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Effect of amphetamine on extracellular acetylcholine and monoamine levels in subterritories of the rat medial prefrontal cortex

Gaël Hedou [a], Judith Homberg [a], Sonsoles Martin [a], Katja Wirth [a], Joram Feldon [a], Christian A. Heidbreder [b,*]

[a] *The Swiss Federal Institute of Technology Zürich (ETH), Laboratory of Behavioral Biology, Zürich, Switzerland*

[b] *SmithKline Beecham Pharmaceuticals, Department of Neuroscience Research, Neurobehavioral Research Group, New Frontiers Science Park (North), Building H25, Room 104a, Third Avenue, Harlow, Essex CM19 5AW, UK*

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Abstract

The present study sought to investigate the contributions of the dorsal prelimbic/anterior cingulate and ventral prelimbic/infralimbic cortices to the reverse microdialysis of amphetamine (1, 10, 100, 500, and 1000 μM) on dialysate acetylcholine, choline, norepinephrine, and serotonin levels. The results demonstrate that basal levels of acetylcholine, choline, and serotonin were homogeneous within subregions of the medial prefrontal cortex. In contrast, dialysate norepinephrine levels were significantly higher in the anterior cingulate cortex compared with the infralimbic cortex. Reverse microdialysis of amphetamine in both subareas of the medial prefrontal cortex produced a dose-dependent increase in norepinephrine and serotonin levels; the magnitude of this effect was similar in both subterritories of the medial prefrontal cortex. Microinfusion of amphetamine increased dialysate acetylcholine levels in a dose-dependent manner only in the infralimbic cortex. Finally, amphetamine decreased choline levels in both subregions of the medial prefrontal cortex. The magnitude of this effect was larger in the anterior cingulate cortex compared with its infralimbic counterpart. Since depletions of frontal cortical acetylcholine result in severe cognitive deficits, the present data raise the possibility that the type of neural integrative processes that acetylcholine mediates depends, at least in part, on the subterritories that characterize the medial prefrontal cortex.

Keywords: Acetylcholine; Choline; Cortex, medial prefrontal; Norepinephrine; Serotonin

1. Introduction

The medial prefrontal cortex can be defined anatomically both in terms of neural connectivity (Berendse et al., 1992; McDonald, 1996; Gorelova and Yang, 1997; Groenewegen et al., 1997) and cytoarchitectonic subterritories (medial precentral, medial orbital, anterior cingulate, prelimbic and infralimbic cortices) (Berendse and Groenewegen, 1991; Jay and Witter, 1991; Conde et al., 1995; Sesack et al., 1998). The anterior cingulate and dorsocaudal prelimbic cortices are usually referred to as the dorsal medial prefrontal cortex, and the ventral prelimbic and rostral infralimbic cortices as the ventral medial prefrontal cortex (Morgan and LeDoux, 1995; Morgan et al., 1993; Neafsey et al., 1993). Functionally, the medial prefrontal cortex can no longer be considered as a whole entity. Rather, the recent literature points to the relevance of conducting a functional analysis of prefrontal subregions and supports the idea that the frontal midline area is characterized by its own internal functional heterogeneity. For example, selective lesions of the anterior cingulate/dorsal prelimbic cortices increase conditioned fear responses (Morgan and LeDoux, 1995), enhance tachycardia to an excitatory conditioned stimulus (Fryszak and Neafsey, 1994), impair working memory for egocentric space (Ragozzino et al., 1998), and block the expression of cocaine sensitization (Pierce et al., 1998). In contrast, selective lesions of the ventral prelimbic/infralimbic cortices increase resistance to extinction of conditioned fear (Morgan et al., 1993), decrease tachycardia to an excitatory conditioned stimulus (Fryszak and Neafsey, 1994), impair passive avoidance (Jinks and McGregor,

* Corresponding author. Tel.: +44-1279-622-457; fax: +44-1279-622-660.

E-mail address: Christian_A_Heidbreder@sbphrd.com (C.A. Heidbreder).

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1997), impair working memory for allocentric space (Ragozzino et al., 1998), attenuate the development of cocaine sensitization (Tzschentke and Schmidt, 1998), and impair reversal learning in a visual discrimination task (Li and Shao, 1998).

Despite these new findings, little is known about the neurochemical dissociations between subareas of the medial prefrontal cortex. We have recently reported (Hedou et al., 1999b) that reverse microdialysis of amphetamine in the ventral medial prefrontal cortex produced a significant dose-dependent decrease in dialysate dopamine levels, whereas no significant alterations in dopamine levels were observed following amphetamine microinfusion in the dorsal medial prefrontal cortex. Furthermore, we have demonstrated a significant negative relationship between dopamine levels in both the ventral and dorsal medial prefrontal cortex and locomotor activity in response to the acute systemic administration of cocaine (Hedou et al., 1999a). These observations are consistent with the discovery that the deep layers of the medial prefrontal cortex along the medial edge of the corpus callosum correspond to the portion of the medial prefrontal cortex that has the greatest dopamine innervation density (Ciliax et al., 1995). Furthermore, the dopamine transporter is densely distributed in the dorsal anterior cingulate cortex and distributed only sparsely in the deep layers of the prelimbic cortex (Sesack et al., 1998). In addition to dopamine, the cholinergic system has also been involved in the neural integrative processes in the medial prefrontal cortex. Many lines of evidence suggest that, anatomically, the rat medial prefrontal cortex receives cholinergic neurons originating from the nucleus basalis magnocellularis and the mesopontine laterodorsal tegmental nucleus (Lehmann et al., 1980; Satoh and Fibiger, 1986; Luiten et al., 1987; Gaykema et al., 1990). Functionally, the activation of these cholinergic neurons and the resulting release of acetylcholine in the target prefrontal cortical areas are associated with electroencephalographic desynchronization (Kainai and Szerb, 1965; Celesia and Jasper, 1966) and locomotor activity (Day et al., 1991). Thus, the activation of the basal forebrain cholinergic neurons projecting toward the medial prefrontal cortex results in arousal, which in turn, is required for the processing of sensory, motor, and cognitive information (Durkin, 1994; Durkin and Toumane, 1992; Pirch, 1993; Sarter and Bruno, 1997; Ragozzino and Kesner, 1998).

