LONG-TERM POTENTIATION IN VISUAL CORTICAL PROJECTIONS TO THE MEDIAL PREFRONTAL CORTEX OF THE RAT

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Abstract—In order to investigate neural mechanisms by which the prefrontal cortex adaptively modifies its activities based on past experience, we examined whether or not sensory cortical projections to the medial prefrontal cortex support long-term potentiation (LTP) in rats. Monosynaptic projections from the secondary visual cortex, mediomodal area (V2MM) to the infralimbic cortex were confirmed by orthodromic as well as antidromic activation of single units. High-frequency stimulation (50 Hz, 2 s) induced LTP (approximately 45% increase over the baseline) in the V2MM projection to the infralimbic cortex. LTP induction in this pathway was completely blocked by an injection (i.p.) of CPP, an N-methyl-D-aspartate receptor antagonist. LTP was also induced in the ventral hippocampal projection to the infralimbic cortex by the same high-frequency stimulation. The present results suggest that modification of synaptic weights of afferent sensory cortical projections is one mechanism underlying learning-induced changes in prefrontal cortical neural activities. © 2003 IBRO. Published by Elsevier Science Ltd. All rights reserved.

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Modifying behavioral strategies in accordance with changes in environment is extremely important for survival. The prefrontal cortex (PFC) is likely to play a crucial role in this adaptive process considering that one important function of the PFC is the planning of future behaviors (Goldman-Rakic, 1987; Fuster, 1997). In this regard, one of the well-known deficits following damage in the PFC is inability of an animal to adapt to changes in behavioral tasks (Fuster, 1997). Results from single-unit recording studies also indicate that the activities of PFC units are altered by learning (Watanabe, 1990; Maxwell et al., 1994; Asaad et al., 1998; Schoenbaum et al., 1999; Mulder et al., 2000; Rainer and Miller, 2000; Baeg et al., 2001). These studies indicate that learning is an essential component of the PFC functions. The mechanisms of learning-induced changes in PFC activity are currently unclear. The changes in PFC activity may reflect learning-induced alterations in the input structures. On the other hand, considering that long-term synaptic plasticity has been shown in the PFC, it is likely that synaptic plasticity within the PFC plays an important role in this process. In rats, long-term potentiation (LTP) and long-term depression have been demonstrated in PFC slices (Hirschi and Crepel, 1990), hippocampal projections to the PFC (Laroche et al., 1990; Doyere et al., 1993; Mulder et al., 1997; Takita et al., 1999), thalamic projections to the PFC (Herry et al., 1999; Gemmell and O’Mara, 2002), and commissural PFC projections to the contralateral PFC (Gemmell and O’Mara, 2000).

The PFC is extensively interconnected with cortical areas that are involved in sensory processing. In monkeys, the primary sensory cortices of the visual, somatosensory, and auditory systems do not send direct projections to the PFC. Instead, they first project to adjacent sensory associative areas. In parallel with sequential projections to higher-order sensory-association areas, each area in the sequence projects to discrete regions of the PFC (Pandya and Yeterian, 1990; Barbas, 1992; Fuster, 1997). The PFC of the rat is also extensively connected with sensory-association areas for these modalities (Conde et al., 1995). Regarding smell and taste, the primary olfactory and gustatory cortices send direct projections to the PFC (Rolls, 1989; Barbas, 1993; Carmichael et al., 1994; Conde et al., 1995). Thus, external sensory information is richly provided to the PFC from primary or associational sensory cortices. Although synaptic plasticity has been reported in various projection systems in the PFC (see above), it is currently unknown whether or not sensory cortical projections to the PFC support synaptic weight changes. Synaptic plasticity in these pathways would provide the PFC with the flexibility of encoding specific sensory inputs and adaptively modifying its activities in response to previously meaningful stimuli.

In this study, LTP induction was examined in the projection from the secondary visual cortex, mediomodal area (V2MM) (Paxinos and Watson, 1998) to the infralimbic cortex (ILC), which was previously shown by a retrograde tracing study (Conde et al., 1995), in adult rats. Parts of the results have been reported in abstract form (Kim and Jung, 1999).

EXPERIMENTAL PROCEDURES

Subjects

Experiments were performed with male Sprague–Dawley rats (approximately 9–11 weeks old, 250–330 g, n = 49). Animals were
housed in the colony room with 12-h light/dark cycle and allowed free access to food and water. The experimental protocol was approved by the Ethics Review Committees for Animal Experimentation of the Ajou University School of Medicine. All efforts were made to minimize the number of animals used and their suffering.

