Exp Brain Res (1994) 98:44-52

ORIGINAL PAPER

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Local pharmacological manipulation of extracellular dopamine levels in the dorsolateral prefrontal cortex and caudate nucleus in the rhesus monkey: An in vivo microdialysis study

Received: 24 February 1993 / Accepted: 5 October 1993

Abstract The prefrontal cortex, caudate nucleus, and their dopaminergic innervations have been implicated in complex information processing. The present study utilized the in vivo microdialysis technique to characterize the extracellular dopamine levels in the prefrontal cortex and the caudate nucleus in the rhesus monkey. Basal levels of dopamine were consistently found in the caudate nucleus, while levels in the prefrontal cortex were less reliably measured. Manipulation of dopamine levels using tetrodotoxin and high potassium demonstrated that dopamine measured was dependent on neuronal firing. Administration of indirect dopamine agonists *d*-amphetamine and cocaine into the prefrontal cortex and the caudate nucleus increased extracellular dopamine levels 250% and 5000%, respectively. Amphetamine and cocaine had greater effects on dopamine levels in the caudate than in the prefrontal cortex. Cocaine induced increases appeared to be less than that of amphetamine and the actions of cocaine lasted longer than amphetamine. This study demonstrates the feasibility of using in vivo microdialysis in monitoring neurochemicals in different regions of the rhesus monkey brain.

Key words Microdialysis · Dopamine · Caudate Prefrontal cortex · Monkey

Introduction

The development of a microdialysis technique to measure extracellular levels of different neurotransmitters and related metabolites in living animals has made it possible to monitor regional neurotransmitter activity in the functioning brain (Delgado et al. 1972, 1984; Ungerstedt and Pycock 1974; Ungerstedt 1984). The levels of neurotransmitters in the extracellular space provide a reliable reflection of neurochemical events occurring in the synaptic region (Zetterstrom et al. 1983; Westerink et al. 1987). The method involves insertion of dialysis probes into restricted brain regions, perfusion with artificial cerebrospinal fluid, diffusion of chemical substances across a semipermeable membrane in the direction of lowest concentration, collection of the dialysate, and analysis with liquid chromatography. Since the dialysis probe can simultaneously act as a distribution as well as a collection device, a complete pharmacological study can be carried out in a restricted brain region.

Brain microdialysis also has the potential to examine neurochemical changes associated with information processing and behavior. Previous experimental neurobehavioral studies with monkeys and clinical findings with psychiatric and neurological illnesses have implicated the prefrontal cortex, the caudate nucleus, and their dopaminergic innervations in complex information processing and behavior (Fuster 1980; Goldman-Rakic 1987; Weinberger 1988). Normal functioning of both prefrontal cortex and the caudate nucleus may depend in part upon dopaminergic transmission. Similarly, the hippocampal formation and the basal forebrain region and cholinergic innervation with areas of the neocortex have been implicated in memory processes. With these enticing regional-functional relationships in mind, we explored using the microdialysis procedure in the non-human primate as a tool for elucidating neurochemical changes associated with complex information processing.

The microdialysis technique has been extensively applied to the study of dopaminergic systems in the rat where the roles of neuronal impulses, synaptic release, reuptake, and motor behavior and stress-related responses have been examined (Ungerstedt 1984; Sharp et al. 1986; Benveniste 1989; for review, see Robinson and Justice 1991). Much less has been done in the primate (Skirboll et al. 1990). In the present experiment, we uti-

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lized the microdialysis technique to both monitor and manipulate the levels of neurochemicals in the extracellular fluid in the prefrontal cortex and in the caudate nucleus in the rhesus monkey. Our aims were to determine the feasibility and reliability of this method in this species, to measure dopamine in cortical and subcortical areas, and to manipulate the levels of dopamine by infusing tetrodotoxin (TTX), Potassium (K^+)-enriched CSF, amphetamine and cocaine directly through the dialysis probes. A preliminary abstract of this work has appeared elsewhere (Kolachana et al. 1991).

Materials and methods

Subjects

Four male adult rhesus monkeys (*Macaca mulatta*) weighing between 5 and 8 kg were used. The monkeys were housed individually with a 12 h light/dark cycle with food and water available ad lib. Care and use of the animals were in strict compliance with the NIH Guide for the Care and Use of Laboratory Animals (1985).

Probes

Concentric dialysis probes were constructed using two lengths of fused silica and polyethylene tubing (Wang et al. 1990). The two lengths of silica tubing made up an inner barrel (i.d. 100 μm, o.d. 170 μm) which extended 4–6 mm beyond the end of the outer barrel (i.d. 320 μm, o.d. 450 μm). A short piece of dialysis tubing (molecular weight cut-off limit of 40 000 Da, AN 69 polyacrylonitrile membrane, Hospal Medical Co) sealed at one end was placed over the extended end of the inner barrel and attached to the inner surface of the outer barrel with cynoacrylic glue. This left an exposed membrane tip of 4–6 mm. A short piece of PE 50 polyethylene tubing (2 cm) was attached to the remaining open end of the outer barrel through which the inner barrel extended. The inner barrel was attached to a piece of PE 10 tubing. A 30 ga stainless steel tube, (with a short piece of PE 10 attached to one end) was placed inside the PE 50 tube situated adjacent to the inner barrel and continuous with the outer barrel. The inner barrel connected to a pump served as inflow while the PE 10 from the outer tube served as outflow and led to the collection vials.

Before each experimental session, the probes were tested in vitro to estimate the recovery of dopamine through the dialysis membrane, in order to determine the efficiency of an individual dialysis probe. The probes were placed in an airtight beaker containing 10^{-7} M or 10^{-8} M dopamine (Sigma Co, St. Louis) in artificial cerebrospinal fluid (aCSF) (Na^+ 147 mM, K^+ 3mM, Ca^{2+} 1.3 mM, Mg^{2+} 1.0 mM, Cl^- 155 mM, 0.15 mM ascorbate buffered with 1.0 mM phosphate, pH 7.4) at 37° C and were perfused continuously with aCSF at 1 μl/min. The probes were perfused for 1 h before three 25 min collections were made. The mean recovery rate for the three collections, expressed as percentage recovery with respect to the dopamine concentration in the aCSF solution outside the probe, was determined in order to select a probe for experimental use. Probes used for in vivo experiments had recovery rates above 40%.

