Neuroscience 153 (2008) 1309–1319

MUSCARINIC ACETYLCHOLINE NEUROTRANSMISSION ENHANCES THE LATE-PHASE OF LONG-TERM POTENTIATION IN THE HIPPOCAMPAL–PREFRONTAL CORTEX PATHWAY OF RATS *IN VIVO*: A POSSIBLE INVOLVEMENT OF MONOAMINERGIC SYSTEMS

C. LOPES AGUIAR,[a1] R. NEVES ROMCY-PEREIRA,[a1]* R. ESCORSIM SZAWKA,[b] O. YINETH GALVIS-ALONSO,[c] J. APARECIDA ANSELMO-FRANCI[b] AND J. PEREIRA LEITE[a]

[a]*Department of Neurology, Psychiatry and Medical Psychology, Ribeirão Preto School of Medicine, University of São Paulo, Av. Bandeirantes, 3900, Ribeirão Preto, SP 14049-900, Brazil*

[b]*Ribeirão Preto School of Odontology, University of São Paulo, Av. Bandeirantes, 3900, Ribeirão Preto, SP 14049-900, Brazil*

[c]*Department of Molecular Biology, São José do Rio Preto School of Medicine, Av. Brigadeiro Faria Lima, 5416, São José do Rio Preto, SP 15090-000, Brazil*

Abstract—The prefrontal cortex is continuously required for working memory processing during wakefulness, but is particularly hypoactivated during sleep and in psychiatric disorders such as schizophrenia. Ammon's horn CA1 hippocampus subfield (CA1) afferents provide a functional modulatory path that is subjected to synaptic plasticity and a prominent monoaminergic influence. However, little is known about the muscarinic cholinergic effects on prefrontal synapses. Here, we investigated the effects of the muscarinic agonist, pilocarpine (PILO), on the induction and maintenance of CA1-medial prefrontal cortex (mPFC) long-term potentiation (LTP) as well as on brain monoamine levels. Field evoked responses were recorded in urethane-anesthetized rats during baseline (50 min) and after LTP (130 min), and compared with controls. LTP was induced 20 min after PILO administration (15 mg/kg, i.p.) or vehicle (NaCl 0.15 M, i.p.). In a separate group of animals, the hippocampus and mPFC were microdissected 20 min after PILO injection and used to quantify monoamine levels. Our results show that PILO potentiates the late-phase of mPFC LTP without affecting either post-tetanic potentiation or early LTP (20 min). This effect was correlated with a significant decrease in relative delta (1–4 Hz) power and an increase in sigma (10–15 Hz) and gamma (25–40 Hz) powers in CA1. Monoamine levels were specifically altered in the mPFC. We observed a decrease in dopamine, 5-HT, 5-hydroxyindolacetic acid and noradrenaline levels, with no changes in 3,4-hydroxyphenylacetic acid levels. Our data, therefore, suggest that muscarinic activation exerts a boosting effect on mPFC synaptic plasticity and possibly on mPFC-dependent memories, associated to monoaminergic changes.

Key words: prefrontal cortex, hippocampus, synaptic plasticity, long-term potentiation, acetylcholine, monoamines.

The hippocampus plays an essential role in the establishment of declarative memories encoding context representations of emotional and non-emotional experiences (Scoville and Milner, 1957; Squire, 1992; Fanselow, 2000). Mnemonic information processed in its circuitry has two direct cortical outputs: the entorhinal cortex and the prefrontal cortex (Swanson and Cowan, 1977; Swanson, 1981; Ferino et al., 1987; Jay et al., 1989; Jay and Witter, 1991; Caballero-Bleda and Witter, 1994; Barbas and Blatt, 1995; Witter and Amaral, 2004). The prefrontal cortex, in particular, lies on a privileged position to access online information from the thalamus and primary sensory cortices, body-state inputs from the brainstem and memory traces encoded by the hippocampus (Groenewegen and Uylings, 2000; Dalley et al., 2004; Vertes, 2006). In fact, this particular merging of information in the prefrontal cortex is thought to be essential to the development of adaptive behavioral strategies and prospective plans in rodents, macaques and humans (Fuster, 1973; Funahashi et al., 1989; Goldman-Rakic, 1995, 1996; Vertes, 2006). Moreover, both the hippocampus and the prefrontal cortex are implicated in the expression of several psychiatric disorders such as schizophrenia, bipolar disorder and major depression, in which neurotransmitter unbalances are observed (Goldman-Rakic, 1999; Lyons, 2002).

The cholinergic system is an important modulator of memory performance involving the hippocampus and the prefrontal cortex (Hasselmo, 1999; Gu, 2002; Chudasama et al., 2004; Dalley et al., 2004). Cholinergic neurons from the septum project to the hippocampus, whereas neurons in the basal forebrain and laterodorsal tegmental nucleus project to the medial prefrontal cortex (mPFC) (McKinney et al., 1983; Mayo et al., 1984; Satoh and Fibiger, 1986). The activity of cholinergic neurons is distinctively regulated across the sleep–wake cycle, showing higher levels during rapid-eye movement (REM) sleep, attention-demanding tasks and learning, but reduced levels during slow–wave sleep and quiet wakefulness (Detari et al., 1984). Besides, the cognitive decline observed in Alzheimer's disease, schizophrenia and normal aging is known to be associated with a reduction of cholinergic activity in subcortical and cortical brain regions (Giacobini, 1990; Barnes et al., 2000; Messer, 2002; Dunbar and Kuchibhatla, 2006).

[1] Authors contributed equally to this study.

*Corresponding author. Tel: +55-16-3602-2556 or +55-16-3602-4535.
E-mail address: rnrpereira@gmail.com (R. Neves Romcy-Pereira).

Abbreviations: CA1, Ammon's horn CA1 hippocampus subfield; DOPAC, 3,4-dihydroxyphenylacetic acid; fPSP, field post-synaptic potential; HPLC-ED, high-performance liquid chromatography with electrochemical detection; LFP, local field potential; LTP, long-term potentiation; mEsc, methyl-scopolamine; mPFC, medial prefrontal cortex; PILO, pilocarpine; REM, rapid-eye movement; 5-HIAA, 5-hydroxyindole-3-acetic acid.

0306-4522/08$32.00+0.00 © 2008 IBRO. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuroscience.2008.02.040

Several studies have shown that monosynaptic projections from both the dorsal and ventral Ammon's horn CA1 hippocampus subfield (CA1) to the mPFC can undergo paired-pulse facilitation, long-term potentiation (LTP) and long-term depression (LTD) (Laroche et al., 1990; Jay et al., 1995; Takita et al., 1999; Izaki et al., 2002; Kawashima et al., 2006). A number of reports have also demonstrated that mPFC synaptic plasticity is modulated by glutamatergic, dopaminergic and serotonergic neurotransmission and can be altered by drugs such as cocaine and clozapine (Gurden et al., 1999; Ohashi et al., 2002; Gemperle and Olpe, 2004; Jay et al., 2004; Dupin et al., 2006; Chen et al., 2007; Huang et al., 2007). Although much effort has been focused on the monoaminergic control of CA1-mPFC synaptic plasticity, very little is known about the cholinergic modulation and its implications to psychiatric disorders and learning. Recently, Couey et al. (2007) showed that nicotine increases the threshold for spike-timing-dependent potentiation in layer V pyramidal neurons of the prefrontal cortex. In addition, we have found indirect evidence for the involvement of the cholinergic system in the bimodal regulation of hippocampal and prefrontal cortical synaptic plasticity. We observed that short-term (4 h) REM sleep deprivation impairs the late-phase maintenance of LTP in the hippocampus but prolongs it in the projections from the posterior dorsal CA1 to the prefrontal cortex (Romcy-Pereira and Pavlides, 2004). Such changes could underlie memory impairments after REM sleep deprivation, since both the dorsal hippocampus and the prefrontal cortex have prominent roles in spatial memory processing. In the present study, therefore, we investigated the effects of the muscarinic cholinergic agonist, pilocarpine (PILO), on the induction and maintenance of LTP in the projections from the posterior dorsal CA1 to the mPFC, as well as its influence on monoamine levels in the hippocampus and prefrontal cortex. In order to monitor the effects of PILO on brain activity before and after LTP, we also analyzed the oscillatory patterns of the hippocampus and mPFC after drug administration. Our results show that PILO prolongs the maintenance of CA1-mPFC plasticity *in vivo* but has no effect on its induction. This modulatory function is accompanied by a clear change in the spectral composition of CA1 and mPFC neuronal oscillations toward high frequencies (>10 Hz). Besides, PILO administration specifically changes monoamine levels in the mPFC, without affecting their levels in the hippocampus.

EXPERIMENTAL PROCEDURES

Subjects

Forty-two male Wistar rats (250–450 g) were housed in standard rodent cages in a vivarium maintained at 24 °C under a 12-h light/dark cycle with lights on at 07:00 h. Food and water were freely available during all phases of the experiment. All procedures were performed according to the Brazilian College of Animal Experimentation (COBEA) guidelines for animal research, affiliated with the International Council for Laboratory Animal Science (ICLAS), and approved by the Ethical Commission at the University of São Paulo. Experiments were designed to minimize the number of animals used and their suffering.

