Plasticity in the Mediodorsal Thalamo-Prefrontal Cortical Transmission in Behaving Mice

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Herry, Cyril, Rose-Marie Vouimba, and René Garcia. Plasticity in the mediodorsal thalamo-prefrontal cortical transmission in behaving mice. J. Neurophysiol. 82: 2827–2832, 1999. We studied changes in thalamo-prefrontal cortical transmission in behaving mice following both low-frequency stimulation of the mediodorsal thalamus (MD) and during extinction of a conditioned fear response. Electrical stimulation of the MD induces a field potential in the medial prefrontal cortex (mPFC) characterized by two initial negative-positive complexes (N1-P1 and N2-P2) followed by two positive-negative complexes (P2-N3 and P3-N4). The N1-P1 and N2-P2 complexes were identified as resulting from orthodromic and antidromic prefrontal activation, respectively. Because the two complexes were not often easily dissociated, plasticity in the prefrontal synaptic transmission was considered to result from changes in N1-P2 amplitude. Low-frequency thalamic stimulation (1,200 pulses at 2 Hz) produced either long-term (at least 32 min) depression or potentiation of the N1-P2 amplitude. Mice submitted to fear conditioning (tone-shock association), displayed on the first day of extinction (tone-alone presentation) a strong freezing behavior, which decreased progressively, but was still high the following day. Extinction of conditioned fear was accompanied by a depression of prefrontal transmission, which was converted into potentiation the following day. Potentiation of prefrontal transmission lasted at least 24 h following the second day of the fear extinction procedure. In conclusion, low-frequency thalamic stimulation can produce, in behaving mice, either depression or potentiation of prefrontal synaptic transmission. Decrease in prefrontal synaptic transmission observed during the first day of extinction may reflect processing of the high degree of predictiveness of danger (unconditioned stimulus: US) by the aversive conditioned stimulus (CS). However, the subsequent potentiation of transmission in the mPFC may be related to processing of cognitive information such as the CS will no longer be followed by the US, even if emotional response (freezing) to the CS is still high.

METHODS

The experiments were performed using young (4–6 mo) male mice of the C57BL/6 J Co strain (IFFA Credo, Arbesle, France) weighing between 28 and 32 g at the time of surgery. They were individually housed in Plexiglas cages and maintained on a free feeding regimen with a 12-h light:dark cycle. They were implanted under anesthesia via a mixture of sodium pentobarbital-atropine (60 mg/kg, 0.04 ml of a 1-mg/ml solution ip), followed by conventional surgery techniques. The stimulating and recording electrodes were made of two twisted platinum-iridium wires (90 μm diam) insulated except at the tip. These were respectively positioned in the MD (0.8 mm posterior to bregma and 0.3 mm lateral to midline) and the mPFC (2.4 mm anterior to bregma, 0.4 mm lateral to midline) at a location generating a maximum amplitude of the prefrontal field potential. The entire...
miniature system was fixed in place with dental cement on the skull. Animals were handled daily from the 3rd day postsurgery to the end of the experiment. The experiments were performed in accordance with the European Communities Council Directive (86/609/EEC).

Prefrontal field potentials evoked by single-pulse thalamic stimulation (0.1-ms rectangular biphasic pulses) were recorded through JFET operational amplifiers placed on the mouse head and amplified (×1,000; 1–1,000 Hz) and recorded (pClamp6 software; Texas Instruments) for off-line analysis. Stimulus intensity was chosen (from the baseline input/output curves: 60–600 μA) according to that which produced a response representing ~60–70% of the maximal level. To determine whether the initial components of the field potential are generated by activation of target cells, extracellular single-unit activity was recorded on behaving mice (n = 7) implanted in similar conditions as subjects of the main experiment except that recording electrodes of 50 μm instead of 90 μm diam were used.

Electrophysiological recordings were performed in a gray cylindrical chamber (16 cm diam; 14 cm high) with a plastic floor. The base of the cylinder was adapted for freezing recording through infrared beans (Imetronic, Pessac, France). The chamber was washed with 1% acetic acid before and after each session. All animals were adapted for 4 days to transportation (from the animal house to the experimental room) and to connection and disconnection of their miniature headstage to the electrophysiological stimulating-recording system. After this habituation period, single-unit activity study was performed in mice implanted with 50-μm diam recording electrodes. The remaining animals were divided into two groups (main experiment). In the first group [low-frequency MD stimulation (LFS); n = 9], five baseline recordings were performed every 2 min (each recording corresponding to an average of 5 field potentials recorded at 0.2 Hz). Two minutes after this baseline recording, animals of the LFS group were submitted to low-frequency MD stimulation (a train of 1,200 pulses at 2 Hz). Post-LFS recordings were performed 2, 4, 16, 18, 30, and 32 min later (5 field potentials at 0.2 Hz per recording). In the second group (Fear extinction; n = 7), a baseline was established over a 3-day period (1 recording session per day). Three days after this period, a fourth recording was performed before the auditory fear conditioning.