Amphetamine, which releases primarily dopamine but also norepinephrine and serotonin from neuronal terminals (Azzaro and Rutledge, 1973; Kuczenski, 1983), increases dialysate acetylcholine levels in the frontal cortex (Day and Fibiger, 1992). Furthermore, the effect of amphetamine on cortical acetylcholine release may be related to arousal as measured by amphetamine-induced behavioral hyperactivity and electroencephalographic desynchronization (Fairchild et al., 1967). Given the growing body of evidence indicating a functional segregation between different laminae of the medial prefrontal cortex, the present study was designed to examine the effect produced by the reverse microdialysis of several doses of amphetamine on acetylcholine release in the anterior cingulate/dorsal prelimbic and ventral prelimbic/infralimbic cortices in the anaesthetized rat. The effect of amphetamine on both norepinephrine and serotonin in these two subregions of the medial prefrontal cortex was also assessed.

2. Materials and methods

2.1. Subjects

Male Wistar rats (Institute of Toxicology, Schwerzenbach, Switzerland) weighing 300 g were housed in a temperature- and humidity-controlled environment. They had free access to food pellets and water and were kept on a reverse 12 h light/dark cycle. Daily care provided to the animals included changing and cleaning soiled cages, providing food and water, and monitoring the general health of all animals. All the procedures used in this study were approved by the Swiss Federal Veterinary Office.

The animals were anaesthetized with sodium pentobarbital (60 mg/kg i.p.). Each rat was mounted on a stereotaxic apparatus (David Kopf, Topanga, CA) with the upper incisor bar set 3.5 mm below the interaural line. The skull was exposed and a hole drilled for unilateral placement of microdialysis probes (CMA/7, 2 mm cuprophane membrane) into either (1) the dorsal subregion of the medial prefrontal cortex, or (2) the ventral subregion of the medial prefrontal cortex. The coordinates, with respect to bregma, were as follows (Paxinos and Watson, 1986): for the ventral part of the medial prefrontal cortex: +3.2 mm anterior (A) to bregma; 0.5 mm lateral (L) to the midsagittal sinus; 6.0 mm ventral (V) to the dura surface; for the dorsal part of the medial prefrontal cortex: $A = 2.7$ mm, $L = 0.5$ mm; $V = 4.0$ mm). One hourly dose of chloral hydrate (100 mg/kg i.p.) was administered to maintain a constant level of anaesthesia throughout the experiment.

2.2. Brain microdialysis procedure

The inlet and outlet polyurethane tubings of the probes were connected to a dual quartz lined two-channel swivel (Instech Lab., Plymouth Meeting, PA) located on a low mass spring counterbalanced arm. The liquid swivel was connected to a gas-tight syringe on a microinfusion pump (Instech 2000, Instech Lab.). The microdialysis probes used for the detection of norepinephrine and serotonin were flushed at 1.0 μl/min with an artificial cerebrospinal fluid containing 145 mM NaCl, 2.7 mM KCl, 1.0 mM $MgCl_2 6H_2O$, 1.2 mM $CaCl_2 2H_2O$, and 2.0 mM Na_2HPO_4, adjusted to pH 7.4 with 85% H_3PO_4 [high pressure liquid chromatography (HPLC) grade]. The microdialysis probes used for the detection of acetylcholine

were flushed at 1.0 μl/min with an artificial cerebrospinal fluid containing 142 mM NaCl, 3.9 mM KCl, 1.0 mM $MgCl_2 6H_2O$, 1.2 mM $CaCl_2 2H_2O$, 1.35 mM Na_2HPO_4, 0.3 mM NaH_2PO_4, adjusted to pH 7.4 with 85% H_3PO_4 (HPLC grade). To recover detectable levels of acetylcholine in microdialysates, a reversible cholinesterase inhibitor (neostigmine bromide, 0.1 μM) was added to this perfusion solution. The physiological perfusates were filtered through a 0.22 μm filter (Millipore, Bedford, MA). When the experiment started, dialysate samples were collected every 10 min at 2.2 μl/min in polyethylene microcentrifuge vials. Stable baseline measurements were determined before introducing an independent variable for within animal reliability. Amphetamine was dissolved in the artificial cerebrospinal fluid and perfused via the microdialysis probe (reverse microdialysis) to circumvent pharmacokinetic factors and to minimize the effects of these compounds on non-medial prefrontal cortex structures that could indirectly influence prefrontal cortex neurotransmission. For the determination of the dose–response relationship, five increasing concentrations of amphetamine (1, 10, 100, 500, and 1000 μM) were substituted for the dialysis perfusate for 60 min each (six samples). Drug delivery and sample collection time were corrected for the lag time (10 min) resulting from the dead volume of the inlet and outlet tubings.