Electrode implants and LTP

Rats were anesthetized with urethane (1.2–1.5 g/kg, i.p., in NaCl 0.15 M; Sigma-Aldrich, St. Louis, MO, USA) and placed in a stereotaxic frame for the implant of electrodes with body temperature maintained at 37±0.5 °C by using a heating pad. The level of anesthesia was maintained stable by supplementary doses of anesthetic (30% of the initial dose) after checking the tail pinch reflex, respiratory rate and EEG signals. In brief, the skull was exposed and small holes drilled on it to allow access to the mPFC (3.0 mm anterior to bregma, 0.5 mm lateral to midline and 3.2 mm ventral to dura mater) and CA1 (5.7 mm anterior to bregma, 4.6 mm lateral to midline and 2.5 mm ventral to dura mater), according to Paxinos and Watson (1998). Teflon-insulated tungsten wires (60 μm diameter; A-M Systems, Carlsborg, WA, USA) were used to prepare electrodes, which were lowered into the brain through holes made on the skull after removing the dura mater. A twisted bipolar electrode (tip separation 500 μm) was used for constant current stimulation of CA1 and a monopolar electrode was used to record field post-synaptic potentials (fPSP) in the mPFC. A micro-screw was implanted over the parietal cortex and served as the recording ground. The final position of the electrodes was adjusted to obtain the highest negative-going response in the mPFC with latency to the first negative peak around 18 ms and amplitude of at least 250 μV (Laroche et al., 1990; Jay et al., 1995). Monophasic test pulses of 200 ms were delivered every 20 s at increasing intensities (60–500 μA) and used to plot input–output curves. Based on input–output curves, we calculated the minimum intensity necessary to produce maximum mPFC responses and used 50% of this intensity to stimulate CA1 during baseline, LTP and post LTP recordings. Baseline was recorded using test pulses (200 μs duration; every 20 s) for 50 min before LTP induction and was followed by continuous fPSP recordings for additional 130 min (every 20 s). LTP was induced by tetanic stimulation of CA1-mPFC projections with two series of 10 trains (50 pulses at 250 Hz, 200 ms duration) every 10 s, 10 min apart from each other (Romcy-Pereira and Pavlides, 2004). Prefrontal fPSPs were amplified and filtered (×100, 0.3–10 kHz; P55-AC pre-amplifier, Grass Instruments Co., West Warwick, RI, USA) before digitization at 4 kHz (PowerLab/16S, ADInstruments, Colorado Springs, CO, USA). Constant current square pulses used throughout the experiments were delivered through an S88 stimulator (Grass Instruments Co.).

Experimental paradigm

In order to investigate the cholinergic modulation of mPFC synaptic plasticity, rats were grouped according to drug treatment during baseline recordings. (1) Veh-Veh animals received two injections of vehicle (Veh; NaCl 0.15M, i.p.), one at 10 min and one at 30 min after baseline recording onset; (2) methyl-scopolamine (mEsc) –Veh animals received an injection of the peripherally specific muscarinic antagonist mEsc (15 mg/kg, i.p., in NaCl 0.15 M; Sigma-Aldrich) at 10 min and an injection of vehicle at 30 min after baseline recording onset, and (3) mEsc-PILO animals received an injection of mEsc (15 mg/kg, i.p., in NaCl 0.15 M) at 10 min and an injection of the muscarinic agonist PILO (15 mg/kg, i.p., in NaCl 0.15 M; Sigma-Aldrich) at 30 min after baseline recording onset. Baseline recordings were identified as BL1: 0–10 min; BL2: 10–30 min and BL3: 30–50 min. Since, mEsc is a muscarinic antagonist that does not cross the blood–brain barrier (Hughes, 1982; van Haaren and van Hest, 1989), mEsc pretreatment prevented the peripheral actions of PILO, such as lethal parasympathetic effects even at the low doses (1 mg/kg) during urethane anesthesia. After tetanization, LTP was calculated as percentage of baseline levels measured during the first 50 min for Veh-Veh and mEsc-PILO groups, or during the last 20 min of baseline for mEsc-Veh group. Differences in baseline and LTP decay were evaluated by comparing 10-min segments of aver-

aged fPSP amplitude values across baselines and between different time-points after LTP induction for all groups.

Local field potential (LFP) recordings

In order to monitor the brain activation state associated to the cholinergic modulation, we recorded LFPs (or deep EEG) in CA1 and mPFC throughout the experiment, from the same electrodes used to induce and record LTP. LFPs were amplified and filtered (×100, 0.3–300 Hz; P55-AC pre-amplifier, Grass Instruments Co.) before digitization at 1 kHz (PowerLab/16S, ADInstruments). They were recorded at several time points during the LTP paradigm: (1) before Veh or mEsc, at BL1; (2) before Veh or PILO, at BL2; (3) before LTP induction, at BL3 and (4) 10 min after LTP induction. For each animal, power spectra were calculated on 10 s-epoch LFPs at all four time-points. Delta (1–4 Hz), theta (4–10 Hz), sigma (10–15 Hz) and gamma (25–40 Hz) relative powers were calculated and compared across the time to evaluate the effect of mEsc and PILO injections on CA1 and mPFC oscillations. After the electrophysiological procedures, a brief current pulse (1 mA/1 s) was delivered through the stimulating and recording electrodes to mark their tip placements.

Brain micro-dissections

Three new groups of animals were prepared for monoamine quantification. Rats were anesthetized with urethane (1.2–1.5 g/kg, i.p., in NaCl 0.15 M) and received an injection of either Veh or mEsc (15 mg/kg, i.p., in NaCl 0.15 M) 1 h later. After 20 min, they received an injection of either Veh or PILO (15 mg/kg, i.p., in NaCl 0.15 M) and were quickly decapitated 20 min later. All rats were treated in a similar way as the animals used for electrophysiology, including urethane anesthesia and the timing for drug injection and decapitation. After decapitation, the brains were removed, immediately frozen on dry ice and labeled as Veh-Veh, mEsc-Veh or mEsc-PILO, according to drug treatment. They were stored at −70 °C until sectioning.

Thick coronal brain sections were cut in cryostat set to −10 °C with controlled micrometric advance of the specimen (Microm HM 505, Mikron Instruments Inc., San Marcos, CA, USA). According to rat brain atlas, three 1000 μm thick sections were sliced from the frozen brain and mounted onto chilled glass for micro-dissection with a 1.0-mm diameter needle by the punch technique (Palkovits, 1973; Paxinos and Watson, 1998). Hippocampal and mPFC punches were collected from the posterior dorsal CA1 and prelimbic mPFC regions, corresponding to the stimulating and recording sites used in the LTP experiment, respectively. Parietal cortex punches were also obtained and used as a control. The mPFC was dissected from a first section extending from 3.7–2.7 mm anterior to bregma, in two punches vertically placed between 3.0 and 5.0 mm ventral from dura mater. The parietal cortex was dissected from a second section, extending from 1.8–2.8 mm posterior to bregma, in two punches placed laterally at 2.0 and 4.0 mm ventral from dura mater. The dorsal posterior CA1 region was dissected from a third section, extending from 4.8–5.8 posterior to bregma, in two punches placed laterally at 2.6 and 2.8 mm ventral from dura mater. With a micro-ultrasonic cell disrupter, punches were homogenized in a solution containing 0.2 M perchloric acid (Merck, Darmstadt, Germany), 0.1 mM EDTA (Merck) and 8 ng/mL of 3,4-dihydroxybenzylamine (Aldrich, Milwaukee, WI, USA). The homogenates were centrifuged for 20 min at 12,000×*g*. The supernatant was removed, filtered through a 0.22-μm filter (Millex PVDF, Millipore, Belford, MA, USA) and placed into auto-injector vials. Protein content was determined in the remaining pellet by the Bradford method (Bradford, 1976).

