Abstract Pairing a cutaneous electrical stimulus of the hind-paw with stimulation of the basal forebrain produces long-term cholinergic enhancement of the responsiveness to a tactile stimulus. A short period of pairing (20 trials) increased the area of the two main components of the evoked potential by 37.1±13.5% (±SEM) and 37.9±6.8%, respectively. The effects lasted for the duration of the experiment (>2 h). The enhancement could be blocked by either MK-801, an NMDA receptor antagonist or by L-NAME, a nitric-oxide-synthase inhibitor when they were given prior to pairing. Control experiments with skin stimulation alone and basal forebrain stimulation alone had only small long-term effects (~10%) on the size of the evoked potential. Thus, long-term cholinergic enhancement, attributable to disinhibition and increased release of acetylcholine in the cortex during neuronal excitation by other sources, and so named because it is blocked by atropine, may be a form of long-term potentiation. The existence of such a mechanism for the control of cortical neuronal plasticity identifies the basal forebrain as a powerful modulator of long-lasting changes in cortical neuronal excitability.

Keywords LTP · Neuronal plasticity · Evoked potentials · Somatosensory cortex · Acetylcholine

Introduction

Learning, memory, and behavioral compensation following lesions of the nervous system necessarily depend upon long-lasting changes in the excitability of neurons; novel behavior is acquired through functional changes at cortical and subcortical levels (Wolpaw 1997). The principal cellular mechanism thought to underlie neuronal plasticity, long-term potentiation (LTP), is generally studied in hippocampal slices and only more recently in cortical slices (e.g., Castro-Alamancos et al. 1995). Whole-animal models of neuronal plasticity are relatively few (cf. Thompson 1967; Jenkins et al. 1990; Weinberger 1995, 1998), and seldom have these been subjected to the pharmacological manipulations needed to test the hypothesis that LTP plays a role in cortical neuronal plasticity.

We produced a long-lasting enhancement of neuronal excitability in the somatosensory cortex of anesthetized rats by pairing basal forebrain (BF) stimulation with cutaneous stimulation. We hypothesize that BF activation creates conditions favorable for neuronal plasticity, and that the plasticity observed depends upon LTP. This is a twofold hypothesis; the first part concerns the effects of the BF, whereas the second part concerns the implication of LTP in cortical neuronal plasticity. For the first part, previous experiments from this laboratory have demonstrated that, when acetylcholine (ACh) is released in the vicinity of neurons depolarized either by glutamate or by a sensory stimulus, the responsiveness of the treated neurons is enhanced for long periods (Metherate et al. 1988a, 1988b; Tremblay et al. 1990a, 1990b). We believe that this long-term cholinergic enhancement (LTCE) is due to cholinergic facilitation of calcium entry into cortical neurons via ACh-dependent calcium channels and N-methyl-D-aspartate (NMDA) channels. Evidence that ACh can enhance neuronal plasticity of sensory cortex comes from studies in both somatosensory cortex and auditory cortex (Rasmusson and Dykes 1988; Metherate and Ashe 1991; Weinberger 1995, 1998; Bakin and Weinberger 1996).

The BF is stimulated in these experiments because it is the major source of the ACh released in neocortex. It is activated by attention, arousal, and learning (cf. Rasmusson 2000), conditions where ACh should promote neuronal plasticity. At least one third of the projections from the BF are cholinergic (Gritti et al. 1997), and another very significant proportion are GABAergic (Freund and Meskenaite 1992). The other projections from the BF to the cortex use unidentified neurotransmitters. One of us has proposed elsewhere (Dykes 1990,
1997) that activation of either or both the cholinergic fibers and the GABAergic fibers projecting to cortex from the BF will create conditions during which the activation of the classical glutamnergic sensory pathways from the thalamus are more likely to produce LTP. This facilitatory effect from the BF occurs because the signal traveling on the sensory pathways arrives in cortex during the time that GABAergic BF activity has caused disinhibition and cholinergic BF activity has slowed repolarization of action potentials and opened acetylcholine-dependent calcium channels. All of these actions of the BF favor LTP. The BF and ACh have also been implicated in the reorganization that follows peripheral nerve injury (cf. Juliano et al. 1991; Webster et al. 1991). Although the permissive state may be created by the BF, it is not the source of the sensory information that activates the neurons in primary somatosensory cortex. These signals arrive in cortex along the usual afferent pathways to define the nature of the change that will occur while the BF is active.

In the work reported here, we turned to the second part of the hypothesis stating that the long-lasting changes observed during LTCE depend on the activation of NMDA channels. At the level of single cells (Mualouf et al. 1998b), we have demonstrated that neuronal plasticity in somatosensory cortex involves the activation of NMDA channels in an established paradigm of sensory preconditioning (Delacour et al. 1987). Because the NMDA-dependency of neuronal plasticity could only be examined for relatively short intervals in small numbers of cells in that preparation, we returned to an evoked-potential preparation where LTCE could be followed for a longer time in a larger population of neurons. To test the hypothesis that LTCE shares, with some forms of LTP, a dependence upon the activation of NMDA channels, we blocked LTCE with MK-801, an NMDA-channel blocker. To pursue the analogy further, we also used L-NAME, a nitric-oxide-synthase inhibitor known to prevent LTP in hippocampal slices (Hawkins et al. 1998).