Training, which was controlled by a microcomputer program (Imetronic software, Pessac, France), took place in a conditioning context consisting of a Plexiglas box (18 × 18 × 23 cm high) with a shock grid floor made of stainless steel rods and a speaker at the top of the box providing a 20-s, 2.5-kHz, 80-dB tone. The shock grid was connected to a shock generator and scrambler to provide a 1-s, 0.9-ma footshock. Two minutes after being placed in the conditioning chamber, mice of the second group were subjected to five tone-shock pairings (intertrial interval: 60–120 s). This protocol was repeated the following day. The conditioning box and the floor were cleaned with 70% ethanol before and after each session. Twenty-four hours following the second day of conditioning, each conditioned mouse was submitted for 2 days to fear extinction procedure in which 25 trials of the tone-alone was presented per day in the electrophysiological recording chamber. Electrophysiological recordings were performed before each extinction session, during each tone-alone presentation and 24 h after the second day of extinction. In all experiments, the behavior of mice was observed by means of a video camera monitoring system. Extinction of conditioned fear was assessed by measuring freezing behavior, defined as the absence of all movement except for respiratory-related movements (Blanchard and Blanchard 1969). The amount of time attributed to freezing was measured during the 20-s conditioned stimulus (CS) period.

On completion of the experiments, mice were placed under deep anesthesia and the tips of electrode placements were marked by passing 0.3- to 0.5-ma current for 20 s. The placement of the electrodes was then verified by standard histological methods.

All data are expressed as means ± SE and analyzed by ANOVA followed by post hoc Scheffé F-tests.

RESULTS

Location of the stimulating and recording sites in the MD and mPFC is shown in Fig. 1.

MD single stimulation evoked a field response in the mPFC characterized (Fig. 2A) by two initial complexes (N1-P1: 3–7 ms; N2-P2: 8–13 ms) followed by two other complexes (P2-N3: 13–18 ms; P3-N4: 20–33 ms). Single stimuli delivered to the MD also evoked single action potentials in prefrontal neurons, the latency of which (varying from 2 to 6 ms; n = 7) coincided with the N1-P1 complex of the field potential (Fig. 2B). We therefore considered the N1-P1 as representing an envelope of action potentials evoked by the MD stimulation. Because two types of excitatory responses have been distinguished in the MD-mPFC pathway according to their latency (Pirot et al. 1994), we identified the N1-P1 as representing the orthodromic short-latency excitatory response and the N2-P2 as the antidromic long-latency excitatory response, and predominantly as the postsynaptic consequences of the antidromic response (even if in our study on mice single-unit recording did not reveal the presence of action potential at the N2-P2 latency). In addition, in the majority of our field potential recordings the two initial components were not easily dissociated. We therefore did not make in the present study the distinction suggested by Pirot and colleagues (1994). Plasticity in the prefrontal synaptic excitability was then indexed by changes in the N1-P2 (including both N1-P1 and N2-P2 com-
plexes; see Fig. 2) amplitude, which also represents changes in the probability of discharging target cells.

Figure 3 illustrates the effects of LFS on the evoked response in the mPFC. All mice in the LFS group displayed stable baseline responses ($F_{4,28} = 0.12; n.s.$). However, one-way ANOVA (11 levels) indicated an effect of LFS ($F_{10,80} = 3.25; P < 0.01$). Examination of individual change in response revealed that following thalamic LFS, five subjects exhibited a long-lasting (at least 32 min) depression in the N1-P2 amplitude, whereas the remaining subjects ($n = 4$) showed an

![Figure 2](image_url)

**FIG. 2.** *A*: example of prefrontal field potential evoked by electrical stimulation of the mediodorsal thalamus (MD; N1–4: negative components; P1–3: positive components). *B*: example of unit activity during thalamic stimulation–evoked prefrontal field potential. Traces were recorded with the same electrode with different filter settings adjusted to reveal either field potential ($\times 1,000$; 1–1,000 Hz) or single-unit activity ($\times 10,000$; 300–10,000 Hz). Latency of single-unit activity (*) coincided with latency of the N1-P1 complex of the field potential. Synaptic plasticity was indexed by changes in N1-P2 amplitude because N1-P1 (orthodromic activation) and N2-P2 (antidromic activation) complexes were not often easily dissociated. Changes in the nonidentified complexes (P2-N3 and P3-N4) were not considered in the present study. S: stimulus artifact.