High-performance liquid chromatography with electrochemical detection (HPLC-ED)

Tissue concentrations of dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), 5-HT, 5-hydroxyindole-3-acetic acid (5-HIAA) and noradrenaline were measured by HPLC-ED. Fifty microliters of each sample were injected by an auto injector (SIL-10Advp, Shimadzu, Kyoto, Japan). Separation was performed at 35 °C on a 250×4-mm reversed-phase C18 column (Purospher Star, 5 μm, Merck), preceded by a 4×4-mm C18 guard column (Lichrospher, 5 μm, Merck). The mobile phase consisted of 100 mM sodium dihydrogen phosphate monohydrate, 10 mM sodium chloride, 0.1 mM EDTA, 0.20 mM sodium 1-octanesulfonic acid (Sigma-Aldrich) and 15% methanol (Merck). The pH was adjusted to 3.5 with phosphoric acid. The flow rate was set at 0.6 mL/min, pumped by a dual piston pump (LC-10Advp, Shimadzu). The detector potential was 0.60 V versus *in situ* Ag/AgCl (Decade, VT-03 electrochemical flow cell; Antec Leyden, Netherlands). Chromatographic data were plotted using Class-VP software (Shimadzu). Noradrenaline, dopamine, DOPAC, 5-HT and 5-HIAA were identified by their peak retention time and quantified by the internal standard method based on the area under the peak. Intra and inter-assay coefficients of variation were less than 5% and 8%, respectively, for all measured compounds. Neurotransmitter levels (dopamine, 5-HT and noradrenaline) were considered to estimate neurotransmitter stocks in synaptic vesicles, whereas dopamine and 5-HT metabolites (DOPAC and 5-HIAA, respectively) were considered to reflect the amount of transmitter released in the samples. Metabolite to neurotransmitter ratio (DOPAC/dopamine and 5-HIAA/5-HT) was taken as a measure of neurotransmitter turnover.

Histology for electrode positioning determination

After electrolytic lesions at CA1 and mPFC electrode tip positions, the animals received an additional dose of urethane (0.5 g/kg, i.p. in NaCl 0.15 M) and were transcardiacally perfused with 100 ml of NaCl 0.15 M followed by 250 ml of 10% formaldehyde in NaCl 0.15 M. Their brains were removed, post-fixed in the formaldehyde solution for 14 h at 4 °C and cryoprotected for 48 h in 20% sucrose solution. After freezing in dry ice-chilled isopentane, brains were cut in 30 mm slices, mounted on gelatinized slides and processed for Cresyl Violet staining. Electrode tip positions were determined after analysis of the slides under the microscope using bright field (BX-60 Olympus, Center Vally, PA, USA).

Statistical analysis

The analysis of group differences after CA1-mPFC LTP was carried out using a mixed model two-way ANOVA for repeated measures (group: fixed factor vs. time: repeated measures). Baseline fPSP data and power spectrum differences in the mPFC and CA1 were analyzed using one-way ANOVA for repeated measures in each group. Monoamine level differences were evaluated using one-way ANOVA. Newman-Keuls post hoc tests were used following ANOVAs when necessary. The results are expressed as mean±S.E.M. (standard error of the mean) and significance level was set to $P<0.05$.

RESULTS

Effects of PILO on hippocampo–prefrontal cortex LTP and brain oscillations

All animals included in our analysis had the stimulating electrode positioned in the dorsal aspect of the temporal CA1 subfield of the hippocampus and the recording electrode positioned in the medial wall of the prefrontal cortex,

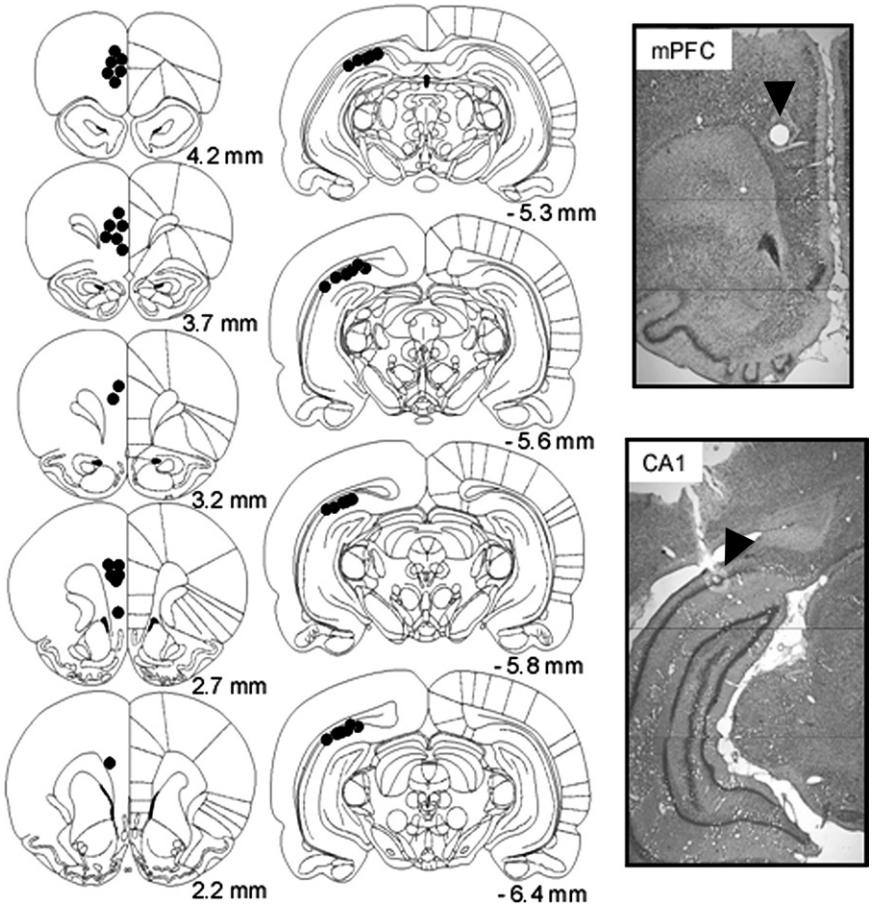

Fig. 1. Electrode placement. Left, schematic representation of final electrode positions in the mPFC and CA1. Right, a typical lesion at electrode tips in the mPFC and hippocampal histological sections. Antero-posterior coordinates are shown in relation to bregma.

most of the time corresponding to the prelimbic area of the mPFC (Fig. 1). Reliable field evoked responses in the mPFC were obtained after CA1 stimulation and consisted of a negative deflection with average latency of 16.8±0.6 ms, amplitude of 250–400 μA and slope of −37.5±2.4 μV/ms. They were consistent with mPFC fPSPs previously reported in the literature and a result of hippocampal monosynaptic projections to the mPFC (Laroche et al., 1990; Degenetais et al., 2003; Romcy-Pereira and Pavlides, 2004).

As shown in the experimental schedule of Fig. 2 (Fig. 2A), baseline fPSPs were recorded during BL1 (before mEsc), BL2 (before PILO) and BL3 (after PILO) and compared within each group in order to monitor mEsc and PILO effects on basal mPFC responses. Veh-Veh and mEsc-PILO animals did not show any difference across baselines (fPSP amplitude: Veh-Veh, $F_{(2,7)}=3.15$, $P>0.05$, mEsc-PILO, $F_{(2,12)}=0.34$, $P>0.05$). However, mEsc-Veh animals showed a slight increase in the amplitude of BL3 fPSPs when compared with BL1 (fPSP amplitude: 14.60±2.20%; $F_{(2,12)}=4.94$, $P<0.05$). For this reason, post-LTP-evoked responses in Veh-Veh and mEsc-PILO groups were normalized against the average of BL1, BL2 and BL3, while mEsc-Veh responses were normalized against BL3 values.

LTP in the mPFC was induced by the application of high frequency trains of stimulation to CA1 and was continuously monitored for 130 min. Fig. 2B shows the effect of PILO treatment on the late-phase of mPFC LTP. A significant group effect was observed in the evoked responses after tetanization (fPSP amplitude: $F_{(2,18)}=3.96$, $P<0.05$). Although similar levels of post-tetanic potentiation (at 0 min) were induced in all three groups (fPSP amplitude, $F_{(2,18)}=1.87$, $P>0.05$), LTP decayed faster in Veh-Veh and mEsc-Veh animals as compared with mEsc-PILO animals. In the mEsc-PILO group, evoked fPSPs were enhanced at 130 min after LTP induction when compared with mEsc-Veh group (148.59±1.06%, mEsc-PILO vs. 122.42±3.32%, mEsc-Veh; $F_{(2,18)}=4.26$, $P<0.01$). No differences were observed in the fPSP amplitude when we compared Veh-Veh and mEsc-Veh animals, at any time-point after LTP induction.