Probe Placement

In order to accurately place the probes in the prefrontal cortex and the caudate nucleus we used stereotactic procedures combined with magnetic resonance imaging (Saunders et al. 1990; Wang et al. 1990). This method produces a unique stereotactic atlas for each individual animal that insures highly reliable and accurate placement of the dialysis probes. In addition, we fabricated a unique array of guide cannulae and fixed it to the skull over the cortical target area, i.e., the medial bank of the principalis sulcus. The guide consisted of a small block of plastic (1 cm × 2 cm) in which four rows of small holes (< 1 mm in diameter and 1 mm center to center apart) had been drilled. This guide was placed over and aligned with the banks of the principalis sulcus. In this way we could place probes into the banks of the principalis sulcus in different experimental sessions without a major surgical procedure prior to each collection. In order to confirm the location of the dialysis probes, magnetic resonance images were repeated after a dialysis session, and, after the final session, the monkeys were killed with an overdose of anesthetic and the brains perfused with fixative, cut, stained and the location of the probes determined (Fig. 1).

Surgery

All surgery, implanting of the guides, and microdialysis was carried out under aseptic conditions. The monkeys were initially sedated with ketamine (10–15 mg/kg, i.m.), intubated, and surgical anesthesia achieved with isofluorane gas (1%–4%) for the length of the procedure. The skin was opened and the bone and guides exposed. For probe placement into the caudate nucleus a small bone flap was removed over the targeted area. The probes were then stereotactically placed into the caudate nucleus using the coordinates derived from MRI. Cortical probes were positioned through the previously implanted guide to the desired depth. The animal's vital signs were monitored throughout the surgery and temperature maintained with heating blankets. A collection session would normally last 8–10 h. At the end of the procedure the bone defect was filled with a fitted Teflon piece sewn into place (Saunders and O'Boyle 1993). The teflon flap made it easier and less traumatic to the tissues when re-entering for the next collection session. The skin was then closed in anatomical layers. The monkey was fully awake before being placed back in his home cage.

In vivo microdialysis

In most experimental sessions we had three probes aimed for the prefrontal cortex of one hemisphere and two probes aimed for the ipsilateral caudate nucleus. Continuous perfusion with freshly prepared aCSF at a rate of 1 μl per min using a microinfusion pump (Harvard Apparatus, Model 2400–006) was started as soon as the probes were in position with collections beginning approximately 25 min later. Consecutive samples (25 μl) were collected every 25 min in amber-colored glass vials, capped, and frozen on dry ice. They were assayed for dopamine, in most cases the same day. When this was not possible they were kept at −70° C until analyzed within 48–72 h. After 3–4 h of baseline collections, different drugs were administered together with the aCSF through the dialysis probe (i.e., *d*-amphetamine (50 μM), cocaine (50 μM), tetrodotoxin (TTX, 10 μM), K^+ (60 mM)) for one 25 min collection period and then the system was switched back to perfusion with the standard aCSF for continued collections. In some cases, after a further 3–4 h, the perfusate was again switched to pharmacologically altered aCSF for another collection period. At the end of the experimental session, the probes were carefully removed and retested in vitro before being used again. The minimum time between dialysis sessions for an individual monkey was 7 days.

Analysis of dialysate

The dialysate was injected using an automated, refrigerated injection unit onto a microbore C-18 column (1 × 100 mm, BAS Sepstiks) coupled with electrochemical detection (BAS LC-4B). A pulse free solvent delivery system was used to pump 40–70 μl/min through the microbore column. To minimize band spreading, a thin (10 μm spacer) plastic wrap was used in the amperometric