Materials and methods

Male, Sprague-Dawley rats weighing between 200 and 400 g were anesthetized with urethane (1.6 g/kg i.p.). Temperature was maintained at 37.5°C with a feedback-controlled heating pad. The animal's head was positioned in a stereotaxic frame and a 5×10 mm craniotomy was centered 2 mm posterior and 2 mm lateral to bregma, exposing the hindlimb representation in the right hemisphere.

Stimulation of the basal forebrain

The projections from the BF to the somatosensory cortex of the rat have been described by Baskerville et al. (1993). Concentric bipolar stimulating electrodes (Kopf Instruments, Tujunga, Calif., USA) targeting the BF were inserted 6.0 mm below the cortical surface at a 20° angle, 1.3 mm posterior and 5.2 mm lateral to bregma. From that point, the electrode was advanced in 0.5-mm steps. Following each step, the electrode was activated with a train of 50 stimuli at 100 Hz. When a site was found that produced a short-latency, prolonged excitation (≥1 s) in the somatosensory cortex, which Jiménez-Capdeville et al. (1997) have shown is correlated with ACh release, the electrode was fixed there for the duration of the experiment. We recognize that these repeated stimuli delivered during the approach to the BF might produce changes in the cortex. However, if changes occurred because of these search stimuli, they should have made our results more difficult to observe (cf. Howard and Simons 1994).

Recording electrode

Low-impedance (≥0.5–1.0 MΩ) electrodes were prepared by filling a glass pipette (1 mm diameter) with either a carbon fiber (7 µM diameter) or a tungsten wire. After pulling this assembly in a Narishige vertical electrode puller, the part containing the carbon fiber was saved, the protruding fiber was cut or etched to expose only a few microns beyond the glass, and the pipette was then filled with 0.9 M saline just before use. When tungsten-in-glass electrodes were used, they were shaped in a similar manner by a second etching after the pipette had been pulled around the tungsten wire in the vertical pipette puller.

Electrophysiological procedures

The electrophysiological signals were led to a preamplifier (Princeton Applied Research, Princeton, N.J., USA), where they were filtered to accept frequencies between 300 and 3000 Hz. A second stage of amplification (Model 5112; Tektronix; Beaverton, Ore., USA) increased the total gain to 2000×, and the output was led to a laboratory interface (CED 1401; Cambridge, England) controlled by a laboratory 486 computer.

The cortical region located 1.5 mm lateral to the midline and 1.0 mm posterior to bregma was mapped quickly to locate the portion of primary somatosensory cortex responding to light mechanical stimulation of the hindlimb digits. The responsive region was located by inserting the electrode into the middle layers of cortex (0.5–1.0 mm below the surface) and by searching the hindlimb for a site responsive to cutaneous stimuli, as detected with an audioamplifier driven by the amplified neural activity. When a cortical site responding to stimulation of the digits was found, the electrode was left in place and two small pins were inserted into the skin, one on each side of the receptive field, to deliver pulsed electrical stimuli of constant current. Once the preparation was stable, the filters were changed to pass signals between 10 and 300 Hz, allowing the evoked potential generated by a single 0.5-ms cutaneous stimulus to be observed while attenuating the higher-frequency, single-unit activity.

Stimulus paradigm

The level of excitability of the sensory pathway and the somatosensory cortex was tested regularly by identical stimuli delivered every 30 s with a single pulse (0.5 ms). The evoked potential was collected with commercial software (Spike 2, CED; Cambridge, England). Groups of 20 stimuli were recorded as a single data set representing a 10-min sampling period. Once a baseline series had been collected (usually 40 min), the stimulation of the BF was paired with the skin stimulus.

BF stimulation consisted of 0.5 s of a train of 50 stimuli (0.2-ms pulses at 100 Hz) ending 100 ms before the first skin stimulus. The skin was stimulated five times with a single pulse (0.5 ms) at intervals of 0.5 s for every BF stimulus train. The rationale for this pattern comes from the fact that the effects of ACh upon cortical neurons last for several seconds, so the five stimuli to the skin, lasting 2.5 s, were within the period during which the cortex was influenced by the released ACh (Krnjevic et al. 1971). After this pairing procedure, which combined BF and skin stimulation, the baseline series, consisting of a single cutaneous stimulus delivered every 30 s, was resumed and data collection was continued for at least another 120 min (Fig. 1A). In some control experiments, animals did not receive the BF stimulation, but evoked potentials elicited by the cutaneous stimulus were recorded every 30 s for a duration of at least 2 h. In other control experiments, the trains of five cutaneous stimuli were given during the