**Surgery**

All experiments in this study were performed under deep urethane anesthesia (1 g/kg). The anesthesia level was monitored by frequently checking response to tail pinch. Additional administration of urethane was administered by intravenous injection of 0.2 g/kg or 0.3 g/kg as necessary. Deeply anesthetized animals were mounted on a stereotaxic instrument (Narishige, Tokyo, Japan) and small bone flaps were removed over the medial prefrontal cortex (mPFC) and either the ipsilateral V2MM or the ipsilateral ventral hippocampus. Burr holes were drilled and two stainless steel screws were mounted on the skull on top of the opposite parietal cortex and cerebellum serving as a ground and a reference lead, respectively.

**Unit activation**

For confirmation of monosynaptic projections from the V2MM to the ILC, single units in the ILC were activated by stimulating the V2MM (orthodromic stimulation) and, conversely, those in the V2MM were activated by stimulating the ILC (antidromic stimulation). A single unit was recorded with a glass micropipette filled with 2 M NaCl (outer diameter (O.D.): 1 mm, impedance: 5–10 MΩ) in the ILC (A: 2.7–3.2, L: 0.3–0.5 mm to bregma, V: 4.1–4.5 mm to brain surface) or V2MM (A: 4.3, L: 1.7–1.9 mm to bregma, V: 1.0–1.4 mm to brain surface, Fig. 1A) and constant current, rectangular pulses (100–200 μA, 400 μs, 0.1 Hz) were delivered through a bipolar stimulation electrode (twisted strands of teflon-coated stainless steel wire, 113 μm O.D.) placed in the V2MM or ILC, respectively. Two percent Pontamine Sky Blue (Sigma, St. Louis, MO, USA) was added to the pipette solution in some experiments. Spike collision was tested by activating units in the V2MM by ILC stimulation at 0.5 Hz and analyzing all spontaneous spikes that preceded a stimulus pulse within 40-ms time window. The largest interval at which stimulation failed to activate a spike (the maximum tested interval for spike inhibition) and the smallest interval at which stimulation activated a spike (the minimum tested interval for spike activation) were determined for each unit. Responses were amplified 1000 times, band-pass filtered between 300 Hz and 10 kHz, digitized at 80 kHz, and stored on an IBM-compatible personal computer.

**LTP induction**

A bipolar stimulating electrode was placed in the V2MM and a recording electrode (teflon-coated stainless steel wire, 113 μm O.D.) was placed in the ILC (Fig. 1A). For stimulation of the hippocampus, the stimulating electrode was placed in the ventral CA1/subicular area (Fig. 1A). After optimization of electrode placement and stabilization of responses, evoked field potentials were recorded in response to low-frequency stimulation (0.05 Hz) that induced sub-maximal (approximately half maximal) responses. Responses were amplified 1000 times, band-pass filtered between 1 Hz and 3 kHz, digitized at 4 kHz, and stored on an IBM-compatible personal computer. Commercial software (Experimenter’s Workbench; Datavave, Longmont, USA) was used for stimulus control and collection of data. Following establishment of a stable baseline for at least 20 min, an episode of tetanic stimulation (50 Hz, 2 s, the same intensity as in the baseline) was delivered to induce LTP and responses to low-frequency stimulation were collected for an additional hour. An N-methyl-D-aspartate (NMDA) receptor antagonist, 4-(3-phosphonopropyl)-2-piperazine-carboxylic acid (CPP) was purchased from Tocris Cookson (Buckhurst Hill, UK). CPP was dissolved in 0.9% saline solution and injected (10 mg/kg) i.p. 30 min before delivering the tetanic stimulation. The magnitude of LTP was determined by measuring the initial slope of the initial negative field potential component and was expressed as a percentage of the baseline. All data are expressed as mean ± S.E.M. Statistical analysis of the effect of high-frequency stimulation was based on a paired t-test. Statistical comparisons between the magnitudes of LTP in different pathways or under different experimental conditions were done by unpaired t-tests. A P value < 0.05 denoted the presence of a significant statistical difference.

**Histology**

When recordings were completed, an electrolytic current (15 μA anodal, 15 s) was applied through the metal (both stimulating and recording) electrodes. Following recording with a glass micropipette, 2% Pontamine Sky Blue was injected (20 μA, 5 s on/5 s off cycles for 20 min) in all preliminary experiments and some of the main experiments to verify recording locations of single units. The animal was perfused with 0.9% saline and then with buffered 10% formal-saline. The brain was removed, left in 10% formal-saline for 5–6 days, and then transferred to a 30% sucrose solution for 1–2 days until it sank to the bottom. Coronal sections (40 μm thick) were cut on a sliding microtome and stained with Cresyl Violet. Blue staining, tracks, and lesion sites were located by light microscopy.

