Published in final edited form as:

Neuroimage. 2007 October 1; 37(4): 1112–1121.

***In vivo* imaging of disturbed pre- and post-synaptic dopaminergic signaling *via* arachidonic acid in a rat model of Parkinson's disease**

Abesh Kumar Bhattacharjee, Lindsey M. Meister, Lisa Chang, Richard P. Bazinet, Laura White, and Stanley I. Rapoport

Brain Physiology and Metabolism Section, National Institute on Aging, National Institutes of Health, Bethesda, MD, USA

Abstract

Parkinson's disease involves loss of dopamine (DA)-producing neurons in the substantia nigra, associated with fewer pre-synaptic DA transporters (DATs) but more post-synaptic dopaminergic D₂ receptors in terminal areas of these neurons.

Hypothesis—Arachidonic acid (AA) signaling *via* post-synaptic D₂ receptors coupled to cytosolic phospholipase A₂ (cPLA₂) will be reduced in terminal areas ipsilateral to a chronic unilateral substantia nigra lesion in rats given D-amphetamine, which reverses the direction of the DAT, but will be increased in rats given quinpirole, a D₂-receptor agonist.

Methods—D-amphetamine (5.0 mg/kg i.p.), quinpirole (1.0 mg/kg i.v.), or saline was administered to unanesthetized rats having a chronic unilateral lesion of the substantia nigra. AA incorporation coefficients, *k*^{*} (radioactivity/integrated plasma radioactivity), markers of AA signaling, were measured using quantitative autoradiography in 62 bilateral brain regions following intravenous [¹⁻¹⁴C]AA.

Results—In rats given saline (baseline), *k*^{*} was elevated in 13 regions in the lesioned compared with intact hemisphere. Quinpirole increased *k*^{*} in frontal cortical and basal ganglia regions bilaterally, more so in the lesioned than intact hemisphere. D-amphetamine increased *k*^{*} bilaterally but less so in the lesioned hemisphere.

Conclusions—Increased baseline elevations of *k*^{*} and increased responsiveness to quinpirole in the lesioned hemisphere are consistent with their higher D₂-receptor and cPLA₂ activity levels, whereas reduced responsiveness to D-amphetamine is consistent with dropout of pre-synaptic elements containing the DAT. *In vivo* imaging of AA signaling using dopaminergic drugs can identify pre- and post-synaptic DA changes in animal models of Parkinson's disease.

Keywords

Arachidonic acid; PLA₂; D₂ receptors; D-amphetamine; Parkinson Disease; quinpirole; substantia nigra; lesion

Address of correspondence: Abesh Kumar Bhattacharjee, Brain Physiology and Metabolism Section, National Institute on Aging, National Institutes of Health, Bldg. 9, Room 1S126, Bethesda, MD 20892, USA. Tel: + 1 (301) 496 8994; Fax: + 1 (301) 402 0074; E-mail: abeshb@mail.nih.gov

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Introduction

Parkinson's Disease (PD) is a progressive neurodegenerative disorder that affects nigrostriatal dopamine (DA) projections, resulting in depletion of DA in the basal ganglia (Hornykiewicz, 1982). Degeneration of pre-synaptic nigrostriatal DA projections leads to decreased DA synthesis and storage within pre-synaptic striatal nerve terminals (Heiss & Hilker, 2004). PD has been modeled in rats by unilaterally injecting the selective monoaminergic toxin, 6-hydroxydopamine (6-OHDA), into the substantia nigra or medial forebrain bundle (Gerlach & Riederer, 1996; Ungerstedt, 1971). Behavioral deficits occur one week after 6-OHDA. After 4 weeks, the animal is considered a model of late-stage asymmetrical PD (Yuan et al., 2005).

Pre-synaptic DA neurons possess tyrosine hydroxylase, the rate-limiting enzyme for DA synthesis, and the high-affinity DA reuptake transporter (DAT). Reduced levels of DAT have been reported in PD patients and in 6-OHDA lesioned rats (Chalon et al., 1999; Ichise et al., 1999; Ribeiro et al., 2002; Zuch et al., 2000). In contrast, post-synaptic D₂-receptors are upregulated (Cadet & Zhu, 1992; Chalon et al., 1999; Ichise et al., 1999; Nikolaus et al., 2003), particularly in the posterior putamen (Antonini et al., 1995; Ichise et al., 1999). Post-synaptic D₁-receptors have been reported to be unchanged (Corvol et al., 2004; Shinotoh et al., 1993), increased (Corvol et al., 2004), or decreased (Turjanski et al., 1997) in the caudate-putamen of PD patients and in 6-OHDA lesioned rats.