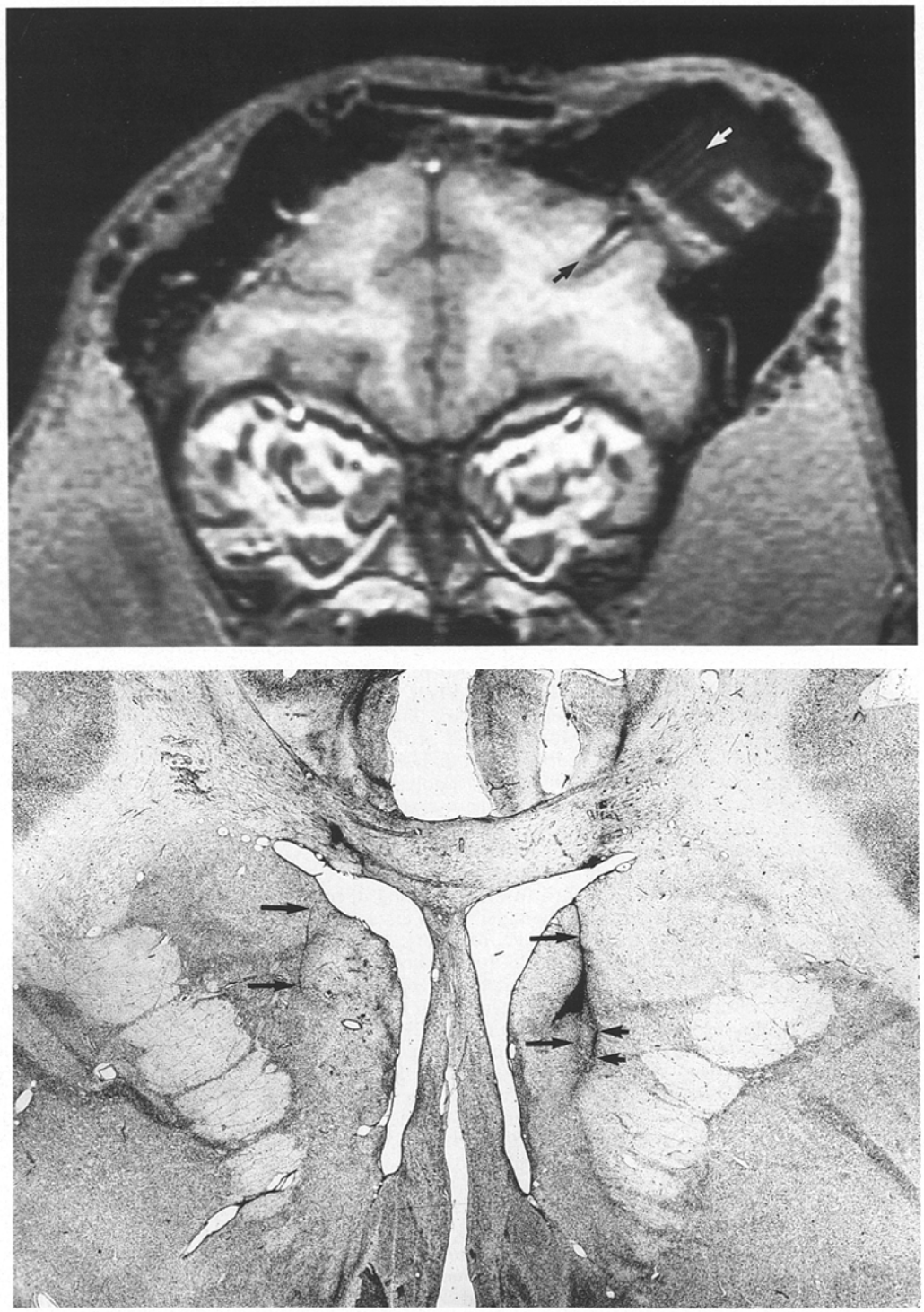

Fig. 1 **A** Magnetic resonance image of coronal section through frontal cortex of the monkey brain. *Black arrow* indicates tract left by dialysis probe insertions. Probe guide with guide holes (*white lines*) can be seen over the right hemisphere aligned with the principalis sulcus. **B** Photomicrograph of cross section at the level of the caudate nucleus. *Arrows* point to a number of tracts left from dialysis probe insertions into the caudate nucleus

electrochemical cell. The pH of the solvent was adjusted to 3.12 and the applied potential at the working electrode was +0.84 V. External standards of dopamine were injected periodically for identification of peaks. The detection limits of our analysis systems ranged from 2 to 15 fmol dopamine per 23 µl injection.

Data analysis

The data is presented for comparisons as a percent of the mean of three consecutive basal values collected immediately prior to infusion of TTX, K^+, amphetamine, or cocaine in the aCSF. Dopamine levels presented here were not corrected for in vitro probe recovery rates because in vivo recovery may vary significantly from in vitro recoveries and therefore may not be a better representation (Benviniste 1989). Nevertheless, it is virtually certain that the actual in vivo concentrations are higher than those recovered. Large variance exists in the dopamine levels from different probes within an individual animal, between experimental sessions in the same animal, and between different animals. Therefore, the data we present in graphic form are from single experiments. It is important to note in every instance the results were replicated within an individual animal as well as between different

monkeys. Every experiment, however, was not repeated in every probe in every animal; therefore, the total number of probes used for analysis varies from one experimental condition to another.

Results

Basal levels of dopamine in the caudate in all four monkeys were well within our limits of detection with HPLC–ED. During the early collections, particularly in the caudate, dopamine levels tended to be high probably as a result of tissue penetration by the dialysis probe. These levels quickly decreased and appeared in most cases to be stable (< 10% variation from adjacent samples) after 2 h. Baseline levels of dopamine were obtained from the caudate in a total of 27 of 31 experimental sessions, with success in 46 of 59 probe insertions. Large differences were found in basal dopamine levels both within animals (between probes and sessions) as well as between animals. These levels ranged from 65 to 135 fmol and from 65 to 1250 fmol per 25 µl, respectively. In two cases (i.e., two monkeys each with one probe) we examined basal levels for over 4 h. The dopamine levels after 4 h (134 and 65 fmol) was nearly the same as that after 1 h (160 and 81 fmol, respectively, see Fig. 2A). In order to increase our chances of obtaining dialysate in multiple sessions, we tried not to aim for the same target site within the caudate nucleus more than twice. We were able to recover dopamine after six sessions with as many as 15 probe penetrations into one caudate nucleus (Fig. 2B). Moreover, in one animal dopamine was recovered five times from probes placed into the same caudate site in different sessions over a 2 month period. In contrast, reliable detection of dopamine in the dorsolateral prefrontal cortex was successful in only 15 out of 50 probe placements. The greatest success occurred with the first probe insertion with little or none in subsequent attempts at the same site. As seen with the caudate, basal levels in the cortex also showed a large range within and between monkeys, 10.5–59 fmol per 25 µl and 6.6 to 83 fmol per 25 µl, respectively (Fig. 3).

Basal levels were examined for six to eight collections (3–4 h) prior to pharmacological manipulation. To explore the relationship of dopamine concentration in the dialysate to impulse dependent neuronal release, TTX (1 µM and 10 µM) was perfused through the dialysis probes in the caudate nucleus in each of the four animals. At 1 µM TTX, no observable reduction of dopamine occurred ($n=2$ probes). However, dopamine levels were greatly reduced in each case ($n=6$ probes) with a 10 µM TTX infusion with at least a 60% reduction ($n=2$), but more often nearly an 80% reduction ($n=4$). In one case the level decreased below detection (see Fig. 4). Basal levels were not reattained for more than five collections following termination of TTX infusion. Similar results were obtained with TTX infusion into the nucleus accumbens ($n=1$).

As a further test for neuronal release of dopamine, K^+ (60 mM)-enriched aCSF was infused in a probe placed in the caudate nucleus in at least one session of each monkey ($n=6$ probes). This resulted in large increases in dopamine levels (1000%–1500%). Dopamine quickly returned to near basal levels with standard aCSF within one to two collection periods (see Fig. 5).