**RESULTS**

**Stimulation and recording sites**

All stimulation/recording sites were found in the intended brain structures (i.e. the ILC, the V2MM and the ventral hippocampus). Examples of brain sections that contain an electrolytic lesion or track in the ILC, V2MM or ventral hippocampus are shown in Fig. 1B.

**Unit activation and spike collision**

Both orthodromic and antidromic activation of single units were tested in this study to confirm the presence of monosynaptic projections from the V2MM to the ILC. Some units in the ILC were driven by stimulation of the V2MM. We recorded four such units out of 25 recorded units (16.7%; each unit recorded from a separate animal), and an example is shown in Fig. 2A. These units were reliably activated by V2MM stimulation, but activation failure was sometimes observed without preceding spontaneous spikes (i.e. activation failure was not because of a spike collision). The mean latency of unit activation was 20.1 ± 2.8 ms (15.7–23.0 ms). Stimulation of the ILC also activated single units in the V2MM. Sixteen such units were recorded (each unit recorded from a separate animal) and an example is shown in Fig. 2B. The mean latency was 16.3 ± 1.0 ms (range: 11.4–25.6 ms). Unlike the ILC units that were driven by V2MM stimulation, the V2MM units were activated at precisely fixed latencies without any failure in response to ILC stimulation (Fig. 2B). These results suggest that the ILC and V2MM units were orthodromically and antidromically activated spikes, respectively.

For further confirmation of antidromic spike activation, we tested collision between spontaneous and evoked spikes in the V2MM in three animals. The mean firing rate
of the three tested units was 1.9±0.2 Hz (1.5, 1.9 and 2.2 Hz) and the numbers of spontaneous spikes that preceded stimulation within 40-ms window were 31, 27 and 32, respectively. In the first animal, a V2MM unit was driven by ILC stimulation at the latency of 14.6 ms; its activation was inhibited by a spontaneous spike that occurred within 16.2 ms before stimulation (the maximum tested interval for spike inhibition), and it was activated when a spontaneous spike occurred more than 17.1 ms before stimulation (the minimum tested interval for spike activation). The latency of spike activation, the maximum tested interval for spike inhibition, and the minimum tested interval for spike activation were 12.1, 13.8 and 14.6 ms, respectively for the second unit. They were 14.3, 13.8 and 16.8 ms, respectively for the third unit. Spike collision of the first unit is shown in Fig. 2B. These results demonstrate that the V2MM units that were driven by ILC stimulation were indeed antidromic spikes. The minimum/maximum intervals for spike activation/inhibition indicate that the refractory period of the stimulated axons is approximately 2 ms.

**LTP in the V2MM projection to the ILC**

Evoked field potentials recorded in the ILC in response to V2MM stimulation typically showed initial negativity, which was sometimes followed by slow polysynaptic components. Field potentials were recorded in both superficial (n=6) as well as deep (n=3) layers of the ILC. The peak latency of the initial negative component was between 16.6 and 23.7 ms (mean=19.3±2.4 ms). This is longer than the latency of antidromic spikes by a few milliseconds (probably due to a synaptic delay) and similar to that of orthodromic spikes, thus it most likely represents a monosynaptic response. LTP, defined as more than 20% increase in the initial slope of the initial negative field potential component over the baseline at 20–25 min following high-frequency stimulation (50 Hz, 2 s), was induced in the majority of animals tested (seven out of nine animals, Fig. 3A). The average magnitude of LTP, including results from all nine animals, was 45.3±10.4% of the baseline response. Statistical analysis indicated that the effect of tetanic stimulation was significant (P<0.01). The potentiated responses were maintained in a stable manner throughout the recording period (60 min).

**LTP in the hippocampal projection to the ILC**

LTP has been reported in the ventral hippocampal projection to the prelimbic cortex (PLC) and ILC in rats (Laroche et al., 1990; Mulder et al., 1997). We examined whether LTP is induced in a similar manner in the ventral hippocampus.