2.3. Chromatographic analysis of brain microdialysates

A chromatography workstation (Millenium, Millipore) was used in conjunction with a solvent delivery pump (Rheos 4000, Flux Instruments, Switzerland) and an electrochemical amperometric detector (Antec-Decade, Leyden, The Netherlands) with a working electrode set at +750 mV vs. Ag/AgCl for the detection of norepinephrine and serotonin. A six-port rotary valve (Model 7125, Rheodyne, Berkeley, CA, USA) was used for sample injection. Chromatographic separations were performed using a Chrompack glass column [100 (L) × 3 (i.d.ø) × 9 (o.d.ø) mm] packed on microparticulate (5 μm) silica gel. The mobile phase consisted of 37.5 mM citric acid, 58.5 mM sodium acetate, 0.72 mM disodium ethylenediamine tetraacetate (Na_2EDTA), and 0.23 mM 1-octanesulfonic acid sodium salt. To this solution, 0.1% diethylamine and 7% methanol (v/v) were added and thoroughly mixed. The pH of the final solution was adjusted to 4.1 with phosphoric acid (85%). The mobile phase was filtered through a 0.22-μm filter (Millipore), degassed under vacuum, and delivered at a flow rate of 1.0 ml/min. The position and height of the peaks of the endogenous components were compared with 22-μl samples of an external calibrating standard solution containing 100, 10 and 1 nM norepinephrine and serotonin. For the detection of choline and acetylcholine, the assay involved a Chrompack Chromspher choline analytical column (100 × 3 × 9 mm) packed with a material based on silica (5 μm particle size), with covalently bonded cation exchange/reversed phase groups. The covalent binding of acetylcholinesterase and choline oxidase onto an Immobilized Enzyme Reactor (Chrompack ChromSep) allowed the sequential and stoichiometric conversion of acetylcholine to betaine and choline, and choline to acetic acid and hydrogen peroxide. The hydrogen peroxide was then electrochemically detected via oxidation on a platinum-working electrode set at +500 mV vs. an Ag/AgCl reference electrode. The mobile phase consisted of a 0.2 M potassium phosphate dibasic buffer (K_2HPO_4). To this buffer, 0.001 M tetramethylammonium hydroxide was added. The pH was adjusted to 8.0 using a 0.2 M potassium phosphate monobasic (KH_2PO_4) solution. The mobile phase was filtered at 0.22 μm and the flow rate of the pump was set at 0.6 ml/min. The position and height of the peaks in dialysates were compared with 22-μl samples of an external calibrating standard solution containing 100, 10 and 1 nM acetylcholine. To compensate for gradual decay in detector sensitivity during a working day, 22 μl of working standard solutions was injected into the HPLC systems after every tenth dialysate samples.

2.4. Drugs

Citric acid, sodium acetate, Na_2EDTA, 1-octanesulfonic acid sodium salt, methanol, diethylamine, tetramethylammonium hydroxide, potassium phosphate, and phosphoric acid were analytical grade and were obtained from Sigma (St. Louis, MO, USA) and Fluka BioChemica (Ronkonkoma, NY, USA). Amphetamine was also obtained from Sigma, dissolved in artificial cerebrospinal fluid at 1.0 mM, and prepared daily. The stock solutions were further diluted with artificial cerebrospinal fluid to tested concentrations.

2.5. Histology

After the final dialysis samples were collected, rats were transcardially perfused with 100 ml NaCl and 500 ml of a 4% paraformaldehyde/1% calcium acetate/100 mM NaCl solution. Histological verification of probe placement was made via serial coronal sections (40 μm thick) using a cryostat. The sections were then processed for Luxol Fast Cresyl violet stain. The final analysis included the following number of rats in each group: anterior cingulate/dorsal prelimbic ($n = 6$ for the acetylcholine/choline assay, $n = 19$ for the norepinephrine and serotonin assays) and ventral prelimbic/infralimbic ($n = 6$ for the acetylcholine/choline assay, $n = 23$ for the norepinephrine and serotonin assays).

2.6. Data analysis

The average level of acetylcholine, choline, norepinephrine, and serotonin in three samples immediately preceding the microinfusion of amphetamine was defined as the

Table 1
Absolute basal levels of acetylcholine (ACh), choline (Ch), norepinephrine (NE), and serotonin (5-HT) obtained from microdialysates of subregions of the medial prefrontal cortex

Subterritory	fmol/μl Sample (±S.E.M.)[a]			
	Ach	Ch	NE	5-HT
Anterior Cingulate	19.9±3.8	256.7±69.8	307.8±32*	0.8±0.3
Infralimbic	36.4±15.2	243.2±43.4	178.4±16.5	0.7±0.1

[a]The average level of acetylcholine, choline, norepinephrine, and serotonin in three samples immediately preceding the microinfusion of amphetamine was defined as the baseline control (100%). Basal values are not corrected for probe recovery.
*$P < 0.05$ vs. infralimbic according to Fischer's test.

baseline control (100%). Basal levels of acetylcholine, choline, norepinephrine, and serotonin were analyzed by an analysis of variance with a main factor of subterritory (infralimbic cortex vs. anterior cingulate cortex) and repeated measurement factor of time with three blocks of 10 min each. Amphetamine-induced changes in dialysate levels of acetylcholine, choline, norepinephrine, and serotonin were expressed as a percentage of baseline control. The raw data were analysed by $2 \times 5 \times 6$ analyses of variance with a main factor of subregion (infralimbic cortex vs. anterior cingulate cortex) and repeated measurements factors of amphetamine dose (1, 10, 100, 500, and 1000 μM) and time (six blocks of 10 min each). The differences between individual means were assessed with the post-hoc Fischer's Protected test. Statistical significance was set at a probability level of 0.05 for all tests.