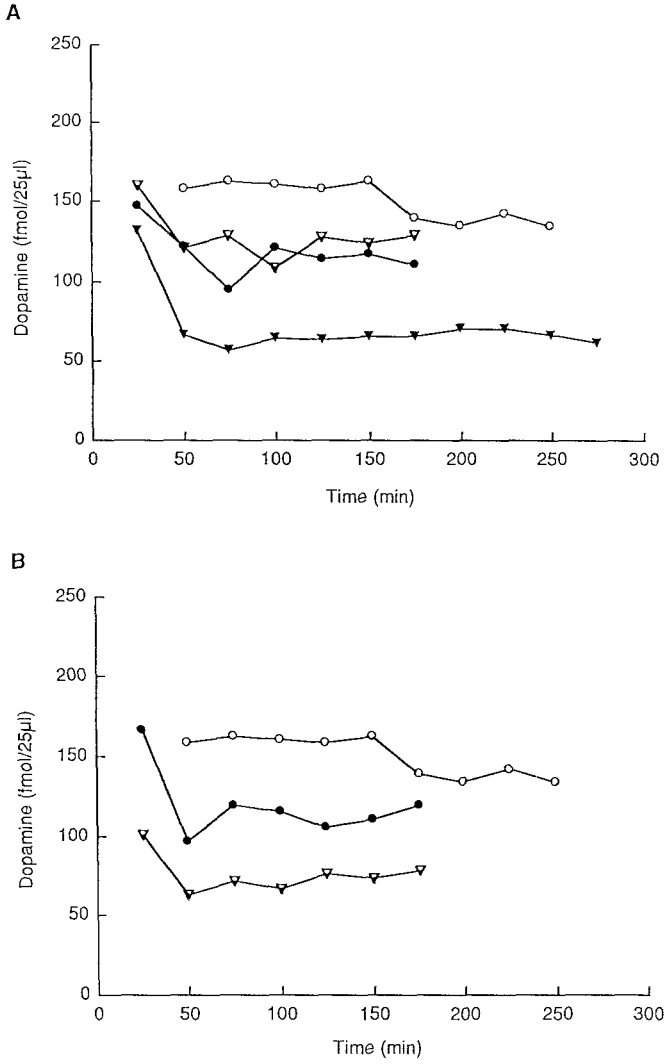

Fig. 2 **A** Extracellular baseline levels of dopamine in the caudate nucleus shown from four different monkeys collected over a 3–4 h period. Stable baseline in each case was achieved in less than 2 h. **B** Three successive experimental sessions in which probes were placed in the same caudate but not the same site. A reduction in baseline dopamine levels was seen with each session

After *d*-amphetamine (50 µM) was infused in the caudate for one 25 min collection period, marked increases in dopamine levels occurred in 29 of 31 probes in 11 different sessions. This increase ranged from 3000 to 5600% over basal dopamine levels. The levels returned to baseline within two to three collection periods after the amphetamine infusion was stopped (Fig. 6). Local *d*-amphetamine infusion into the prefrontal cortex resulted in substantial increases in dopamine levels (250% to 550%) in all four monkeys. A total of 19 cortical probes in ten different dialysis sessions were infused with amphetamine and clear changes in dopamine levels

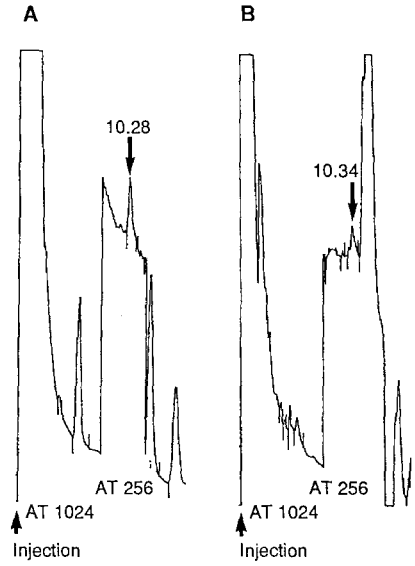

Fig. 3 Representative chromatograms showing **A** standard dopamine of 150 fmol, **B** dialysate sample with 70 fmol collected from the medial bank of the principalis sulcus. *Long arrows* indicate dopamine peaks. *Short arrows* indicate dialysate sample injection time into the HPLC system

occurred in nine sessions. This could be observed in some cases (two out of 11 probes), even when basal levels of dopamine were below detection (Fig. 7). If dopamine was detected during basal collections, we invariably saw an increase in dopamine concentration in the dialysate after the amphetamine infusion. Following termination of the amphetamine infusion, dopamine levels returned to near baseline, sometimes below detection, usually within two to three collection periods.

Cocaine (50 μM), infused through either the caudate or the cortical probes, resulted in changes similar to those seen after amphetamine infusion, in three monkeys and nine probes in six dialysis sessions. Large increases were seen in dopamine in the caudate nucleus (six of six probes) with somewhat smaller increases in the cortex (four probes). Both increases were smaller than that seen after the amphetamine infusion. Dopamine levels in the caudate ranged from 1700% to 2000% above basal levels. After the cocaine infusion was stopped dopamine levels decreased substantially but remained above baseline for four to five collections (Fig. 8). In the cortex the increase ranged from 170% to 280% of basal levels. As with the cocaine infusion in the caudate, but in contrast to that seen after amphetamine in cortex, the elevated cortical dopamine levels following cocaine infusion decreased but remained slightly elevated for at least three collections. In some cases this was above previously undetectable levels.

Discussion

The present study demonstrates the feasibility of using microdialysis techniques for measuring extracellular

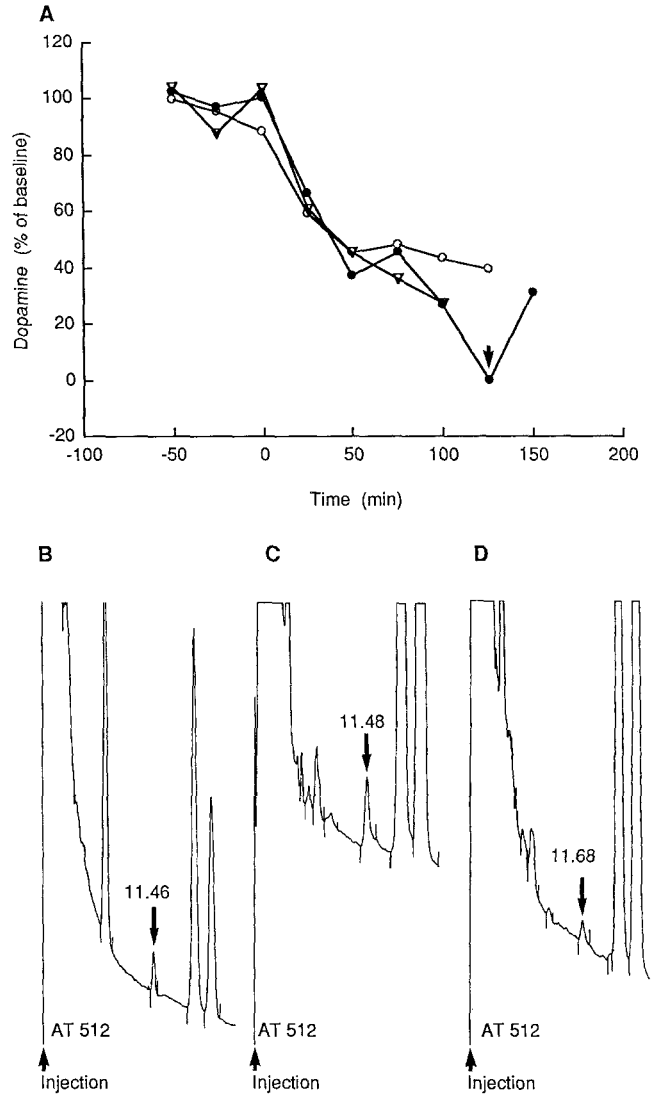

Fig. 4 **A** Local infusion of tetrodotoxin (TTX; 10 μM) for 25 min resulted in a marked reduction of dopamine (60% to 80%) in the caudate nucleus in each of three animals. In one animal (*filled circles*) TTX infusion resulted in dopamine levels dropping below detection limits after 2 h (see *arrow*). Time zero refers to infusion time following 2 h stable baseline. Representative chromatograms showing **B** dopamine standard of 150 fmol used for calibration, **C** caudate dopamine baseline of 246 fmol, and **D** caudate dopamine after TTX infusion, 74 fmol. Assays of dialysate samples depicted in **C** and **D** are from a single probe within an experimental session. *Long arrows* indicate dopamine peaks. *Short arrows* indicate dialysate sample injection time into the HPLC system