![Figure 3](image_url)

**FIG. 3.** *A*: representative example of changes in field potentials recorded before (left) and 32 min after low-frequency thalamic stimulation (right). *B*: mean changes in N1-P2 amplitude ($\pm$SE) during baseline establishment (recordings $-10$, $-8$, $-6$, $-4$, $-2$ min) and following thalamic low-frequency stimulation (LFS; recordings 2, 4, 16, 18, 30, 32 min). Thalamic low-frequency stimulation induced either potentiation (*top traces*) or long-lasting depression (*bottom traces*) of the thalamo-prefrontal transmission.
enhancement of the N1-P2 amplitude from the 16-min post-LFS recording. A two-way ANOVA performed on these data indicated a significant effect of groups ($F_{1,7}^{1,75} = 29.10; P < 0.001$), as well as a time \times group interaction ($F_{10,70}^{10,70} = 10.8; P < 0.001$). Post hoc Scheffé tests revealed that in mice exhibiting synaptic depression, significant modification occurred from the first post-LFS recording (all $P$ values $< 0.01$), whereas in mice exhibiting synaptic potentiation, significant changes occurred from the 16-min post-LFS recording (16, 18, 30, and 32 min: all $P$ values $< 0.05$).

LFS did not induce any significant change in latency of the early component.

Figure 4 shows changes in the MD-mPFC evoked response during the extinction of the conditioned response. The prefrontal evoked activity was stable across the preconditioning recordings ($F_{3,18} = 1.05$; n.s.). Following the two days of conditioning, a slight and no significant decrease in the N1-P2 amplitude was observed [Day 11 (D11), recording a]. Presentation of the tone CS resulted in an additional and significant decrease in N1-P2 amplitude as compared with both preconditioning level (D11, recording b vs. D1–3 and 8; $F_{4,24} = 13.9; P < 0.001$) and the recording performed before the onset of extinction (D11, recording b vs. D11, recording a: $F_{1,24} = 13.9; P < 0.001$). This was followed by a progressive but not significant recovery as compared with the first block of extinction recording (all $P$ values $> 0.05$). The day after, a significant increase in the N1-P2 amplitude was observed before the extinction session as compared with baseline level ($F_{4,24} = 13.9; P < 0.01$). Presentation of the tone CS did not induce any further increase with respect to the presession recording (all $P$ values $> 0.05$). Potentiation of prefrontal synaptic transmission was still present and significant 24 h after the second extinction session (D12 vs. D8: $F_{1,6} = 12.17; P < 0.01$).

No significant changes in latency of the early component were observed during extinction phase.

Behavioral data are shown in Fig. 5. Following the two initial days of auditory fear conditioning, presentation of the tone CS produced a high freezing level as compared with the basal level of freezing (D10, block b vs. D8: $F_{1,6} = 71.52; P < 0.001$). The freezing level progressively decreased over the 2 days of CS-alone presentations. An ANOVA with repeated measures (D10 and D11: 5 blocks of 5 CS-alone presentations each) indicated the effect of blocks ($F_{9,54} = 4.04; P < 0.001$). However, the freezing level in the last block of CS-alone presentations was still significantly elevated as compared with the basal level of freezing (D11, block f vs. D8: $F_{1,6} = 8.96; P < 0.05$).

**DISCUSSION**

The data from the present study show that low-frequency MD stimulation induces either a potentiation or a depression of
synaptic transmission in the mPFC of freely moving mice. Modifications in prefrontal synaptic transmission were also observed during extinction of learned fear. Mice initially displayed a depression of synaptic transmission during the first session of extinction. This was then followed by a significant potentiation of synaptic transmission the following day. This potentiation was maintained during the second session of extinction and was still present the day after.

LTP and LTD of synaptic transmission are considered to represent the neural basis of some forms of learning and memory processes (Ito 1989; Lynch and Baudry 1984; Teyle and DiScenna 1984). Even if the two phenomena share some common characteristics (for review, see Malenka 1994), they are usually induced by different stimulation parameters (brief high-frequency stimulation for LTP induction; continuous low-frequency stimulation for LTD induction). However, it has been shown in the visual cortex that LTP could be elicited by an LTD-inducing stimulation (Berry et al. 1989; Komatsu et al. 1988). The present study shows that low-frequency MD stimulation can also induce potentiation of synaptic transmission in the mPFC. Moreover, we found that low-frequency MD stimulation induced a long-lasting depression (at least for 32 min). An induction of either LTP or LTD at the prefrontal level has also been reported following a patterned high-frequency stimulation (Hirsh and Crepel 1990). Taken together, these data demonstrate that synaptic transmission in the prefrontal cortex may either increase or decrease following the same parameters of stimulation (either LTP-inducing stimulation; see Hirsh and Crepel 1990; or LTD-inducing stimulation, the present study) applied to a prefrontal excitatory input. The direction of the plasticity has been proposed to depend on the postsynaptic Ca$^{2+}$ concentration, which could be mediated by the level of dopamine in the mPFC (Otani et al. 1998).