3. Results

3.1. Basal levels of acetylcholine, choline, norepinephrine and serotonin in subregions of the medial prefrontal cortex

Basal levels of acetylcholine did not differ between the infralimbic and anterior cingulate cortices (Table 1). An analysis of variance with a main factor of subterritory (infralimbic cortex vs. anterior cingulate cortex) and a repeated measurement factor performed on the last three dialysate samples revealed no main effect of subterritory [$F(1,10) = 1.1$; $P = 0.3$], no significant effect of time [$F(2,20) = 0.1$; $P = 0.9$] and no significant subterritory × time interaction [$F(2,20) = 0.9$; $P = 0.4$]. Basal dialysate levels of choline were also similar in both subregions of the medial prefrontal cortex (Table 1). Thus, the analysis of variance failed to reveal any significant effect of subterritory [$F(1,10) = 0.02$; $P = 0.9$], and time [$F(2,20) = 1.3$; P 0.3] as well as a significant subterritory × time interaction [$F(2,20) = 0.9$; $P = 0.4$]. Basal dialysate levels of norepinephrine were significantly higher in the anterior cingulate cortex (307.8 ± 32 fmol/μl sample) compared with the infralimbic cortex (178.4 ± 16.5 fmol/μl sample) (Table 1). The analysis of variance revealed a significant

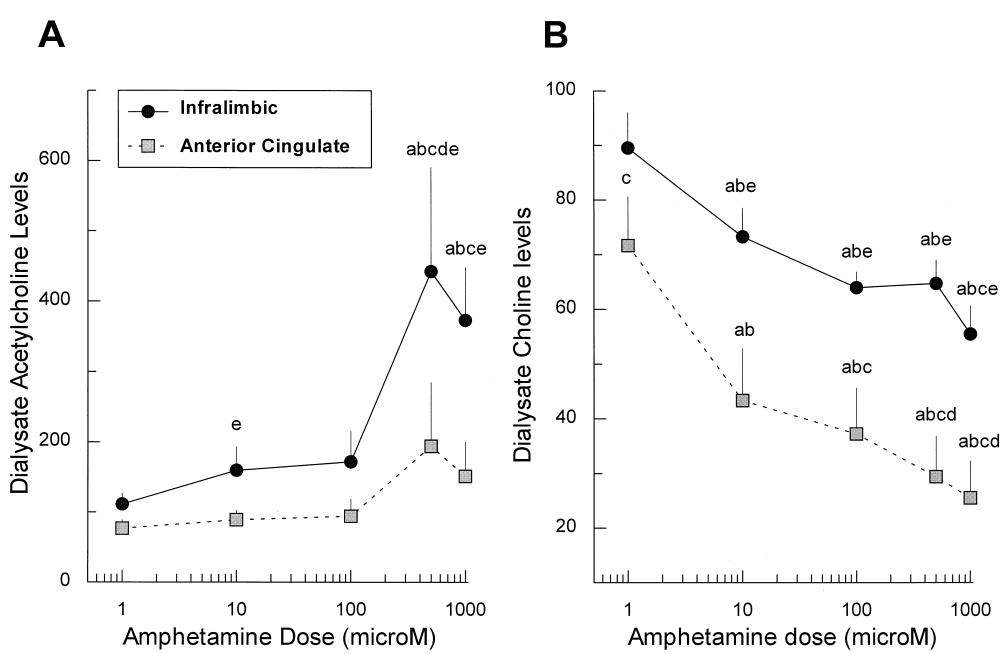

Fig. 1. Effect of local microinfusion of amphetamine into either the infralimbic or anterior cingulate subregions of the medial prefrontal cortex on dialysate acetylcholine (ACh) (Panel A) and choline (Ch) (Panel B) levels. For the determination of the dose–response relationship, five increasing concentrations of amphetamine (1, 10, 100, 500, and 1000 μM) were substituted for the dialysis perfusate for 60 min each (six samples). The data are represented as mean (±S.E.M.) percentage baseline and were summed over each 60 min period. (a) $P < 0.05$ vs. baseline (paired t-test); (b) $P < 0.05$ vs. 1.0 μM (paired t-test); (c) $P < 0.05$ vs. 10.0 μM (paired t-test); (d) $P < 0.05$ vs. 100.0 μM (paired t-test); (e) $P < 0.05$ vs. corresponding point in the other curve (unpaired t-test).

main effect of subterritory [$F(1,40) = 14.1$; $P < 0.0005$], but no significant effect of time [$F(2,80) = 1.2$; $P = 0.3$] and no significant subterritory × time interaction [$F(2,80) = 0.9$; $P = 0.4$]. Finally, no significant differences in basal levels of serotonin were found between the infralimbic and anterior cingulate cortices (Table 1). The analysis of variance confirmed the absence of significant main effects of subterritory [$F(1,19) = 0.1$; $P = 0.7$] and time [$F(2,38) = 0.1$; $P = 0.9$] as well as the absence of a significant subterritory × time interaction [$F(2,38) = 0.6$; $P = 0.5$].