levels of dopamine in the caudate nucleus and to a lesser extent in the prefrontal cortex in the anesthetized rhesus monkey. In vivo microdialysis has proven to be a useful technique for sampling and assessing neurochemicals present in extracellular fluid, making it possible to follow dynamic extracellular chemical events in minimally disturbed neural tissue. Because transfer of neural information depends on anatomical interconnections and chemical transmission, monitoring the regional variations in these chemical events is an important tool for understanding information processing in the brain.

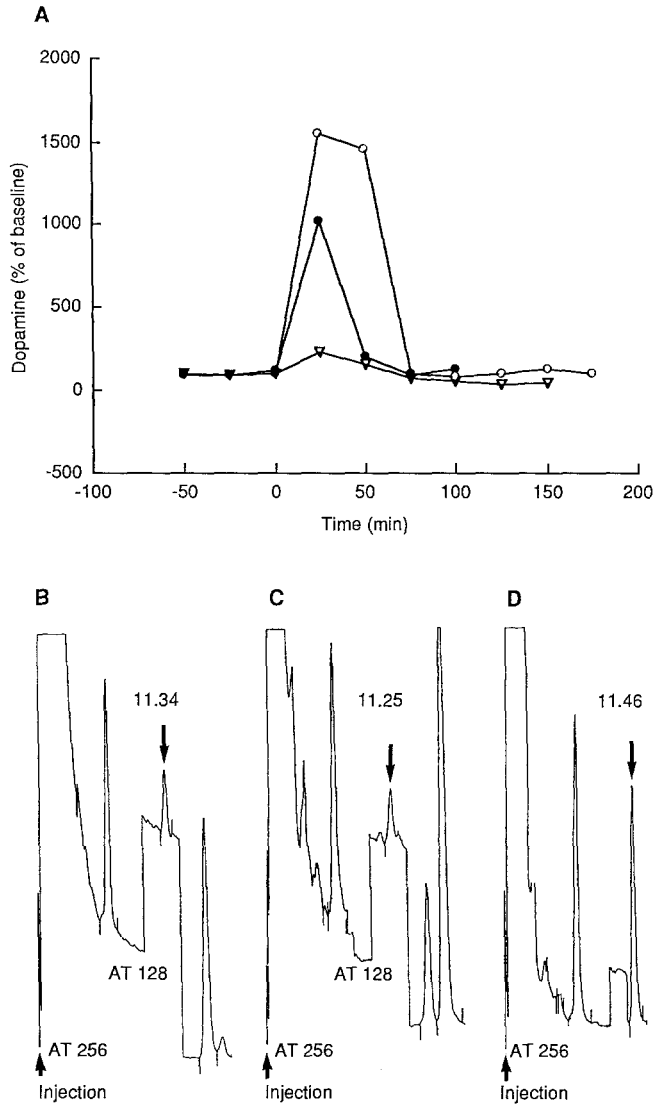

Fig. 5 **A** K^+ (KCl, 60 mM) infusion into the caudate nucleus resulted in a dramatic increase (1000% to 1500%) in dopamine levels which returned to baseline in less than 2 h. Time zero refers to infusion time following 2 h stable baseline. Representative chromatograms showing **B** dopamine standard of 150 fmol used for calibration, **C** caudate dopamine baseline of 120 fmol, and **D** caudate dopamine after K^+ infusion, 1150 fmol. Assays of dialysate samples depicted in **C** and **D** are from a single probe within an experimental session. *Long arrows* indicate dopamine peaks. *Short arrows* indicate dialysate sample injection time into the HPLC system

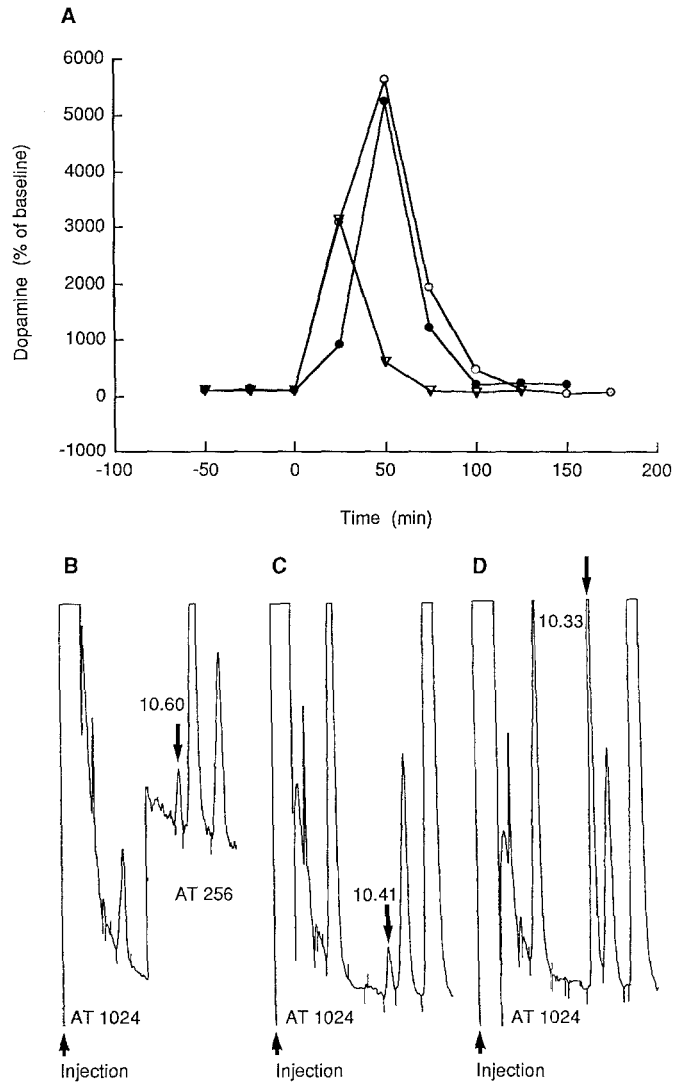

Fig. 6 **A** *d*-Amphetamine (50 μM) infusion into the caudate nucleus for 25 min resulted in marked increase (3000%–5000%) in dopamine. Levels returned to baseline in less than 2 h. Time zero refers to infusion time following 2 h stable baseline. Representative chromatograms showing **B** dopamine standard of 150 fmol, **C** caudate dopamine baseline of 486 fmol, and **D** caudate dopamine after *d*-amphetamine infusion, 4536 fmol. Assays of dialysate samples depicted in **C** and **D** are from a single probe within an experimental session. *Long arrows* indicate dopamine peaks. *Short arrows* indicate dialysate sample injection time into the HPLC system