LFPs were recorded in CA1 and mPFC in order to monitor their oscillatory patterns before and after PILO injection. Slow-wave oscillations in the delta band (1–4 Hz) were dominant throughout the experiment in Veh-Veh and mEsc-Veh animals, but they were reduced after PILO administration, particularly in the hippocampus (Fig. 3A, *top panels*). Twenty minutes after PILO injection (mEsc-PILO animals), the relative delta power in CA1 decreased

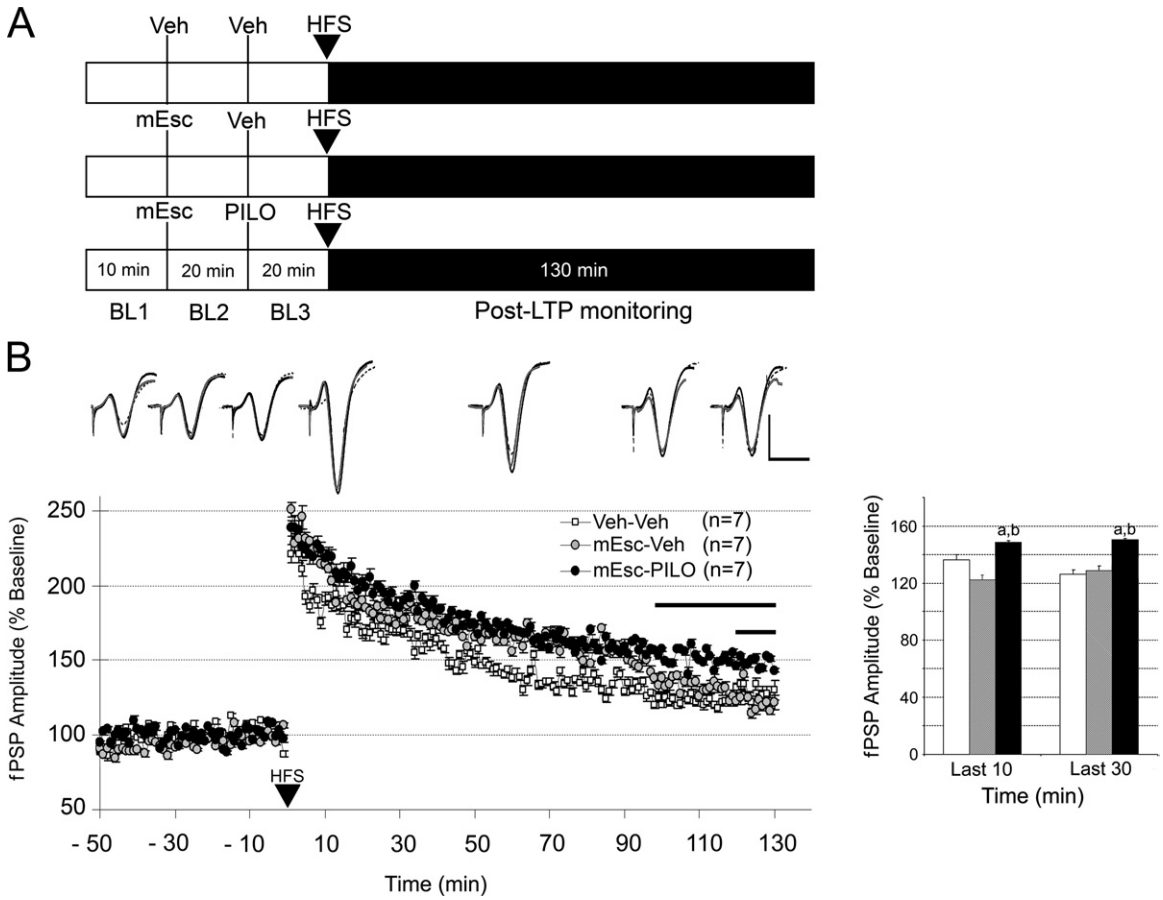

Fig. 2. Experimental paradigm and LTP. (A) Experimental paradigm. Evoked fPSP responses in the mPFC were recorded during baseline (BL1–BL3) and monitored for 130 min after CA1 tetanization (HFS). mEsc-PILO rats received mEsc (15 mg/kg, i.p.) followed by PILO (15 mg/kg, i.p.) before LTP induction. Control rats (Veh-Veh, mEsc-Veh) received sterile saline instead of PILO and mEsc. (B) LTP. PILO treatment did not affect LTP induction but significantly potentiated the late-phase of mPFC evoked fPSP amplitude after HFS. mEsc-PILO rats showed a significant enhancement of the amplitude of mPFC evoked responses 130 min after LTP induction compared with mEsc-Veh animals (see bar graph). Bar graphs show statistical differences in 10 min-epoch (short bar) and 30 min-epoch (long bar) recordings. Top tracings, representative fPSP responses in the mPFC after CA1 stimulation (dashed line, Veh-Veh; gray line, mEsc-Veh; black line, mEsc-PILO). Trace responses are aligned to the time scale in the *x* axis of the graph. Data shown as mean±S.E.M. Differences between experimental groups were determined by two-way ANOVA with repeated measures followed by Neuman-Keuls post hoc test: a $P<0.05$ compared with mEsc-Veh; b $P<0.05$ compared with Veh-Veh. Scale bars=20 ms (horizontal), 250 mV (vertical).

approximately 35% (74.87±10.71% before PILO vs. 37.24±7.28% after PILO; $F_{(3,12)}=4.84$, $P<0.05$) from pre-PILO levels. This pattern could still be observed after LTP induction. The desynchronization of CA1 neuronal activity could also be seen by the concurrent increase in theta, sigma and gamma relative powers (Fig. 3A, *middle and lower panels*). Significant increases in sigma (~100%; 3.36±1.47% before PILO vs. 6.16±0.41% after LTP; $F_{(3,12)}=3.24$, $P<0.05$) and gamma (~200%; 4.48±1.95% before PILO vs. 15.70±1.80% after PILO; $F_{(3,12)}=7.73$, $P<0.001$) powers were detected 20 min after PILO, which lasted at least until 10 min after LTP induction. Although less prominent than in CA1, the cholinergic activation of mPFC promoted a decreasing trend in delta power that was associated with an increasing trend in theta and a significant boosting in gamma power (~400%; 1.32±0.56% before mEsc vs. 6.36±1.93% after PILO; $F_{(3,12)}=4.43$ $P<0.05$) in the mPFC. We also detected small changes in sigma and gamma powers in the Veh-Veh group, which were probably related to the level of anesthesia at the moment of LFP recording. Importantly, mEsc did not affect EEG spectral composition in any frequency band analyzed. Fig. 3B shows illustrative examples of LFP segments recorded in the CA1 and mPFC of control and PILO-treated rats.

Effects of PILO on monoamine levels in CA1 and mPFC

Fig. 4A shows the experimental paradigm used to prepare animals for monoamine quantification. Dopamine, 5-HT, and their metabolites DOPAC and 5-HIAA, as well as noradrenaline, were quantified by HPLC-ED in mPFC, hippocampus and parietal cortex micro-dissections. Fig. 4B depicts the micro-dissection sites where samples were collected from Veh-Veh, mEsc-Veh and mEsc-PILO groups. Fig. 4C–E shows the results of monoamine quantification. In the mPFC, PILO injection promoted a decrease in dopamine (−43.6%; $F_{(2,18)}=3.95$, $P<0.05$),