3.2. Effect of amphetamine applied to subregions of the medial prefrontal cortex on dialysate acetylcholine levels

Reverse microdialysis of amphetamine in the medial prefrontal cortex produced a significant dose-dependent increase in dialysate acetylcholine levels (Fig. 1A). An overall $2 \times 5 \times 6$ analysis of variance with a main factor of subterritory (infralimbic vs. anterior cingulate) and repeated measurements factors of amphetamine dose (1, 10, 100, 500, and 1000 μM) and time (six blocks of 10 min each) revealed significant effects of subterritory [$F(1,9) = 5.4$, $P < 0.04$], amphetamine dose [$F(4,36) = 5.7$, $P < 0.001$], and time [$F(5,45) = 3.5$, $P < 0.01$] as well as a significant time × amphetamine dose interaction [$F(20,180) = 2.05$, $P < 0.01$]. Separate analyses of variance applied to each subterritory revealed the lack of dose-dependent effect of amphetamine in the anterior cingulate cortex [$F(4,20) = 1.7$, $P = 0.2$]. In the infralimbic subregion of the medial prefrontal cortex, however, amphetamine produced a dose-dependent increase in dialysate acetylcholine levels as revealed by a significant effect of amphetamine dose [$F(4,20) = 4.1$, $P < 0.02$].

3.3. Effect of amphetamine applied to subregions of the medial prefrontal cortex on dialysate choline levels

Reverse microdialysis of amphetamine in the medial prefrontal cortex produced a significant dose-dependent decrease in dialysate choline levels (Fig. 1B). An overall $2 \times 5 \times 6$ analysis of variance with a main factor of subterritory (infralimbic vs. anterior cingulate) and repeated measurements factors of amphetamine dose (1, 10, 100, 500, and 1000 μM) and time (six blocks of 10 min each) revealed significant effects of subterritory [$F(1,9) = 11.2$, $P < 0.01$], amphetamine dose [$F(4,36) = 47.4$, $P < 0.0001$], and time [$F(5,45) = 14.7$, $P < 0.0001$] as well as a significant time × amphetamine dose interaction [$F(20,180) = 4.8$, $P < 0.0001$]. Separate analyses of variance applied to each subterritory revealed that amphetamine produced a dose-dependent effect in both the anterior cingulate [$F(4,20) = 97.9$, $P < 0.0001$] and infralimbic [$F(4,20) = 11.7$, $P < 0.0001$] cortices.

3.4. Effect of amphetamine applied to subregions of the medial prefrontal cortex on dialysate norepinephrine levels

Microinfusion of amphetamine into the medial prefrontal cortex by reverse microdialysis produced a signifi-

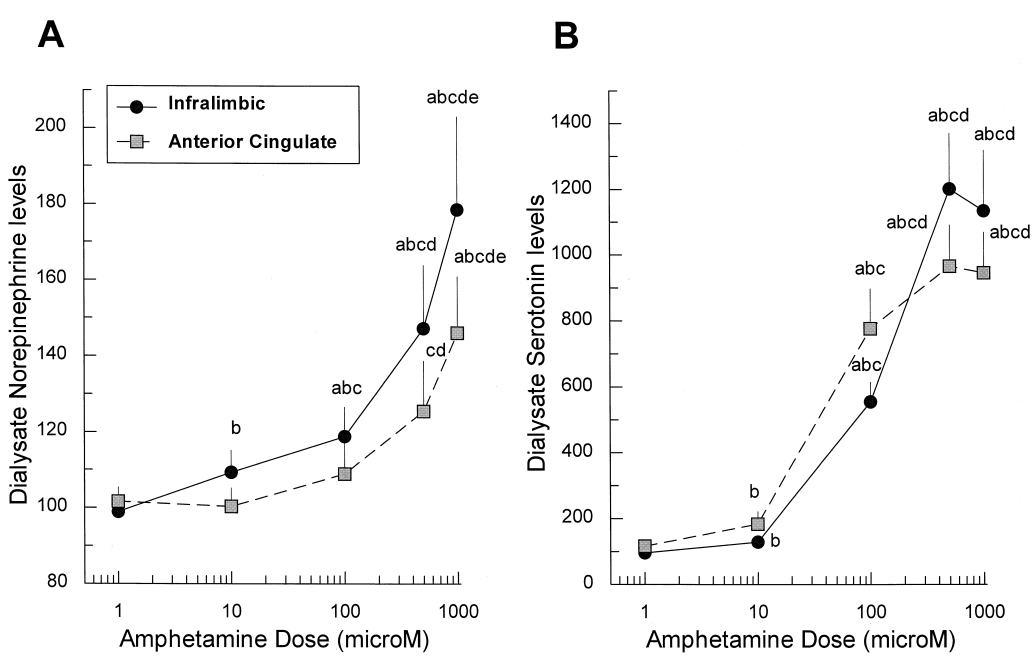

Fig. 2. Effect of local microinfusion of amphetamine into either the infralimbic or anterior cingulate subregions of the medial prefrontal cortex on dialysate norepinephrine (NE) (Panel A) and serotonin (5-HT) (Panel B) levels. For the determination of the dose–response relationship, five increasing concentrations of amphetamine (1, 10, 100, 500, and 1000 μM) were substituted for the dialysis perfusate for 60 min each (six samples). The data are represented as mean (± S.E.M.) percentage baseline and were summed over each 60 min period. (a) $P < 0.05$ vs. baseline (paired *t*-test); (b) $P < 0.05$ vs. 1.0 μM (paired *t*-test); (c) $P < 0.05$ vs. 10.0 μM (paired *t*-test); (d) $P < 0.05$ vs. 100.0 μM (paired *t*-test); (e) $P < 0.05$ vs. 500.0 μM (paired *t*-test).