Baseline extracellular levels of dopamine were relatively easy to measure in the caudate nucleus. We were successful in detecting dopamine in the caudate nucleus in almost every experimental session. Indeed, by the end of this experimental series, perhaps because of inevitable improvements in techniques that accompany experience, we were able to measure dopamine in the caudate with every attempt. In contrast, detection and measurement of baseline cortical dopamine levels in the principalis sulcus was much less reliable. We attempted to measure baseline dopamine in the medial bank of the principalis sulcus in the dorsal-lateral prefrontal cortex and in most cases the levels of dopamine were below the detection limits of our HPLC. When success was achieved, it most often occurred on the first or second attempt. The present results and those of previous efforts (Moghaddam et al. 1993) indicate that consistent detection of dopamine in the dorsal lateral prefrontal cortex may be too unreliable for planned experimentation. This may not be true, however, for other regions of

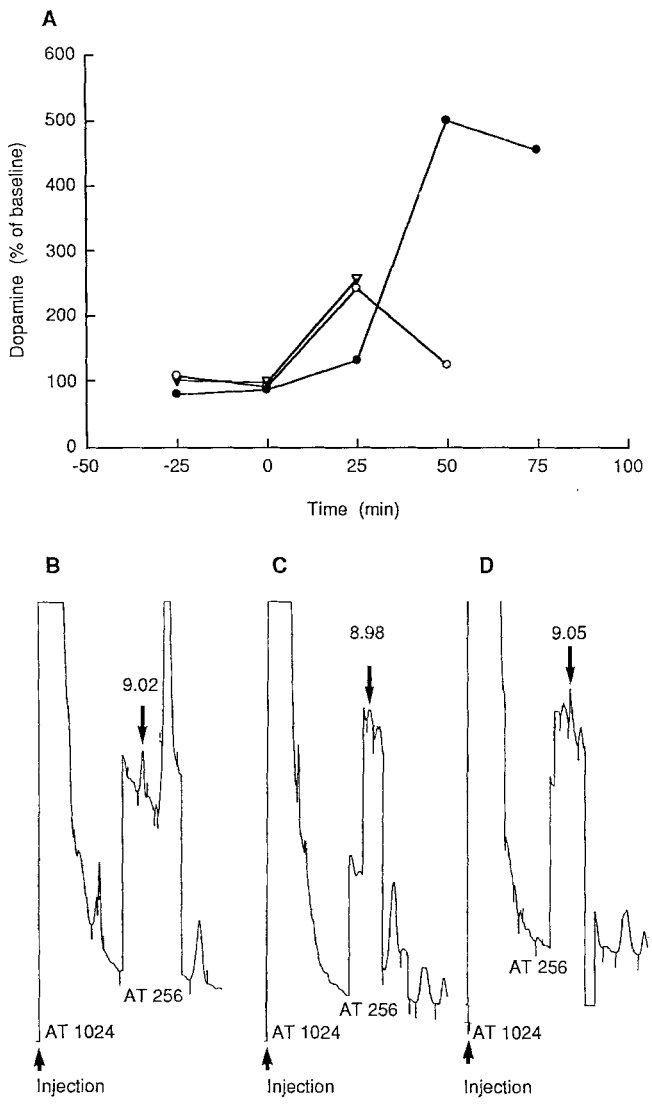

Fig. 7 **A** Infusion of *d*-amphetamine (50 μM) into the prefrontal cortex for 25 min resulted in 200%–500% increases in cortical dopamine levels. Time zero refers to infusion time following 2 h stable baseline. Representative chromatograms showing **B** dopamine standard of 150 fmol, **C** cortical dopamine baseline of 64 fmol, and **D** cortical dopamine after *d*-amphetamine infusion, 143 fmol. Assays of dialysate samples depicted in **C** and **D** are from a single probe within an experimental session. *Long arrows* indicate dopamine peaks. *Short arrows* indicate dialysate sample injection time into the HPLC system