Post-synaptic D₂-receptors can be coupled *via* a G-protein to Ca²⁺-dependent cytosolic phospholipase A₂ (cPLA₂) (Clark et al., 1991; Nilsson et al., 1998; Vial & Piomelli, 1995), which also is localized on post-synaptic membranes and dendrites and which, upon activation, selectively releases AA from membrane phospholipid (Garcia & Kim, 1997; Nilsson et al., 1998; Ong et al., 1999; Pardue et al., 2003; Vial & Piomelli, 1995). D₁-receptors are not normally coupled to AA release (Bhattacharjee et al., 2005), but can become coupled following a chronic lesion of the substantia nigra in rodent brain (Cai et al., 2002; Hayakawa et al., 2001).

We have developed a method to image AA signaling *via* PLA₂ in unanesthetized rodents, in terms of a regional brain AA incorporation coefficient k^* (brain radioactivity/integrated plasma radioactivity) that is measured with quantitative autoradiography following the intravenous injection of radiolabeled AA. k^* is unaffected by changes in cerebral blood flow that might be affected by drugs or stress, thus reflects only changes in brain AA metabolism. The method is presented in detail elsewhere (Bhattacharjee et al., 2005; Chang et al., 1997; DeGeorge et al., 1991; Hayakawa et al., 2001; Ohata et al., 1982; Rapoport, 2003; Robinson et al., 1992; Soncrant et al., 1988). With it, we showed in unlesioned unanesthetized rats that acute administration of quinpirole, a D₂-receptor agonist, increased k^* for AA in brain regions with high densities of D₂-receptors, and that the increases could be blocked by the preferential D₂-receptor antagonist, butaclamol (Bhattacharjee et al., 2005; Bristow et al., 1998; Hayakawa et al., 2001). We also reported that D-amphetamine, which increases synaptic DA by increasing presynaptic DA release and reducing DA reuptake by the DAT, increased k^* for AA in a dose-dependent manner in brain areas rich in D₂-receptors (Bhattacharjee et al., 2006). D-amphetamine's effects could be prevented entirely by pre-administration of raclopride, a selective D₂-receptor antagonist (Bhattacharjee et al., 2006; Kohler et al., 1985), indicating that they were mediated specifically by D₂-receptors.

We thought it of interest in this paper to use our fatty acid method to examine the effects of D-amphetamine compared with quinpirole on AA signaling in unilaterally 6-OHDA lesioned rats. Based on evidence that ipsilateral regions in these animals have decreased DAT but increased D₂-receptor densities (see above), we predicted that the AA signal would be increased following quinpirole but might be reduced following D-amphetamine, depending on the extent of pre-synaptic loss and DAT reduction. We therefore quantified k^* for AA on the intact and

lesioned sides of chronically left-sided 6-OHDA lesioned rats acutely administered saline 1 ml/kg i.p., quinpirole 1.0 mg/kg i.v., or D-amphetamine 5.0 mg/kg i.p. These doses were chosen on the basis of our prior studies in which drug-induced changes in k^* were measured following injection of [$1\text{-}^{14}\text{C}$]AA (Basselin et al., 2005; Bhattacharjee et al., 2005; Bhattacharjee et al., 2006; Hayakawa et al., 2001).

Materials and Methods

Chemicals and drugs

[$1\text{-}^{14}\text{C}$]AA in ethanol (50 mCi/mmol, 99% pure) was purchased from Moravsek Biochemicals (Brea, CA). (–)-Quinpirole (LY-171,555) was obtained from Research Biochemicals International (Natick, MA). D-amphetamine sulfate, HEPES, fatty acid-free bovine serum albumin, paraformaldehyde and propylene glycol were purchased from Sigma Chemicals (St. Louis, MO). Sodium pentobarbital was purchased from Richmond Veterinary Supply (Richmond, VA).