cant dose-dependent increase in dialysate norepinephrine levels (Fig. 2A). An overall $2 \times 5 \times 6$ analysis of variance with a main factor of subterritory (infralimbic vs. anterior cingulate) and repeated measurements factors of amphetamine dose (1, 10, 100, 500, and 1000 μM) and time (six blocks of 10 min each) revealed no significant main effect of subterritory [$F(1,28) = 1.4$, $P = 0.2$], but significant effects of amphetamine dose [$F(4,112) = 17.7$, $P < 0.0001$] and time [$F(5,140) = 4.1$, $P < 0.001$] as well as a significant time × amphetamine dose interaction [$F(20,560) = 2.9$, $P < 0.0001$]. Separate analyses of variance applied to each subterritory revealed that amphetamine produced a dose-dependent effect in both the anterior cingulate [$F(4,52) = 9.4$, $P < 0.0001$] and infralimbic [$F(4,60) = 8.1$, $P < 0.0001$] cortices.

3.5. Effect of amphetamine applied to subregions of the medial prefrontal cortex on dialysate serotonin levels

Reverse microdialysis of amphetamine in the medial prefrontal cortex produced a significant dose-dependent increase in dialysate serotonin levels (Fig. 2B). An overall $2 \times 5 \times 6$ analysis of variance with a main factor of subterritory (infralimbic vs. anterior cingulate) and repeated measurements factors of amphetamine dose (1, 10, 100, 500, and 1000 μM) and time (six blocks of 10 min each) revealed no significant main effect of subterritory [$F(1,17) = 0.06$, $P = 0.8$], but significant effects of amphetamine dose [$F(4,68) = 64.8$, $P < 0.0001$] and time [$F(5,85) = 11.9$, $P < 0.0001$] as well as significant time × amphetamine dose and time × amphetamine dose × subterritory interactions [$F(20,340) = 3.6$, $P < 0.0001$ and $F(20,340) = 2.1$, $P < 0.004$, respectively]. Separate analyses of variance applied to each subterritory revealed that amphetamine produced a dose-dependent effect in both the anterior cingulate [$F(4,36) = 41.8$, $P < 0.0001$] and infralimbic [$F(4,32) = 28.2$, $P < 0.0001$] cortices.

3.6. Histology

Fig. 3 depicts the location of the dialysis membrane in the dorsal prefrontal cortex (dorsal anterior cingulate cortex and dorsal prelimbic cortex) and ventral prefrontal cortex (ventral prelimbic and infralimbic cortex). Silhouettes of probe tracks were drawn onto representative sections of the rat brain (Paxinos and Watson, 1986).