Alternatively, the direction of the changes in prefrontal synaptic plasticity observed in the present study could also be related to the distinct N1-P1 and N2-P2 components. The N1-P1 of the field potential is characterized as the monosynaptic activation of MD-prefrontal target cells, whereas the N2-P2 could mainly represent postsynaptic activation of the intracortical circuit via the cortico-MD recurrent stimulation. Thus using single-unit recording, the absence of action potentials at the N2-P2 latency may be related to the deeper location of cells generating this component. In the lateral septum, it has been reported that stimulation of the hippocamposeptal fimbria
generates a septal field potential comprising an excitatory component representing an envelope of spikes that was recorded at different depths in the lateral septum (Garcia and Jaffard 1992). However, the action potential related to this population spike was recorded only in the area from which the amplitudes of the population spike component were highest and the latencies shortest (Garcia et al. 1997). The N2-P2 component may also correspond to an envelope of action potentials originating in a layer other than the N1-P1 component. It is suggested that if low-frequency stimulation induces potentiation or depression of the N2-P2 component while the same stimulation produces an opposite direction in changes of plasticity of the N1-P1 component, the form of plasticity (potentiation or depression) occurring in one component may mask plasticity taking place in the other component. Specifically, in the present study the N1-P1 and N2-P2 were not easily dissociated; consequently, in each case (subject), the largest change in plasticity induced by low-frequency stimulation may have masked the other change in plasticity. The direct cause that determines the predominant type of plasticity observed in the present study remains, however, to be determined.

Contrary to the effects of low-frequency MD stimulation, the learning processes used in the present study led to the same sequence of changes in the plasticity in prefrontal synaptic transmission in the group of mice tested. Indeed, following the first 2 days of fear conditioning (tone-shock association), all subjects displayed a large decrease in prefrontal transmission during the first session of the tone-alone presentations. All subjects also showed a potentiation of prefrontal transmission the next day and a persistence of this potentiation for at least 24 h. The decrease in prefrontal synaptic strength is suggested to be, in part, generated by prefrontal dopaminergic activation for three main reasons. First, psychological stress (related to the perception of an auditory CS) produces activation of dopaminergic system in the mPFC (Goldstein et al. 1996). Second, electrical stimulation of ascending neurons containing dopamine blocks the prefrontal excitatory responses evoked by the MD stimulation (Ferron et al. 1984; Mantz et al. 1988). Third, dopamine application in the mPFC depresses glutamatergic transmission and lowers the threshold for LTD induction in glutamatergic synapses (Otani et al. 1998). Taking into account the role of the prefrontal cortex in cognitive function (Fuster 1989), the decrease in prefrontal synaptic transmission may be related to processing of cognitive information such as the occurrence of danger [unconditioned stimulus (US)]. However, during repeated presentations of the CS-alone, the subject learns that the CS will be no longer followed by the US. Consequently, this could be accompanied by the increase in synaptic strength observed in the mPFC, which may be, this time, involved in long-term cognitive memory of the weakness of the CS to predict the US, even if the behavioral response to the CS is still high. The possible involvement of dopaminergic system in prefrontal LTP-like changes observed in the present study remains also to be determined. As a whole, this study suggests that synaptic plasticity in the mPFC may encode the degree of predictiveness of danger by the CS rather than the emotional consequences of the CS. Similar conclusions were also made concerning changes in synaptic plasticity in the lateral septum (Garcia and Jaffard 1996). We reported that when the capacity of a contextual CS to predict a footshock US is strong, a decrease in synaptic excitability is observed in the...
lateral septum, whereas when the degree of contextual predictiveness is weakened following a tone-shock association in the context, subsequent reexposure of mice to the contextual CS failed to induce the decrease in septal excitability. However, both types of contextual CS (with either strong or weak capacities to predict the aversive US) produced the same level of freezing behavior (Garcia and Jaffard 1996).

In conclusion, bidirectional plasticity (either potentiation or depression) may occur in mPFC transmission following the same pattern of plasticity-inducing stimulation. In contrast, learning (at least learning that occurs during extinction of a learned fear response) induces a constant sequence of changes in synaptic plasticity in all trained mice. Moreover, conditioned freezing seems to be dissociated from plasticity that takes place in the mPFC, which may encode earlier the nonreinforcement of the CS during the extinction of conditioned fear.

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