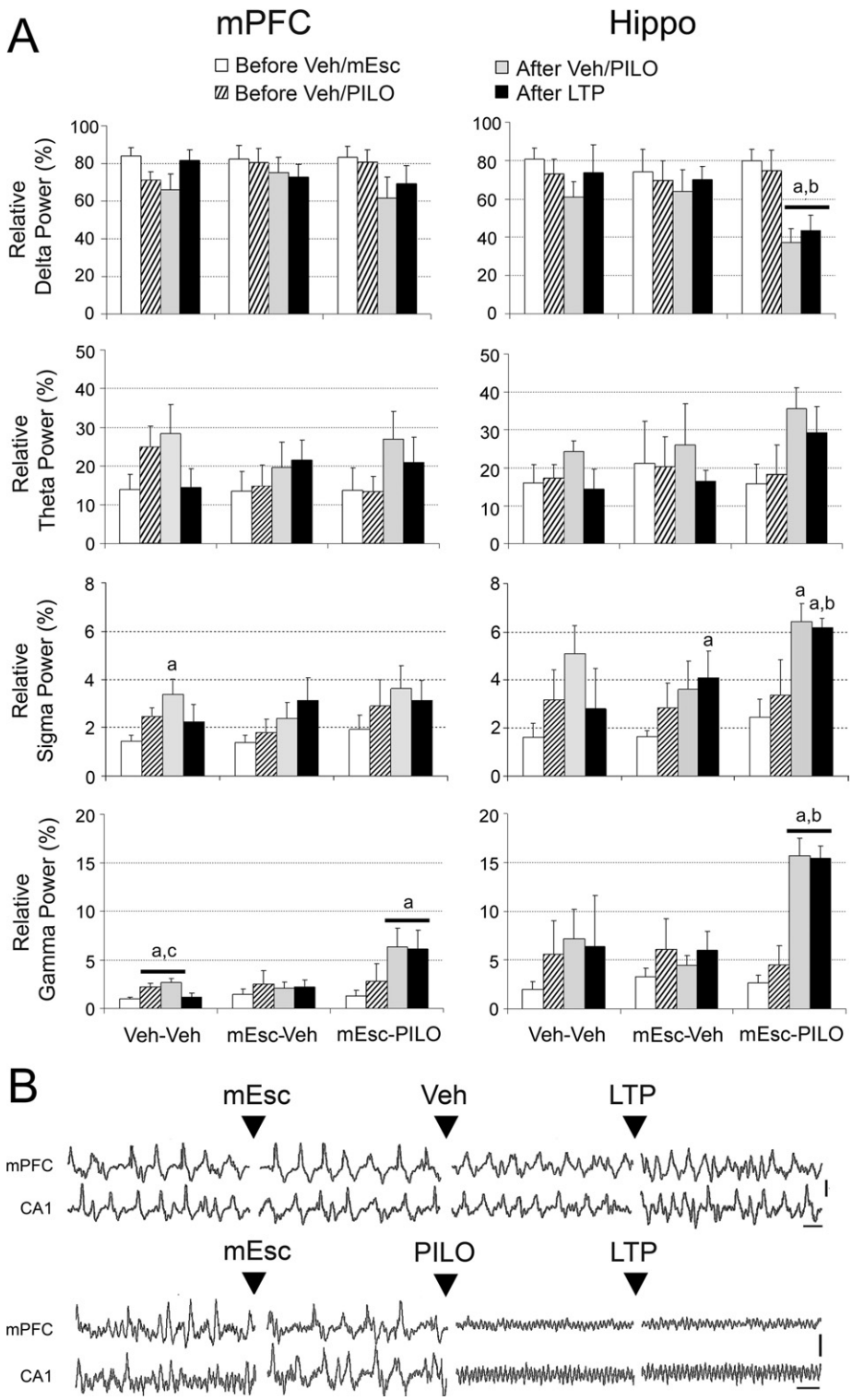

Fig. 3. Oscillatory brain activity after PILO treatment. (A) Relative power spectrum changes in CA1 and mPFC oscillations before and after drug treatment (see also Fig. 2A). Top, PILO injection significantly decreased delta (1–4 Hz) power in CA1 (−35%, $P<0.05$) and set a trend toward reduction in the mPFC (mEsc-PILO group). mEsc did not affect delta oscillatory activity either in CA1 or mPFC (mEsc-Veh group). Middle-top, PILO produced a non-significant increase in theta (4–10 Hz) power in both areas. Middle-bottom, PILO significantly increased sigma (10–15 Hz) power in CA1, and promoted a similar trend toward augmentation in the mPFC. Drifts in sigma power were seen in the mPFC of Veh-Veh rats, and in CA1 of mEsc-Veh rats. Bottom, PILO strongly increased gamma (25–40 Hz) power in CA1 (+200%, $P<0.05$) and mPFC (+400%, $P<0.05$). Importantly, mEsc did not affect gamma oscillatory activity either in CA1 or mPFC. (B) Representative mPFC and CA1 EEG tracings from mEsc-Veh and mEsc-PILO animals. Data shown as mean±S.E.M. Differences between time-points were determined by one-way ANOVA with repeated measures followed by Neuman-Keuls post hoc test: a, $P<0.05$ compared with before Veh/mEsc; b, $P<0.05$ compared with before Veh-PILO. Scale bars=1 s (horizontal), 400 mV (vertical).

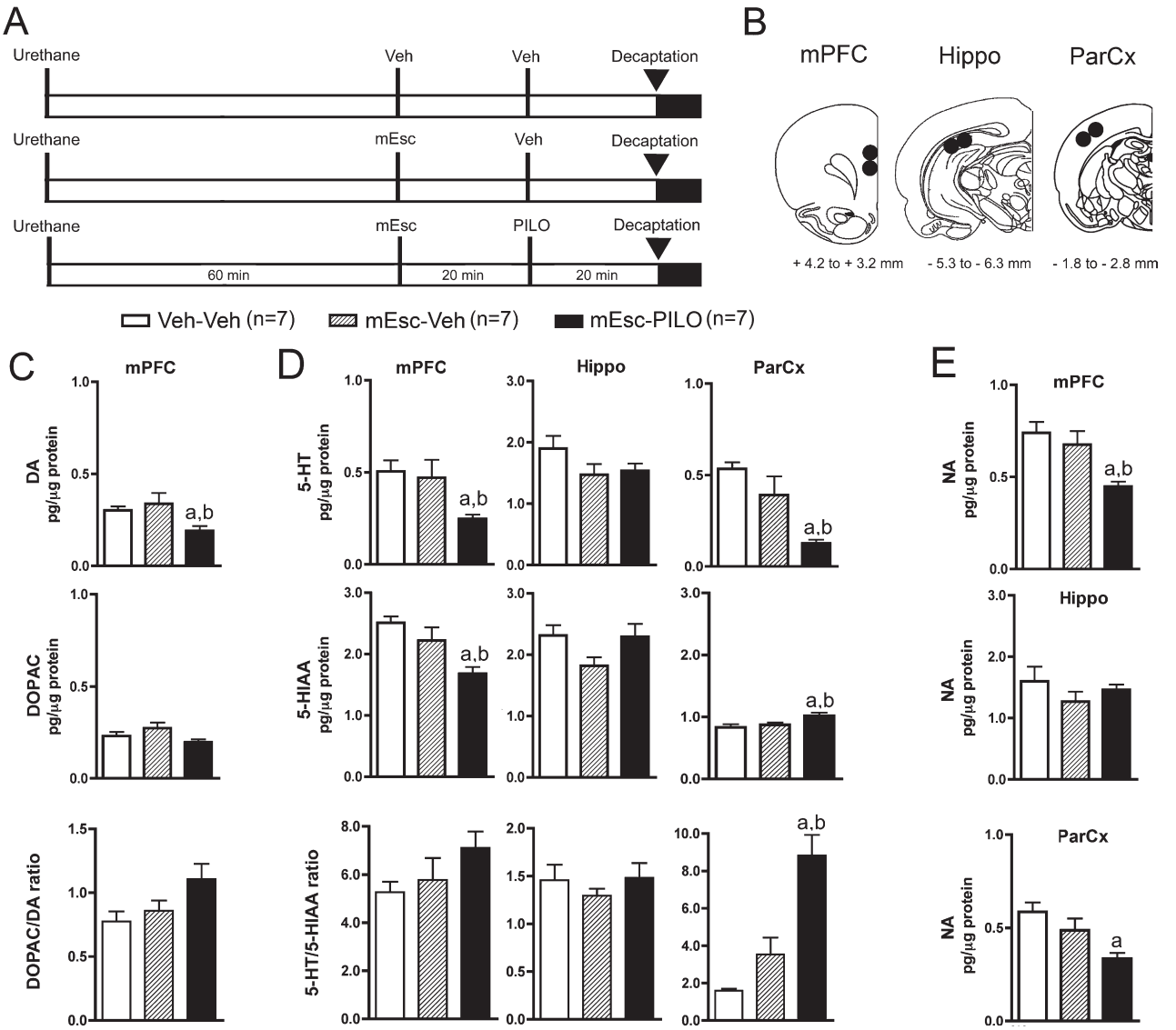

Fig. 4. Monoamine and metabolite levels in the mPFC, hippocampus and parietal cortex (ParCx) 20 min after PILO injection. (A) Rats were anesthetized with urethane (1.5 g/kg, i.p.) and received Veh, mEsc (15 mg/kg, i.p.) or PILO (15 mg/kg, i.p.) injections according to the paradigm. (B) Punch samples (1000 mm thick×1.0 mm diameter) from the mPFC, hippocampus and ParCx (control) were obtained and prepared for HPLC-ED quantification of DA, 5-HT, noradrenaline and metabolites. Antero-posterior coordinates are shown in relation to bregma. (C–E) DA, 5-HT and NA quantification results. Metabolite to neurotransmitter ratios for DA and 5-HT are shown in the lower graphs of C and D, respectively. Data are shown as mean±S.E.M. Differences between experimental groups were determined by one-way ANOVA followed by Newman-Keuls test. a $P<0.05$ compared with Veh-Veh group; b $P<0.05$ compared with mEsc-Veh group. DA levels in the hippocampus and ParCx were not quantified as they were below detection limit (approximately 20 pg). DA, dopamine; NA, noradrenaline.

5-HT (−47.2%; $F_{(2,18)}=4.34$, $P<0.05$), 5-HIAA (−24.3%; $F_{(2,17)}=6.96$, $P<0.01$) and noradrenaline (−33.6%; $F_{(2,18)}=7.42$, $P<0.01$) levels when compared with the control mEsc-Veh group. DOPAC levels were unchanged in the mPFC ($F_{(2,18)}=2,52$, $P=0.11$). As a result, dopamine turnover displayed a trend toward an increase in mEsc-PILO animals (+27.9% vs. mEsc-Veh; $F_{(2,18)}=3.30$, $P=0.06$) and 5-HT turnover did not change ($F_{(2,17)}=1.73$, $P=0.21$). In the hippocampus, on the other hand, we did not detect any change in 5-HT, 5-HIAA or noradrenaline levels (ANOVA, $P>0.05$). PILO also did not alter 5-HT turnover in the hippocampus ($F_{(2,18)}=0.56$, $P=0.58$). Dopamine and DOPAC levels were below the assay detection limit (approximately 20 pg). In the parietal cortex, however, PILO induced a distinct pattern of neurochemical changes from that observed in the mPFC. There was a reduction in 5-HT (−67.5%; $F_{(2,17)}=9.13$, $P<0.01$) and noradrenaline (−31.2%; $F_{(2,17)}=5.72$, $P<0.05$) levels, and an increase in 5-HIAA (+16.9%; $F_{(2,17)}=4.54$, $P<0.05$) as compared with mEsc-Veh animals. PILO also significantly increased 5-HT turnover as compared with controls (+250.1% vs. mEsc-Veh, +555.3% vs. Veh-Veh; $F_{(2,17)}=21.00$, $P<0.001$). Dopamine and DOPAC levels were below detection limit in the parietal cortex.