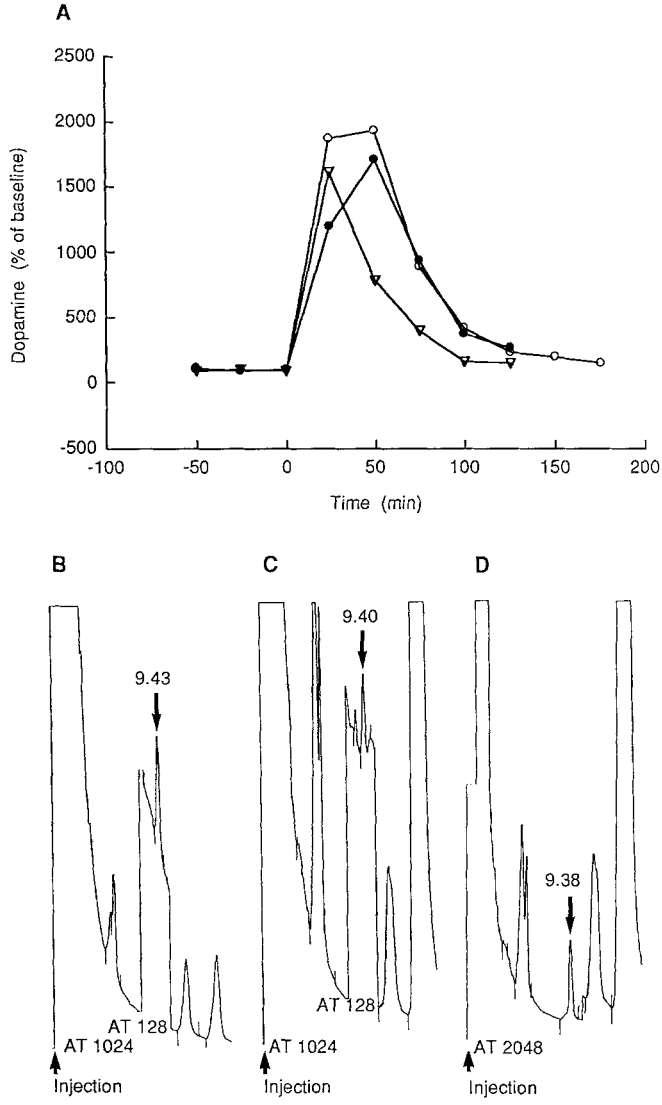

Fig. 8 **A** Infusion of cocaine hydrochloride (50 μM) into the caudate nucleus elevated dopamine levels by 1500%–2000%. Levels returned to baseline in 3 h after infusion stopped. Time zero refers to infusion time following 2 h stable baseline. Representative chromatograms showing **B** dopamine standard of 150 fmol, **C** caudate dopamine baseline of 101 fmol, and **D** caudate dopamine after cocaine infusion, 1858 fmol. Assays of dialysate samples depicted in **C** and **D** are from a single probe within an experimental session. *Long arrows* indicate dopamine peaks. *Short arrows* indicate dialysate sample injection time into the HPLC system

neocortex, for other neurotransmitters (Smith et al. 1992; Moghaddam et al. 1993), or for other experimental conditions (Kolachana et al. 1993).

While reliable measurement of dopamine in the caudate nucleus was demonstrated, the levels were variable both within an animal, (i.e., either from different probes within a session, or from similar site placements between sessions), as well as between monkeys. This variability may result from a number of methodological factors, including variations in probe efficiency, probe placement, recovery function, pathological changes caused by probe insertions, and potentially the anesthetic level of the animal. Similar variability in baseline levels has been noted in rat striatum (Westerink et al. 1987), where it had been suggested that the large fluctuation is due to biological variation rather than variable probe recovery. Results from multiple insertions of probes within a given targeted structure also suggested that levels of dopamine tended to decrease somewhat after the initial dialysis session. Nevertheless, it was also clear that multiple insertions were possible and reliable data could be collected. This was particularly true for the caudate nucleus in which we were able to collect and measure dopamine after five sessions with as many as 15

probe penetrations into the same caudate nucleus (though not all aimed for the identical site) (see Fig. 1).

It is important to note that despite the fluctuations in dopamine levels found between as well as within individual animals stable baseline levels were achievable and the effects of the pharmacological manipulation were consistent. In two previous reports assessing dopamine levels in the monkey, the baseline levels were similar to those reported here (Skirboll et al. 1990; Moghaddam et al. 1993).

To manipulate neuronal activity and determine whether the dopamine we were measuring was a result of neuronal firing, Tetrodotoxin (TTX) was administered locally via the dialysis probe. Since TTX blocks voltage-dependent Na^+ channels, its administration will depress electrical events and thus cause a drop in the neurotransmitter levels that are derived from stimulus dependent neuronal activity (Narahashi 1974; Westerink et al. 1987). Dramatic decreases in dopamine (80%) were seen after administration of TTX in the caudate nucleus. These decreases in the monkey parallel that found in the rat, both in the quantity and in their time course (Westerink et al. 1987; Westerink and DeVries 1988). Manipulation of K^+ levels was also carried out to further address whether dopamine concentrations measured were a result of neuronal release. Increasing K^+ levels in the extracellular tissue increases dopamine levels because of increase depolarization effects (Westerink and Tuinte 1986; Zetterstrom et al. 1988). In contrast to that seen after the TTX infusion, increases in extracellular K^+ levels resulted in large increases in dopamine levels. These changes resulting from the TTX and K^+ infusion demonstrate that the dopamine levels represented stimulus dependent dopamine release from presynaptic transmitter pools.

Amphetamine and cocaine, indirect dopaminergic agonists, were administered through the dialysis probes to examine more specific effects of pharmacological manipulation of synaptic dopamine levels. In rats it has been demonstrated that both systemic and localized administration of amphetamine and cocaine result in large increases in dopamine in the caudate nucleus (Zetterstrom et al. 1983; Imperato and DiChiara 1984; Westerink and Tuinte 1986; Hurd and Ungerstedt 1989; Moghaddam and Bunney 1989). In the present study amphetamine infusion in the caudate resulted in more than 300 fold increase in dopamine. In cortex a dramatic increase – while substantially less (25 to 50 fold) – could nonetheless be seen, even in some cases where basal levels were below detection. A similar differential effect has also been demonstrated in rats (Moghaddam and Bunney 1989; Maisonneuve et al. 1990). Amphetamine appeared to have a greater effect than cocaine in both the caudate and cortex; however, this may be a relative potency effect and not a true drug difference (Moghaddam and Bunney 1989). After both drug administrations, the increase in dopamine was seen in the first collection after delivery with the highest levels obtained most often in the second. The levels then decreased over the next two to three collections (50–75 min). As also shown in the rat, levels tended to stay elevated longer after cocaine than after amphetamine (Moghaddam and Bunney 1989; Maisonneuve et al. 1990), perhaps in part because of the local anesthetic properties of cocaine. It should be noted that any differences between the cortex and the caudate nucleus in percent response due to drug might actually reflect differences in dopamine release from vesicular and non-vesicular pools.

There have been two previous reports of microdialysis assessment of dopamine in the caudate nucleus in the primate (Skirboll et al. 1990; Moghaddam et al. 1993). Skirboll et al. (1990) used microdialysis to measure dopamine in the caudate and putamen of monkeys that were hemiparkisonian after unilateral MPTP administration. Baseline dopamine levels in the caudate in the normal hemisphere were found to be higher than the levels in the MPTP-treated hemisphere. Moghaddam et al. (1993) examined baseline levels of dopamine in the premotor cortex and the caudate nucleus. Similar to the present results, levels of dopamine were higher in the caudate nucleus than in the premotor cortex, and in both regions dopamine increased following systemic amphetamine administration.