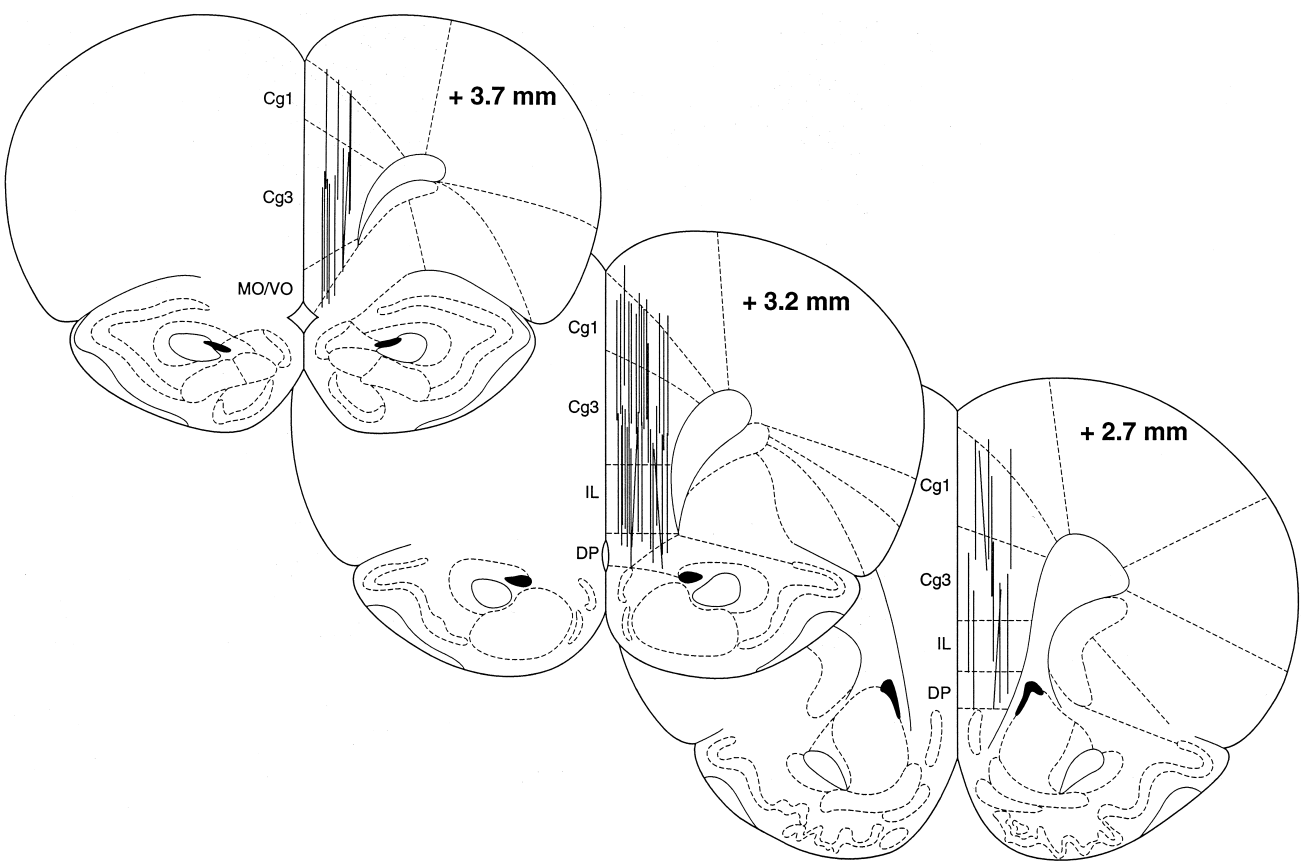

Fig. 3. Location of microdialysis membranes in the dorsal and ventral prefrontal cortices. Silhouettes of probe tracks were drawn onto representative sections of the rat brain (Paxinos and Watson, 1986). The numbers indicate millimeters rostral to bregma. Cg1: cingulate cortex, area 1 or area 24b; Cg3: cingulate cortex, area 3 or area 32; MO/VO: medial orbital cortex/ventral orbital cortex; IL: infralimbic cortex or area 25; DP: dorsal peduncular cortex.

4. Discussion

The results of the present study demonstrate that basal levels of acetylcholine, choline, and serotonin were homogeneous between the anterior cingulate and infralimbic subregions of the medial prefrontal cortex. In contrast, basal dialysate norepinephrine levels were significantly higher in the anterior cingulate cortex compared with the ventral medial prefrontal cortex. Reverse microdialysis of amphetamine in both subareas of the medial prefrontal cortex produced a dose-dependent increase in norepinephrine and serotonin levels; the magnitude of this effect was similar in both subterritories of the medial prefrontal cortex. Microinfusion of amphetamine increased dialysate acetylcholine levels in a dose-dependent manner only in the infralimbic cortex. Finally, amphetamine decreased choline levels in both subregions of the medial prefrontal cortex. The magnitude of this effect was larger in the anterior cingulate cortex compared with its infralimbic counterpart.

Basal levels of acetylcholine and serotonin had similar patterns in both the anterior cingulate and infralimbic subregions of the medial prefrontal cortex. The cholinergic innervation of the cortex originating from the caudal area of the basal forebrain has been shown to be diffuse in nature and to terminate in all cortical layers (Lamour et al., 1984; Woolf, 1991; Woolf et al., 1984; Luiten et al., 1987; Eckenstein et al., 1988; Sarter and Bruno, 1997). The serotonergic innervation of the cerebral cortex originates mainly from the raphe nuclei (Kosofsky and Molliver, 1987; Wilson and Molliver, 1991a,b), which send two morphologically distinct classes of fibers: fine axons with small varicosities originate from the dorsal raphe nucleus whereas beaded axons characterized by large, spherical varicosities arise from the median raphe nucleus. Fine serotonin axon terminals are widely distributed among all cortical layers, although variations in density and laminar distribution are observed between different cortical areas (Kosofsky and Molliver, 1987; Wilson and Molliver, 1991a,b). Beaded serotonin axon terminals are found primarily in the outer cortical layers (Wilson and Molliver, 1991a,b). Thus, despite evidence for a topographical organization of serotonergic fibers, and to a lesser extent cholinergic fibers, the results of the present study indicate that both acetylcholine and serotonin are released in a relatively uniform manner in the anterior cingulate and infralimbic cortices. These findings are also supported by recent studies indicating that both basal and stimulated acetylcholine releases are similar in the prefrontal and frontoparietal cortices (Himmelheber et al., 1998).

In the present study, dialysate norepinephrine levels were significantly higher in the dorsal medial prefrontal cortex compared with its ventral counterpart. It has been demonstrated that the anterior cingulate cortex has the lowest density of norepinephrine innervation in the neocortex, whereas the granular retrosplenial cortex (i.e., posterior cingulate cortex) receives the densest norepinephrine axon terminals (Morrison et al., 1979). The density of norepinephrine projections in the prelimbic cortex falls between that of the anterior and posterior cingulate cortices (Morrison et al., 1979). Despite the low norepinephrine innervation in the anterior cingulate cortex, our results show that there is a gradient in the extracellular concentrations of norepinephrine in the medial prefrontal cortex and that the highest basal dialysate norepinephrine levels are found in the dorsal medial prefrontal cortex. Further investigation is required to study this apparent discrepancy.

In the present study, reverse dialysis of amphetamine in both the anterior cingulate and infralimbic cortices produced a dose-dependent increase in norepinephrine and serotonin levels; the magnitude of this effect was similar in both subterritories of the medial prefrontal cortex. In addition to its well-known effects on dopamine neurotransmission, amphetamine also competitively inhibits the uptake of both norepinephrine (Azzaro and Rutledge, 1973; Harris and Baldessarini, 1973) and serotonin (Taylor and Ho, 1978). It has been demonstrated that amphetamine increases dialysate serotonin levels in both the medial prefrontal cortex (Kuroki et al., 1996) and nucleus accumbens (Hernandez et al., 1987), and that stimulation of serotonin$_{1A}$ receptors inhibits this amphetamine-induced enhanced serotonin neurotransmission (Kuroki et al., 1996). Systemic amphetamine (0.25–0.50 mg/kg i.v.) also increases dialysate norepinephrine levels in the medial prefrontal cortex (Tanda et al., 1997). Thus, our results indicate that changes in norepinephrine and serotonin efflux in response to the microinfusion of amphetamine follow similar patterns in both the anterior cingulate and infralimbic cortices. However, the magnitude of the serotonin response to amphetamine was greater than the norepinephrine response in two main cytoarchitectonic subterritories of the rat medial prefrontal cortex.