DISCUSSION

Cholinergic projections from the septum, basal forebrain and laterodorsal tegmental nucleus to limbic and neocortical areas modulate a variety of brain processes including arousal, attention and memory. Despite the importance of hippocampo-prefrontal cortex communication to emotional and cognitive processing, very little has been reported about the cholinergic control of CA1-mPFC synaptic transmission. In this study, we demonstrated that the muscarinic agonist PILO enhances synaptic plasticity in the hippocampal inputs to mPFC in intact animals, which is also associated with cortical monoaminergic changes and fast oscillatory brain rhythms. Our main findings show that (1) PILO strengthened mPFC LTP late-phase without affecting its induction phase. PILO sustained mPFC LTP above control levels at 130 min after LTP induction; (2) the effects of PILO on mPFC LTP were paralleled by a decrease in the relative power spectrum of delta oscillations and an increase of sigma oscillations in CA1, as well as an increase of gamma oscillations in CA1 and mPFC; (3) PILO specifically altered monoamine levels in the mPFC compared with control groups. It decreased dopamine levels without changing the levels of its metabolite, DOPAC; decreased both 5-HT and its metabolite, 5-HIAA tissue levels; and it also decreased noradrenaline levels; but (4) PILO did not affect monoamine levels in the hippocampus.

The cholinergic enhancement of mPFC LTP observed in our study is consistent with previous reports using intact animals showing a muscarinic potentiation of LTP maintenance in the motor (Boyd et al., 2000), somatosensory (Verdier and Dykes, 2001) and visual cortices (Dringenberg et al., 2007), as well as in CA1 (Iga et al., 1996) and dentate gyrus (Frey et al., 2003) of the hippocampus. Although muscarinic agonists were also shown to enhance LTP induction in brain slices of CA1, dentate gyrus (Blitzer et al., 1990; Abe et al., 1994) and, piriform and perirhinal cortices (Hasselmo and Barkai, 1995; Cheong et al., 2001), we did not observe any effect of PILO on mPFC LTP induction. This discrepancy could either reflect a difference in the animal preparation used (brain slice vs. intact animal), a particular response to our LTP protocol or a specific property of the muscarinic modulation of CA1-mPFC synaptic plasticity. On the other hand, we could also consider the possibility that a small amount of mEsc may have leaked across the blood–brain barrier following systemic mEsc pre-treatment, counteracting PILO central effects (Pakarinen and Moerschbaecher, 1993). However, in spite of the small effect of mEsc pre-treatment on baseline mPFC responses in mEsc-Veh animals, it is unlikely that mEsc leakage inhibited a hypothetical PILO-induced increase in LTP induction. In support of this, we found that the dose of mEsc used in our study did not alter LTP levels in mEsc-Veh animals when compared with Veh-Veh animals, as would be expected after mild muscarinic blockade (Dringenberg et al., 2007). Therefore, these findings indicate that PILO promotes strengthening of mPFC LTP by sustaining its late-phase without directly affecting its induction.

Three important findings suggest that mPFC is particularly sensitive to cholinergic inputs and support the effects of PILO on mPFC plasticity. First, mPFC cells have a particularly strong fast-hyperpolarizing response to acetylcholine, which is followed by a long-lasting depolarization (Gulledge et al., 2007). Second, pyramidal prefrontal neurons treated with PILO lack the initial fast-hyperpolarizing response and have a very potent slow depolarization possibly associated to the activation of M1 muscarinic postsynaptic receptors (McCormick and Prince, 1985). Third, M1 receptor activation promotes tonic firing and enhances the temporal integration of dendritic excitatory fPSPs in mPFC neurons, attenuating isolated single fPSPs and potentiating fPSPs generated by spatially and temporally coherent inputs (Carr and Surmeier, 2007).

In addition, we also observed that the PILO-enhancement of mPFC LTP was associated with a desynchronization of LFPs recorded in the hippocampus and cortex. PILO significantly shifted the oscillations from a high-amplitude low-frequency pattern (delta oscillations) to a low-amplitude high-frequency pattern with higher contribution of sigma and gamma oscillations in CA1 and mPFC. These results confirm that PILO affected brain oscillatory state by the time LTP was induced and suggest that the late-phase of mPFC LTP is favored during desynchronized states, as seen in behaving animals during wakefulness and REM sleep (Bramham and Srebro, 1989; Leung et al., 2003). In fact, CA1-mPFC evoked responses are modulated by the behavior state in rats (Romcy-Pereira and Pavlides, unpublished observations). Recent evidence also indicate that mPFC neuronal firing is entrained by the cholinergic-associated theta oscillation of the hippocampus and increases its synchrony with CA1 neurons during working memory tasks (Jones and Wilson, 2005; Siapas et al., 2005). Such hippocampo-cortical interactions may be important mechanisms for gating information flow and promoting plastic changes thought to underlie the storage of information within this network.

It is possible that the muscarinic enhancement of CA1-mPFC LTP may have been influenced by monoaminergic changes induced by PILO at the time of LTP induction, 20 min after injection. The trend toward an increase in DOPAC/dopamine ratio detected in the mPFC suggests that PILO induced an enhancement of dopamine release. This is in agreement with studies showing that systemic and midbrain administration of muscarinic agonists, including PILO, augment the release of dopamine in the mPFC (Gronier et al., 2000; Stanhope et al., 2001; Ichikawa et al., 2002). It is also possible that PILO modulates a supposed dopamine release induced by the electrical stimulation of the hippocampus, since NMDA stimulation of the ventral hippocampus increases DA release in the mPFC (Peleg-Raibstein et al., 2005). It was shown that although dopamine inhibits normal synaptic transmission, it enhances early and late mPFC LTP *in vivo* (Jay et al., 1996; Gurden et al., 1999). In contrast to what we observed in the mPFC, in the hippocampus and parietal cortex, the undetected low levels of dopamine suggest that it does not play a major influence on the PILO-induced mPFC LTP strengthening.

As for 5-HT, the simultaneous decrease in neurotransmitter and metabolite levels observed in the mPFC indicates that PILO may have inhibited raphe neurons. In fact, iontophoretic application of acetylcholine into the dorsal raphe of anesthetized rats inhibits about one-half of its neurons (Koyama and Kayama, 1993), which could indicate a reduction in cortical 5-HT release. Considering that a single dose of fluvoxamine treatment seems to have no effect on CA1-mPFC LTP *in vivo* (Ohashi et al., 2002), we do not believe that the serotonergic changes observed here affected PILO-induced mPFC LTP enhancement. Besides, PILO did not alter 5-HT turnover in the mPFC. In the parietal cortex, however, PILO altered 5-HT and 5-HIAA levels as compared with mPFC and hippocampus. 5-HT turnover was strongly enhanced in the parietal cortex as compared with the other areas. These data suggest that the serotonergic transmission may undergo specific muscarinic modulation, presumably associated to particular distributions of cholinergic receptors in cortical areas. Although we observed specific noradrenaline changes in the mPFC, noradrenaline also tended to be reduced in the parietal cortex.

Considering that LTP is a synaptic correlate of memory storage, our result of muscarinic strengthening of mPFC LTP agrees with reports that prefrontal-dependent memories are enhanced by muscarinic activation. Muscarinic agonists enhance the performance in tasks requiring attentional and working memory capacities in rats, monkeys and humans (Granon et al., 1995; Sarter and Bruno, 1997; Ragozzino and Kesner, 1998; Gill et al., 2000; Chudasama et al., 2004; Hasselmo and Stern, 2006). Besides, working memory tasks induce an increase in acetylcholine efflux in the prefrontal cortex of rats (Hironaka et al., 2001). Our findings also support the idea of an important cholinergic modulation of the synaptic plasticity in prefrontal circuits involved in cognitive and emotional processes underlying normal and psychiatric behaviors (Sarter et al., 2005). In one case, it was recently reported that REM sleep, a cholinergically-driven behavioral state, has an enhancing effect on hippocampal LTP but a dampening effect on mPFC LTP (Romcy-Pereira and Pavlides, 2004). In agreement to that, REM sleep is correlated with a reactivation of temporal lobe structures along with a deactivation of the prefrontal cortex (Maquet et al., 1996). In addition, the muscarinic receptor system seems to be altered in the pathophysiology of schizophrenia (Raedler et al., 2007). The atypical antipsychotic drug clozapine, which possibly exerts its cognitive-enhancing effects by increasing acetylcholine release in the prefrontal cortex (Ago et al., 2006), promotes an enhancement of synaptic plasticity in the mPFC (Gemperle et al., 2003). It has also been shown that acetylcholine levels specifically increase in the hippocampus and prefrontal cortex of rats during an inescapable stress model of depression (Mark et al., 1996). Therefore, the muscarinic neurotransmission has an important modulatory influence on prefrontal cortex functions. Further investigations should address the role of specific muscarinic and nicotinic receptor modulators on synaptic plasticity at connections between the mPFC and other relevant cortical and subcortical structures involved in cognitive and neuropsychiatric disorders.