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Fig. 1. Recording and stimulation locations. (A) Three sagittal sections (0.4, 1.9, 5.5 mm lateral from the midline) of the rat brain that show the three brain structures (ILC, V2MM, and ventral hippocampus) stimulated or recorded in the present study. (B) These examples show histological verification of recording electrode placement in the ILC and stimulating electrode placements in the V2MM and ventral hippocampus. Sub: subiculum. Calibration: 1 mm.
pocampus–ILC pathway by the same high-frequency stimulation (50 Hz, 2 s) that induced LTP in the V2MM-ILC pathway. In general, ventral hippocampal stimulation evoked field potential responses that contained multiple components in the ILC. The peak latency of the initial negative component ranged from 17.6–20.3 ms (mean=18.2±0.7 ms), which is similar to the previously reported latency in the ventral hippocampus-PLC pathway (Laroche et al., 1990). LTP was induced in a reliable manner (five out of five animals) by tetanic stimulation of the ventral hippocampus (Fig. 3B). The average magnitude of LTP was 64.6±4.9%. Statistical analysis indicated that the effect of tetanic stimulation was significant (P<0.01). When the magnitude of LTP in this pathway was compared with that in the V2MM-ILC projection, there was no significant difference, indicating that the same tetanic stimulation induced similar amounts of potentiation in the two pathways. Although LTP in the two projection systems was similar, there was a difference in the time course of LTP development; LTP developed more slowly in the V2MM projection compared with that in the hippocampal projection.

Effect of CPP on LTP induction

We then examined whether or not NMDA receptor activation is required for the LTP observed in the V2MM projection to the mPFC. For this, CPP, which is an NMDA receptor antagonist, was injected (i.p.) 30 min before delivering the tetanus. Injection of CPP induced no detectable changes in the baseline responses. The initial slope of field potential responses following CPP injection was 102.4±1.4% of that before the injection. When delivered to the CPP-injected animals, tetanic stimulation did not enhance evoked field potential responses (n=five animals; Fig. 4B). The average magnitude of potentiation was 2.4±1.4%, which was not a significant enhancement over the baseline. In contrast, LTP was reliably induced in vehicle (0.9% saline)-injected animals in all cases tested (n=five animals; Fig. 4A). The magnitude of LTP was
65±9.7%, which was a significant increase over the baseline (P<0.01). There was no significant difference in the magnitudes of LTP between vehicle-injected and control (uninjected) animals. These results provide evidence that LTP in the V2MM–mPFC projection requires activation of NMDA receptors.

**DISCUSSION**

**Monosynaptic projections from the V2MM to the ILC**

Previous studies have reported conflicting results on the monosynaptic projection from the V2MM to the ILC. Whereas a retrograde labeling study found evidence for direct projections from the V2MM (Conde et al., 1995), an anterograde labeling study did not (van Eden et al., 1992). In the present study, we could activate single units in the V2MM by stimulating the ILC, and, conversely, activate single units in the ILC by stimulating the V2MM. The V2MM units were driven at precisely fixed latencies without failure by ILC stimulation and inhibited by a previous spontaneous spike (spike collision), indicating that they were antidromic spikes. These results provide compelling evidence that there exist monosynaptic projections from the V2MM to the ILC. Stimulation of the V2MM evoked field potentials in the ILC with a similar latency to that of orthodromically activated units, strongly suggesting that they represent monosynaptic responses.

Then why was this projection not detected in a previous anterograde labeling study? This may be due to that extensive versus focal injections were made in the previous retrograde- and anterograde-labeling studies, respectively (van Eden et al., 1992; Conde et al., 1995). It is possible that the cells projecting to the ILC were not well labeled by a focal injection of anterograde tracers. Our results are consistent with this possibility. It was quite difficult to obtain sizable (>0.2 mV) field potentials in the ILC by stimulating the V2MM, suggesting that V2MM cells projecting to the mPFC are not diffusely distributed, but may be concentrated at some hot spots. In such a configuration, it would be difficult to hit the right spot with a focal injection of anterograde tracer. We expect that injection of anterograde tracers in a number of locations within the V2MM will result in labeling in the ILC.