In our study, the microinfusion of amphetamine by reverse microdialysis increased dialysate acetylcholine levels in a dose-dependent manner only in the infralimbic cortex. Although it has been reported that the systemic administration of both amphetamine (2.0 mg/kg i.p.) and apomorphine (1.0 mg/kg i.p.) increases acetylcholine efflux in the medial prefrontal cortex (Day and Fibiger, 1992), the local application of amphetamine by reverse microdialysis at a concentration of 10 μM fails to significantly increase acetylcholine levels above baseline (Day and Fibiger, 1992). It should be noted that the Day and Fibiger's study was not designed to discriminate between subregions of the medial prefrontal cortex. Although the same concentration of amphetamine also failed to significantly increase acetylcholine levels above baseline values in the present study, our results demonstrate that the effect of amphetamine may be partly masked unless a subterritory factor is taken into account in the experimental design. In fact, our data indicate that amphetamine at 10, 500 and 1000 μM has a more potent effect on dialysate

acetylcholine levels in the infralimbic subregion of the medial prefrontal cortex compared with its dorsal anterior cingulate counterpart. Moreover, the sensitivity of neurons to the microiontophoretic application of acetylcholine in the dorsolateral prefrontal cortex of the monkey is not homogeneous between cortical layers (Sawaguchi and Matsumura, 1985), suggesting that acetylcholine may differentially influence the neuronal activity of specific laminae of the dorsolateral prefrontal cortex. Finally, acetylcholine induces both excitatory (Aou et al., 1983; Inoue et al., 1983; Sawaguchi and Matsumura, 1985) and inhibitory (Randic et al., 1964; Phillis and York, 1967, 1968) responses of neurons in the dorsolateral prefrontal cortex. Although both responses are mediated via a muscarinic mechanism, the existence of low- and high-affinity muscarinic receptor sites may account for either inhibitory or excitatory responses to acetylcholine (Lang and Henke, 1983). The distribution of the low-affinity muscarinic receptors seems to correspond with the distribution of neurons inhibited by acetylcholine (Sawaguchi and Matsumura, 1985). The determination of whether or not the laminar release of acetylcholine in the rat medial prefrontal cortex in response to amphetamine is related to a laminar distribution of low- and high-affinity muscarinic receptors requires further investigation.

One controversial methodological issue related to the electrochemical detection of acetylcholine in microdialysates from the rat brain concerns the addition of acetylcholinesterase inhibitors to the perfusion fluid to improve basal recovery of acetylcholine by hindering its enzymatic degradation (Damsma et al., 1987). This artificial increase in basal dialysate acetylcholine levels may lead to changes in the mechanisms by which both cholinergic (Damsma et al., 1987; Quirion et al., 1994; DeBoer and Abercrombie, 1996; Marchi and Raiteri, 1996) and dopaminergic (Acquas and Fibiger, 1998; DeBoer et al., 1990) compounds affect striatal and cortical acetylcholine release. Recent studies have demonstrated that the concentration of neostigmine in the perfusion solution can quantitatively and even qualitatively influence the manner in which dopaminergic agents regulate acetylcholine overflow in the striatum (DeBoer et al., 1990; Acquas and Fibiger, 1998). In both the prefrontal and frontoparietal cortices, the concentration of neostigmine (0.5 vs. 0.05 μM) had no effect on the magnitude or temporal dynamics of the increased acetylcholine efflux observed following tactile stimulation (Himmelheber et al., 1998). As can be predicted, however, the higher concentration of neostigmine used in the Himmelheber's study significantly increased basal acetylcholine outflow (26.5 vs. 85.0 fmol/μl). Thus, it is reasonable to suggest that, in the present study, the relatively low level of neostigmine that was added to the perfusion fluid (i.e., 0.1 μM) lead to an overestimation of basal dialysate acetylcholine levels in both subregions of the medial prefrontal cortex. Although it is unlikely that the differential dynamics of acetylcholine release that we observed in the infralimbic vs. anterior cingulate cortices in response to amphetamine depend on the levels of acetylcholinesterase inhibitors in the perfusion fluid, this requires further investigation.

Recent studies have elegantly demonstrated that microinfusions of the muscarinic cholinergic antagonist scopolamine into the prelimbic/infralimbic cortices, but not the anterior cingulate cortex, impair spatial working memory in a dose-dependent manner (Ragozzino and Kesner, 1998). The scopolamine-induced effect on spatial working memory is also attenuated by the concomitant administration of the muscarinic agonist oxotremorine in the same subregion of the medial prefrontal cortex (Ragozzino and Kesner, 1998), suggesting that the working memory impairment produced by scopolamine is likely due to the blockade of muscarinic cholinergic receptors in the prelimbic/infralimbic cortices. Given the functional segregation between the dorsal and ventral subregions of the medial prefrontal cortex and the increasing body of evidence suggesting the involvement of the prelimbic/infralimbic cortex in the use of mnemonic information to generate and revise planned actions or foraging strategies (Seamans et al., 1995), our data support the contention that whereas the release of acetylcholine in the medial prefrontal cortex heightens arousal, which in turn is required for the processing of sensory and motor information (Sarter and Bruno, 1997) as well as spatial working memory (Ragozzino and Kesner, 1998), the type of cognitive processes that acetylcholine enhances depends, at least in part, on the subterritories that characterize the medial prefrontal cortex.

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