Acknowledgments—We would like to thank Renata Scandiuzzi and Renato Meirelles for their excellent technical support. We would also like to thank Ana Claudia Zanetti, Graziela Bachiega and Raquel do Val for valuable discussions and advice. The work was supported by grants from CAPES-PRODOC, FAPESP and CNPq.

REFERENCES

Abe K, Nakata A, Mizutani A, Saito H (1994) Facilitatory but nonessential role of the muscarinic cholinergic system in the generation of long-term potentiation of population spikes in the dentate gyrus in vivo. Neuropharmacology 33:847–852.

Ago Y, Sato M, Nakamura S, Baba A, Matsuda T (2006) Lack of enhanced effect of antipsychotics combined with fluvoxamine on acetylcholine release in rat prefrontal cortex. J Pharmacol Sci 102:419–422.

Barbas H, Blatt GJ (1995) Topographically specific hippocampal projections target functionally distinct prefrontal areas in the rhesus monkey. Hippocampus 5:511–533.

Barnes CA, Meltzer J, Houston F, Orr G, McGann K, Wenk GL (2000) Chronic treatment of old rats with donepezil or galantamine: effects on memory, hippocampal plasticity and nicotinic receptors. Neuroscience 99:17–23.

Blitzer RD, Gil O, Landau EM (1990) Cholinergic stimulation enhances long-term potentiation in the CA1 region of rat hippocampus. Neurosci Lett 119:207–210.

Boyd TE, Trepel C, Racine RJ (2000) Cholinergic modulation of neocortical long-term potentiation in the awake, freely moving rat. Brain Res 881:28–36.

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254.

Bramham CR, Srebro B (1989) Synaptic plasticity in the hippocampus is modulated by behavioral state. Brain Res 493:74–86.

Caballero-Bleda M, Witter MP (1994) Projections from the presubiculum and the parasubiculum to morphologically characterized entorhinal-hippocampal projection neurons in the rat. Exp Brain Res 101:93–108.

Carr DB, Surmeier DJ (2007) M1 muscarinic receptor modulation of Kir2 channels enhances temporal summation of excitatory synaptic potentials in prefrontal cortex pyramidal neurons. J Neurophysiol 97:3432–3438.

Chen L, Bohanick JD, Nishihara M, Seamans JK, Yang CR (2007) Dopamine D1/5 receptor-mediated long-term potentiation of intrinsic excitability in rat prefrontal cortical neurons: Ca^{2+}-dependent intracellular signaling. J Neurophysiol 97:2448–2464.

Cheong MY, Yun SH, Mook-Jung I, Joo I, Huh K, Jung MW (2001) Cholinergic modulation of synaptic physiology in deep layer entorhinal cortex of the rat. J Neurosci Res 66:117–121.

Chudasama Y, Dalley JW, Nathwani F, Bouger P, Robbins TW (2004) Cholinergic modulation of visual attention and working memory: dissociable effects of basal forebrain 192-IgG-saporin lesions and intraprefrontal infusions of scopolamine. Learn Mem 11:78–86.

Couey JJ, Meredith RM, Spijker S, Poorthuis RB, Smit AB, Brussaard AB, Mansvelder HD (2007) Distributed network actions by nicotine increase the threshold for spike-timing-dependent plasticity in prefrontal cortex. Neuron 54:73–87.

Dalley JW, Cardinal RN, Robbins TW (2004) Prefrontal executive and cognitive functions in rodents: neural and neurochemical substrates. Neurosci Biobehav Rev 28:771–784.

Degenetais E, Thierry AM, Glowinski J, Gioanni Y (2003) Synaptic influence of hippocampus on pyramidal cells of the rat prefrontal cortex: an in vivo intracellular recording study. Cereb Cortex 13:782–792.

Detari L, Juhasz G, Kukorelli T (1984) Firing properties of cat basal forebrain neurones during sleep-wakefulness cycle. Electroencephalogr Clin Neurophysiol 58:362–368.

Dringenberg HC, Hamze B, Wilson A, Speechley W, Kuo MC (2007) Heterosynaptic facilitation of in vivo thalamocortical long-term potentiation in the adult rat visual cortex by acetylcholine. Cereb Cortex 17:839–848.

Dunbar GC, Kuchibhatla R (2006) Cognitive enhancement in man with ispronicline, a nicotinic partial agonist. J Mol Neurosci 30:169–172.

Dupin N, Mailliet F, Rocher C, Kessal K, Spedding M, Jay TM (2006) Common efficacy of psychotropic drugs in restoring stress-induced impairment of prefrontal plasticity. Neurotox Res 10:193–198.

Fanselow MS (2000) Contextual fear, gestalt memories, and the hippocampus. Behav Brain Res 110:73–81.

Ferino F, Thierry AM, Glowinski J (1987) Anatomical and electrophysiological evidence for a direct projection from Ammon's horn to the medial prefrontal cortex in the rat. Exp Brain Res 65:421–426.

Frey S, Bergado JA, Frey JU (2003) Modulation of late phases of long-term potentiation in rat dentate gyrus by stimulation of the medial septum. Neuroscience 118:1055–1062.

Funahashi S, Bruce CJ, Goldman-Rakic PS (1989) Mnemonic coding of visual space in the monkey's dorsolateral prefrontal cortex. J Neurophysiol 61:331–349.

Fuster JM (1973) Unit activity in prefrontal cortex during delayed-response performance: neuronal correlates of transient memory. J Neurophysiol 36:61–78.

Gemperle A, Olpe HR (2004) Effects of subchronic clozapine treatment on long-term potentiation in rat prefrontal cortex. Eur Neuropsychopharmacol 14:340–346.

Gemperle AY, Enz A, Pozza MF, Luthi A, Olpe HR (2003) Effects of clozapine, haloperidol and iloperidone on neurotransmission and synaptic plasticity in prefrontal cortex and their accumulation in brain tissue: an in vitro study. Neuroscience 117:681–695.

Giacobini E (1990) Cholinergic receptors in human brain: effects of aging and Alzheimer disease. J Neurosci Res 27:548–560.

Gill TM, Sarter M, Givens B (2000) Sustained visual attention performance-associated prefrontal neuronal activity: evidence for cholinergic modulation. J Neurosci 20:4745–4757.

Goldman-Rakic PS (1995) Cellular basis of working memory. Neuron 14:477–485.

Goldman-Rakic PS (1996) The prefrontal landscape: implications of functional architecture for understanding human mentation and the central executive. Philos Trans R Soc Lond B Biol Sci 351: 1445–1453.

Goldman-Rakic PS (1999) The physiological approach: functional architecture of working memory and disordered cognition in schizophrenia. Biol Psychiatry 46:650–661.

Granon S, Poucet B, Thinus-Blanc C, Changeux JP, Vidal C (1995) Nicotinic and muscarinic receptors in the rat prefrontal cortex: differential roles in working memory, response selection and effortful processing. Psychopharmacology (Berl) 119:139–144.

Groenewegen HJ, Uylings HB (2000) The prefrontal cortex and the integration of sensory, limbic and autonomic information. Prog Brain Res 126:3–28.

Gronier B, Perry KW, Rasmussen K (2000) Activation of the mesocorticolimbic dopaminergic system by stimulation of muscarinic cholinergic receptors in the ventral tegmental area. Psychopharmacology (Berl) 147:347–355.

Gu Q (2002) Neuromodulatory transmitter systems in the cortex and their role in cortical plasticity. Neuroscience 111:815–835.

Gulledge AT, Park SB, Kawaguchi Y, Stuart GJ (2007) Heterogeneity of phasic cholinergic signaling in neocortical neurons. J Neurophysiol 97:2215–2229.

Gurden H, Tassin JP, Jay TM (1999) Integrity of the mesocortical dopaminergic system is necessary for complete expression of in vivo hippocampal-prefrontal cortex long-term potentiation. Neuroscience 94:1019–1027.

Hasselmo ME (1999) Neuromodulation: acetylcholine and memory consolidation. Trends Cogn Sci 3:351–359.

Hasselmo ME, Barkai E (1995) Cholinergic modulation of activity-dependent synaptic plasticity in the piriform cortex and associative memory function in a network biophysical simulation. J Neurosci 15:6592–6604.

Hasselmo ME, Stern CE (2006) Mechanisms underlying working memory for novel information. Trends Cogn Sci 10:487–493.

