evoked potentials over the somatosensory and motor cortices of mice epicranially, i.e. by electrodes placed on the skull. First we recorded in the naïve state, then following a three-week trial-session in the radial arm maze. On the same group of animals the third electrophysiological experiments followed a period of some weeks of whisker cutting. Our conclusions were drawn based on the parameters of the evoked responses. We measured latencies, amplitudes, the width of the response complexes, differences and temporal relations of the sensory and motor peaks.

**3**. In the third set of experiments we tried to approach the physiological background of the above changes by pharmacological methods. We did this by applying cholinergisc pharmacons intracortically by pressure-microinjection and – like in all previous experiments – naturally stimulated the vibrissae, while extracellularly recorded the activity of neurons of the primary somatosensory cortex. We intended to answer the question of how these drugs modulate the evoked activity of the cortical neurons. We examined the different latency components of the multi-unit responses and their changes after drug application, using different stimulus parameters.

## METHODS

### Behavioural experiments

On the course of these experiments the animals had to explore a new, enriched environment. For this purpose we employed a modified version of the eight-arm radial maze. The goal was to ensure an increased use of the vibrissal apparatus throughout a three week period. Every day, every animal could spend 10 minutes in the maze. The radial maze itself is a plexi-glass instrument fixed 50 cm above the ground. There is a 15 cm diameter central platform, and 8 identical arms placed 6 cm away from the central platform. This gap size is optimal for the animals to palpate the entrance of the arms after being placed in the middle. They cannot use their forelimbs for palpation, but after making a decision they coud step over the gap. The entrance of each arm was covered by sandpaper of the same roughness, except for one, that was different. In this one arm the animal could get access to food reward. Under these conditions the animals used excessively their vibrissal system to explore the new environment as well as to get acces to alimentation.



## RESULTS

### Cortical plasticity after infraorbital nerve crush

Cells of the barrel cortex responded to the stimulation of the principal whisker intensivela, with short latency (less than 15 ms). On these cells responses could not be evoked by stimulatig the forelimb digits, neitheir we came across cells with receptive fields on different body parts during the same penetration (in case we went perpendicular to the cortical surface). Moving medio-rostrally the cells became sensitive to forelimb stimulation.

We found that the effect of ION crush is seen shortly after the intervention as a reorganization of cortical topography. This took the form of expansion of the neighbouring cortical area, that is responsible for the representation of the forepaw digits. This new topography exists for at least three days after which regeneration of the nerve brings forth the reappearance of whisker evoked responses. Thus the two neighbouring area does not separate from each other as the extent of the digits' representation is still greater than in controls. The representation areas of whiskers and digits has an overlapping zone during this regeneration period. Two months after the nerve crush the representational areas are very similar to the control conditions.

**Pharmacological experiments**

Throughout these experiments we attempted to test the effects of cholinergic drugs on evoked unit activity of the barrel cortex. To avoid systemic effects we applied the substances by pressure micro-injection in the close vicinity of the recorded cells. To accomplish this we attached a low impedance glass capillary to the side of the recording electrode so as their tips were not farther than 30-50 μm from each other. By a hydraulic pressure injector system we could inject amounts as small as 20-40 pl into the IV-V layer of the cerebral cortex. Acetyl-choline, acetyl-L-carnitine, carbachol, scopolamine and atropin were used in concentrations of $10^{-4}$-$10^{-3}$ M, dissolved in physiologic saline. We started recordings some minutes before application of the drug and continued for 16-20 minutes afterwards. Responses were processed as described above.



**Sensory deprivation and denervation**

Throughout our experiments we did not only intend to answer the questions of the increased use of a sensory system, but also of the consequences of the elimination of a sensory input. To accomplish this we crushed the infraorbital nerve that innervates the whiskers, or cut the vibrissae on the whisker-pad.

We performed the nerve crush on adult rats. In short anaesthesia we cut the common fur on the muzzle between the eyes and the caudalmost whisker row. Here we opened the skin and bluntly dissected the tissue overlying the infraorbital nerve. After making it visible, we subjected the nerve fibres to 30 seconds of pressure by a fine pair of pincers. Thus by preserving the continuity of the nerve we made it lose its function without any bleeding so the animal was deprived of the right side vibrissal input.

On mice we attempted to produce sensory deprivation while leaving the nervous system intact. We reached this by cutting the whiskers continuously for three weeks under ether anaesthesia.

**Electrophysiological experiments**

In the experiments carried out on rats we opened the skull to get access to the cortical surface so as to be able to record from the cortical surface as well as intracortically. These recordings were done simultaneously in order to map the activity of the somatosensory cortex evoked by naturally stimulating two different inputs. The contralateral whiskers and forelimb digits were mechanically stimulated by an electromechanical stimulator with adjustable frequency, stimulus width, amplitude and slope. For recording macropotentials we employed a silver macro-electrode that we placed over the cortical surface under an operating microscope. We searched for the *punctum maximum* of signals by this electrode. Then we inserted the microelectrode into the cortex at the same location. Recordings were made at 300-500 μm intervals. We constructed a schematic stereotaxic map of the cortical surface, where we put down each recording points. Thirty on-off cycles were averaged.

During intracortical recordings we measured extracellular unit-activity. The capillary used for recording had a diameter of 2 μm, and a resistance of 5-10 MΩ. Recordings took place in 600-800 μm deep in the cortex.



Following amplifying and filtering the signals (50 Hz, 5 kHz) we digitalized them (Digidata 1200, software pClamp6, Axon Instruments) and saved on disk. From the average of 30 sweeps we generated PSTHs.

In the experiments that were done on mice minimal invasivity had priority, because the animals were supposed to survive the experiment in a good condition to be able to take part in latter phases of the study. Thus we employed the epicranial evoked potential recording method. Following the exposure of the skull we placed 2 electrodes over the somatosensory and motor cortices, contralateral to stimulation, right on the bone over the corresponding cortical area. The gained responses were treated the same way as above. After the experiment the scalp was closed and the animals were kept under observation until waking-up.

Signals were termed “on” responses in case they followed the stimulus onset within 100 ms, and “off” responses if they had the same temporal relation with the stimulus offset. Peaks of the response complexes were designated by letters from a to *g*.

**TÁRSSZERZŐI NYILATKOZAT**