**Encoding of external sensory information in the PFC**

Previous studies have shown that PFC units change their activity patterns through experience (Watanabe, 1990; Maxwell et al., 1994; Asaad et al., 1998; Schoenbaum et al., 1999; Mulder et al., 2000; Rainer and Miller, 2000; Baeg et al., 2001). These changes probably reflect adaptive modification of behavioral strategies. What underlies such alterations in PFC activity? It is likely that modification of synaptic weights is responsible for such alterations. Synaptic weight changes may occur within the PFC or in the brain areas that send afferent projections to the PFC. Alternatively, synaptic weight changes in the PFC projections back to the input structures may modify processing of...
incoming sensory information so that PFC unit responses to learned stimuli are altered. It is also possible that reciprocal projections between the PFC and downstream brain structures, such as the premotor cortex, undergo synaptic weight changes so that PFC unit activities are modified by the influence of altered interactions between the PFC and downstream brain structures. Currently very little is known about the nature of changes that underlie learning-induced alterations in PFC neural activity. To this end, we first need to elucidate synaptic plasticity rules in identified projections to the PFC. The present results demonstrate that LTP can be induced in a reliable manner in visual cortical projections to the ILC in adult rats. This is the first demonstration that sensory cortical projections to the PFC support LTP. As there exist extensive projections from sensory afferential areas to the PFC (see introduction), assuming that other sensory cortical projections to the PFC also support LTP, the present results suggest that a large fraction of external sensory information is not only processed but also registered in the PFC. This will broaden the range of adaptive changes the PFC can make based on past experience. Based on this mechanism, for example, when an animal encounters a previously important sensory stimulus again, the PFC may come up with an appropriate behavioral plan quickly, without referring to memory systems to evaluate its significance. Future investigations should address whether or not synaptic plasticity shown in identified projection pathways to the PFC actually contributes to learning-induced alterations in PFC neural activity in behaving animals.

**Dependence of LTP on NMDA receptor activation**

This study also shows that LTP induction in the V2MM–ILC projection is dependent upon activation of NMDA receptors, suggesting that the ILC registers important external sensory information following Hebbian learning rule. This conclusion is not strong, however. I.p. injection of CPP may have induced unknown side effects, completely apart from the blockade of NMDA receptors in the ILC, which disturbed the LTP induction process. For example, let us assume that an optimal level of dopamine release is critical for LTP induction in the V2MM projection to the ILC. Dopamine is known to influence synaptic plasticity in the mPFC (Otani et al., 1998; Gurden et al., 1999, 2000; Blond et al., 2002). I.p.-injected CPP may alter discharges of dopaminergic neurons in the ventral tegmental area (VTA) so that dopamine release in the mPFC deviates from the optimal range. Previous studies have shown that systemic injection of an NMDA receptor antagonist (MK-801) enhanced discharge rates of VTA dopaminergic neurons (French and Ceci, 1990) and increased dopamine release in the mPFC of the rat (Mathe et al., 1999). Another possibility is that CPP alters baseline synaptic responses, such as by acting on presynaptic terminals, so that sufficient depolarization is not achieved during tetanic stimulation. This is not likely, however, because CPP did not alter baseline synaptic responses. That baseline responses were not altered by CPP suggests that side effects of CPP were minimal. Because we cannot completely rule out other possibilities, however, more controlled experiments such as local infusions of an NMDA receptor antagonist will be required in future investigations to clearly resolve this issue.

**Associative encoding of sensory information and hippocampal memories in the PFC**

Although the time course of LTP development was different, LTP induction was similar in the projections from the visual cortex and the hippocampus to the PFC, suggesting that both external sensory information and hippocampal memories are registered in the PFC in a similar manner. Previous studies have shown that LTP in the ventral hippocampal projection to the PFC is dependent upon activation of NMDA receptors (Jay et al., 1995). Although convergent synaptic inputs from the V2MM and ventral hippocampus to the same PFC neurons are yet to be shown, these results suggest that external sensory information and hippocampal memories interact cooperatively for LTP induction in the PFC (McNaughton et al., 1978). A sensory stimulus that activates a memory stored in the hippocampus may be registered better and be associated with the activated hippocampal memory in the PFC.

One caveat of the above suggestions is that the present findings have not been confirmed in chronic preparations. Racine and colleagues (Racine et al., 1995; Trepel and Racine, 1998) have shown that neocortical LTP is preferentially induced by repeated stimulation trains that are spaced over several days in chronic preparations, suggesting that the neocortex may operate with slow learning (McClelland et al., 1995). If the same rule stands in the PFC, it is unlikely that LTP in the PFC is behind the rapid modification of PFC unit activities. In both monkeys and rats, PFC units can rapidly change their activities after a few exposures to a sensory stimulus (Rainer and Miller, 2000; Baeg et al., 2001). It is also unlikely that external sensory information and hippocampal memories are rapidly associated in the PFC. On the other hand, rapid induction of LTP has been reported in the ventral hippocampal projection to the PFC in chronic preparations (Doyere et al., 1993; Jay et al., 1996), raising the possibility that synaptic plasticity rules in the PFC may be different from those in other neocortical areas. Additional work is needed to determine whether or not rapid induction of LTP is achieved in the V2MM projection to the PFC in chronic preparations.

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