Hironaka N, Tanaka K, Izaki Y, Hori K, Nomura M (2001) Memory-related acetylcholine efflux from rat prefrontal cortex and hippocampus: a microdialysis study. Brain Res 901:143–150.

Huang CC, Lin HJ, Hsu KS (2007) Repeated cocaine administration promotes long-term potentiation induction in rat medial prefrontal cortex. Cereb Cortex 17:1877–1888.

Hughes RA (1982) Anticholinergic drugs, blood-brain-barrier and tonic immobility in chickens. Physiol Behav 29:67–71.

Ichikawa J, Chung YC, Li Z, Dai J, Meltzer HY (2002) Cholinergic modulation of basal and amphetamine-induced dopamine release in rat medial prefrontal cortex and nucleus accumbens. Brain Res 958:176–184.

Iga Y, Arisawa H, Ise M, Yasuda H, Takeshita Y (1996) Modulation of rhythmical slow activity, long-term potentiation and memory by muscarinic receptor agonists. Eur J Pharmacol 308:13–19.

Izaki Y, Takita M, Nomura M (2002) Local properties of CA1 region in hippocampo-prefrontal synaptic plasticity in rats. Neuroreport 13: 469–472.

Jay TM, Burette F, Laroche S (1995) NMDA receptor-dependent long-term potentiation in the hippocampal afferent fibre system to the prefrontal cortex in the rat. Eur J Neurosci 7:247–250.

Jay TM, Burette F, Laroche S (1996) Plasticity of the hippocampal-prefrontal cortex synapses. J Physiol Paris 90:361–366.

Jay TM, Glowinski J, Thierry AM (1989) Selectivity of the hippocampal projection to the prelimbic area of the prefrontal cortex in the rat. Brain Res 505:337–340.

Jay TM, Rocher C, Hotte M, Naudon L, Gurden H, Spedding M (2004) Plasticity at hippocampal to prefrontal cortex synapses is impaired by loss of dopamine and stress: importance for psychiatric diseases. Neurotox Res 6:233–244.

Jay TM, Witter MP (1991) Distribution of hippocampal CA1 and subicular efferents in the prefrontal cortex of the rat studied by means of anterograde transport of Phaseolus vulgaris-leucoagglutinin. J Comp Neurol 313:574–586.

Jones MW, Wilson MA (2005) Theta rhythms coordinate hippocampal-prefrontal interactions in a spatial memory task. PLoS Biol 3:e402.

Kawashima H, Izaki Y, Grace AA, Takita M (2006) Cooperativity between hippocampal-prefrontal short-term plasticity through associative long-term potentiation. Brain Res 1109:37–44.

Koyama Y, Kayama Y (1993) Mutual interactions among cholinergic, noradrenergic and serotonergic neurons studied by ionophoresis of these transmitters in rat brainstem nuclei. Neuroscience 55: 1117–1126.

Laroche S, Jay TM, Thierry AM (1990) Long-term potentiation in the prefrontal cortex following stimulation of the hippocampal CA1/subicular region. Neurosci Lett 114:184–190.

Leung LS, Shen B, Rajakumar N, Ma J (2003) Cholinergic activity enhances hippocampal long-term potentiation in CA1 during walking in rats. J Neurosci 23:9297–9304.

Lyons DM (2002) Stress, depression, and inherited variation in primate hippocampal and prefrontal brain development. Psychopharmacol Bull 36:27–43.

Maquet P, Peters J, Aerts J, Delfiore G, Degueldre C, Luxen A, Franck G (1996) Functional neuroanatomy of human rapid-eye-movement sleep and dreaming. Nature 383:163–166.

Mark GP, Rada PV, Shors TJ (1996) Inescapable stress enhances extracellular acetylcholine in the rat hippocampus and prefrontal cortex but not the nucleus accumbens or amygdala. Neuroscience 74:767–774.

Mayo W, Dubois B, Ploska A, Javoy-Agid F, Agid Y, Le Moal M, Simon H (1984) Cortical cholinergic projections from the basal forebrain of the rat, with special reference to the prefrontal cortex innervation. Neurosci Lett 47:149–154.

McCormick DA, Prince DA (1985) Two types of muscarinic response to acetylcholine in mammalian cortical neurons. Proc Natl Acad Sci U S A 82:6344–6348.

McKinney M, Coyle JT, Hedreen JC (1983) Topographic analysis of the innervation of the rat neocortex and hippocampus by the basal forebrain cholinergic system. J Comp Neurol 217:103–121.

Messer WS Jr (2002) The utility of muscarinic agonists in the treatment of Alzheimer's disease. J Mol Neurosci 19:187–193.

Ohashi S, Matsumoto M, Otani H, Mori K, Togashi H, Ueno K, Kaku A, Yoshioka M (2002) Changes in synaptic plasticity in the rat hippocampo-medial prefrontal cortex pathway induced by repeated treatments with fluvoxamine. Brain Res 949:131–138.

Pakarinen ED, Moerschbaecher JM (1993) Comparison of the effects of scopolamine and methylscopolamine on the performance of a fixed-ratio discrimination in squirrel monkeys. Pharmacol Biochem Behav 44:815–819.

Palkovits M (1973) Isolated removal of hypothalamic or other brain nuclei of the rat. Brain Res 59:449–450.

Paxinos G, Watson C (1998) The rat brain in stereotaxic coordinates. New York: Academic Press.

Peleg-Raibstein D, Pezze MA, Ferger B, Zhang WN, Murphy CA, Feldon J, Bast T (2005) Activation of dopaminergic neurotransmission in the medial prefrontal cortex by N-methyl-d-aspartate stimulation of the ventral hippocampus in rats. Neuroscience 132: 219–232.

Raedler TJ, Bymaster FP, Tandon R, Copolov D, Dean B (2007) Towards a muscarinic hypothesis of schizophrenia. Mol Psychiatry 12:232–246.

Ragozzino ME, Kesner RP (1998) The effects of muscarinic cholinergic receptor blockade in the rat anterior cingulate and prelimbic/infralimbic cortices on spatial working memory. Neurobiol Learn Mem 69:241–257.

Romcy-Pereira R, Pavlides C (2004) Distinct modulatory effects of sleep on the maintenance of hippocampal and medial prefrontal cortex LTP. Eur J Neurosci 20:3453–3462.

Sarter M, Bruno JP (1997) Cognitive functions of cortical acetylcholine: toward a unifying hypothesis. Brain Res Brain Res Rev 23:28–46.

Sarter M, Nelson CL, Bruno JP (2005) Cortical cholinergic transmission and cortical information processing in schizophrenia. Schizophr Bull 31:117–138.

Satoh K, Fibiger HC (1986) Cholinergic neurons of the laterodorsal tegmental nucleus: efferent and afferent connections. J Comp Neurol 253:277–302.

Scoville WB, Milner B (1957) Loss of recent memory after bilateral hippocampal lesions. J Neurol Neurosurg Psychiatry 20:11–21.

Siapas AG, Lubenov EV, Wilson MA (2005) Prefrontal phase locking to hippocampal theta oscillations. Neuron 46:141–151.

Squire LR (1992) Memory and the hippocampus: a synthesis from findings with rats, monkeys, and humans. Psychol Rev 99: 195–231.

Stanhope KJ, Mirza NR, Bickerdike MJ, Bright JL, Harrington NR, Hesselink MB, Kennett GA, Lightowler S, Sheardown MJ, Syed R, Upton RL, Wadsworth G, Weiss SM, Wyatt A (2001) The muscarinic receptor agonist xanomeline has an antipsychotic-like profile in the rat. J Pharmacol Exp Ther 299:782–792.

Swanson LW (1981) A direct projection from Ammon's horn to prefrontal cortex in the rat. Brain Res 217:150–154.

Swanson LW, Cowan WM (1977) An autoradiographic study of the organization of the efferent connections of the hippocampal formation in the rat. J Comp Neurol 172:49–84.

Takita M, Izaki Y, Jay TM, Kaneko H, Suzuki SS (1999) Induction of stable long-term depression in vivo in the hippocampal-prefrontal cortex pathway. Eur J Neurosci 11:4145–4148.

van Haaren F, van Hest A (1989) The effects of scopolamine and methylscopolamine on visual and auditory discriminations in male and female Wistar rats. Pharmacol Biochem Behav 32:707–710.

Verdier D, Dykes RW (2001) Long-term cholinergic enhancement of evoked potentials in rat hindlimb somatosensory cortex displays characteristics of long-term potentiation. Exp Brain Res 137: 71–82.

Vertes RP (2006) Interactions among the medial prefrontal cortex, hippocampus and midline thalamus in emotional and cognitive processing in the rat. Neuroscience 142:1–20.

Witter MP, Amaral DG (2004) Hippocampal formation. In: The rat nervous system (Paxinos G, ed), pp 637–703. San Diego: Elsevier Academic Press.

(Accepted 26 February 2008)
(Available online 29 February 2008)