The use of monkeys for dialysis necessitates repeated experimental sessions in which the probes must be accurately placed in sites of interest. This presents at least two potential problems. First, is the ability to reliably position the probe into the targeted site. In the present study we used the combination of MRI and specially designed probe guides. This facilitated reliable probe placement and minimized the likelihood that a probe aimed at the caudate nucleus might inadvertently end up in the putamen, in which case different levels of dopamine might have resulted, as has been reported previously (Skirboll et al. 1990; Moghaddam et al. 1993). Second, is the problem of repeated dialysis from the same or immediately adjacent sites. While we do not have sufficient data to fully analyze this question, it appears that placing a probe into a particular site reduces the likelihood of being successful in neurotransmitter recovery in future sessions. Nevertheless, as mentioned previously, we have been successful in recovering detectable baseline dopamine levels with repeated placement into the same sites as many as six separate times. Similar results were reported by Skirboll et al. (1990).

The present results have demonstrated the ability to assess neurotransmitter levels in the prefrontal cortex and caudate nucleus in the non-human primate and to manipulate these levels via local pharmacological intervention. Using this technique it is possible to monitor a number of neurotransmitters in many different regions of the brain. We expect that this will prove to be a practical and sensitive tool in characterizing neurochemical interactions between different anatomical regions and neurochemical systems, especially in the study of primate cognition.

References

Benveniste H (1989) Brain microdialysis. J Neurochem 52:1667–1679

Delgado JMR, DeFreudis FV, Roth RH, Ryugo DK, Mitruka BK (1972) Dialytrode for long term intracerebral perfusion in awake monkeys. Arch Int Pharmacodyn 198:9–21

Delgado JMR, Lerma J, Martin del Rio R, Soilis JM (1984) Dialytrode technology and local profiles of amino acids in the awake cat brain. J Neurochem 42:1218–1228

Fuster JM (1980) The prefrontal cortex: anatomy, physiology, and neuropsychology of the frontal lobe. Raven Press, New York

Goldman-Rakic PS (1987) Circuitry of the primate prefrontal cortex and regulation of behavior by representational memory. In: Plum F (ed) Handbook of physiology, the nervous system, higher functions of the brain. American Physiological Society, Bethesda, MD, pp 373–417

Hurd YL, Ungerstedt U (1989) Cocaine: an in vivo microdialysis evaluation of its acute action on dopamine transmission in rat striatum. Synapse 3:48–54

Imperato A, DiChiara G (1984) Trans-striatal dialysis coupled to reverse phase high performance liquid chromatography with electrochemical detection: a new method for the study of the in vivo release of endogenous dopamine and its metabolites. J Neurosci 4:966–977

Kolachana BS, Saunders RC, Weinberger DR (1991) Effects of amphetamine and cocaine on extracellular dopamine levels in the dorsolateral prefrontal cortex and caudate nucleus in the rhesus monkey: a microdialysis study. Soc Neurosci Abstr 17:682

Kolachana BS, Saunders RC, Weinberger DR (1993) Corticostriatal regulation in the rhesus monkey: an in vivo neurochemical assessment of dopamine and glutamate interactions. Soc Neurosci Abstr 19:1175

Maisonneuve IM, Keller RW, Glick SD (1990) Similar effects of *d*-amphetamine and cocaine on extracellular dopamine in medial prefrontal cortex in the rat. Brain Res 535:221–226

Moghaddam B, Bunney BS (1989) Differential effect of cocaine on extracellular dopamine levels in the rat medial prefrontal cortex and nucleus accumbens: comparison to amphetamine. Synapse 4:156–161

Moghaddam B, Berridge CW, Goldman-Rakic PS, Bunney BS, Roth RH (1993) In vivo assessment of basal and drug-induced dopamine release in cortical and subcortical regions of the anesthetized primate. Synapse 13:215–222

Narahashi T (1974) Chemical tools in the study of excitable membranes. Physiol Rev 54:813–889

Robinson TE, Justice Jr JB (1991) Microdialysis in the neurosciences. Elsevier, New York

Saunders RC, O'Boyle V (1993) Repair of cranial defects with teflon: a method of cranioplasty in monkeys. J Neurosci Methods 47:163–167

Saunders RC, Aigner TA, Frank JA (1990) Magnetic resonance imaging of the rhesus monkey brain: use for stereotactic neurosurgery. Exp Brain Res 81:443–446

Sharp T, Zetterstrom T, Ungerstedt U (1986) A direct comparison of amphetamine-induced behaviours and regional brain dopamine release in the rat using intracerebral dialysis. Brain Res 401:322–330

Skirboll S, Wang J, Mefford I, Hsiao J, Bankiewicz KS (1990) In vivo changes of catecholamines in hemiparkinsonian monkeys measured by microdialysis. Exp Neurol 110:187–193

Smith TD, Saunders RC, Weinberger DR (1992) Manipulation of acetylcholine and dopamine levels in hippocampus and prefrontal cortex in the conscious primate as measured by microdialysis. American College of Neuropsychopharmacology Annual Meeting, p 111

Ungerstedt U (1984) Measurement of neurotransmitter release by intracranial dialysis. In: Marsden CA (ed) Measurement of neurotransmitter release in vivo, Wiley, New York, pp 81–107

Ungerstedt U, Pycock C (1974) Functional correlates of dopamine neurotransmission. Bull Schweiz Akad Med Wiss 1278:1–13

Wang J, Skirboll S, Aigner TA, Saunders RC, Hsiao J, Bankiewicz KS (1990) Methodology of microdialysis of neostriatum in hemiparkinsonian nonhuman primate. Exp Neurol 110:181–186

Weinberger D (1988) Schizophrenia and frontal lobe. Trends Neurosci 11:367–370

Westerink BHC, Tuinte HJ (1986) Chronic use of intracerebral dialysis for the in vivo measurement of 3,4-dihydroxyphenylethylamine and its metabolite 3,4-dihydroxyphenylacetic acid. J Neurochem 46:181–185

Westerink BHC, De Vries JB (1988) Characterization of in vivo dopamine release as determined by brain microdialysis after acute and subchronic implantations: methodological aspects. J Neurochem 51:683–687

Westerink BHC, Tuntler J, Damsma G, Rollema H, De Vries JB (1987) The use of tetrodotoxin for the characterization of drug-enhanced dopamine release in conscious rats studied by brain dialysis. Naunyn-Schmiedebergs Arch Pharmacol 336:502–507

Zetterstrom T, Sharp T, Marsden C, Ungerstedt U (1983) In vivo measurement of dopamine and its metabolites by intracerebral dialysis: Changes after d-amphetamine. J Neurochem 41:1769–1773

Zetterstrom T, Sharp T, Collins AK, Ungerstedt U (1988) In vivo measurement of extracellular dopamine and dopac, in rat striatum after various dopamine-releasing drugs: implications for the origin of extracellular dopac. Eur J Pharmacol 148:327–334