Animals

This study was approved by the National Institute of Child Health and Human Development Animal Care and Use Committee (Protocol 03-012). The experiments were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* (NIH Publication 86-23). We obtained male 6-OHDA lesioned Fischer-CDF rats from Charles River Laboratories (Wilmington, MA). Briefly, 8.5 ± 0.5 -week-old rats were anesthetized with 43 mg/kg ketamine and 8.77 mg/kg xylazine, and the left substantia nigra was lesioned by a 3-min infusion of 12 $\mu\text{g}/4 \mu\text{l}$ 6-OHDA in 0.9% saline (w/v) at the following coordinates: AP = -1.5 , ML = $+1.8$, and DV = -7.5 mm from bregma (Hayakawa et al., 2001). Two weeks after lesioning, the rats were shipped to our controlled animal facility having constant temperature, humidity, and lighting cycle (6:00 AM – 6:00 PM), and had *ad libitum* access to food pellets and water. To assess the efficacy of the lesion, rats were tested 9 ± 1 days and 25 ± 3 days later for their response to S-(+)-apomorphine HCl, 0.5 mg/kg i.p. (Hayakawa et al., 2001; Ungerstedt, 1971). Only rats that completed at least 100 contralateral rotations in 20 min during both testing sessions were studied.

Arterial and venous catheter placement

Prior to surgery, the 14 ± 1 week-old rats (5.5 ± 1 week after 6-OHDA lesion) weighed 270 ± 25 g. Polyethylene catheters (PE 50) (Becton Dickinson, Sparks, MD) filled with heparin (50 IU) in 0.9% saline were surgically implanted into the right femoral artery and vein under halothane (1–2.5% v/v in O_2) anesthesia. The incision was infiltrated with 1% lidocaine hydrochloride and closed with clips. The rat was loosely bound in a fast-setting cast with its upper body free, and the cast was attached to a wooden block. The rat was allowed to recover from anesthesia in a sound-dampened, temperature-controlled box for 3–4 hours, to remove any effect of anesthesia on brain metabolism (Kimes et al., 1985; Sokoloff et al., 1977). Arterial blood pressure, heart rate, and rectal temperature were measured prior to and 11 min after quinpirole, and 55 min after saline or D-amphetamine.

Radiolabeled arachidonic acid infusion

An unanesthetized rat was administered 1.0 ml/kg saline i.p. ($n = 11$), 5.0 mg/kg D-amphetamine in saline i.p. ($n = 7$), or 1.0 ml/kg quinpirole in saline i.v. ($n = 7$). Forty five min after saline or D-amphetamine or 1 min after quinpirole, the rat was infused intravenously with 170 $\mu\text{Ci}/\text{kg}$ [$1\text{-}^{14}\text{C}$]AA in 2 ml of 5 mM HEPES buffer, pH 7.4, containing 50 mg/ml fatty acid free bovine serum albumin. Infusion was performed for 5 min and at a rate of 400 $\mu\text{l}/\text{min}$, using a pump (Model 22, Harvard Apparatus, Natick, MA), and timed arterial aliquots (70–100

μl) were collected and centrifuged during and for after 15 min following infusion. At 20 min following the start of infusion, the rat was killed with i.v. sodium pentobarbital (60 mg/kg) and its brain was removed, quickly frozen in 2-methyl butane at -40°C , and then stored at -80°C until sectioning.

Radiolabeled unesterified AA in plasma

Plasma was extracted from the arterial samples with 3 ml chloroform/methanol (2:1 v/v) and 1.5 ml 0.1 M KCl (Folch et al., 1957). Using a Liquid Scintillation Counter (Model 2200CA, Packard Instruments, Downers Grove, IL), 100 μl of the lower organic phase was counted to determine plasma radioactivity.

Autoradiography

Brains were sectioned in the coronal plane at -20°C using a cryostat (Hacker Instruments, Fairfield, NJ). At 100 μm intervals, sets of 3 adjacent 20 μm slices were placed on 22 x 44 mm coverslips and dried for at least 5 min on a 55°C hot plate. The coverslips and calibrated [^{14}C] methylmethacrylate standards (Amersham, Arlington Heights, IL) were exposed for 6 weeks to autoradiographic film (EMC1, Eastman Kodak Company, Rochester, NY), and the film was developed following the manufacturer's instructions. Radioactivity in each of 62 identified brain regions (Paxinos & Watson, 1987) was measured in six times bilaterally with quantitative densitometry using the public domain NIH Image program 1.62 (developed at the U.S. National Institutes of Health and available on the Internet at <http://rsb.info.nih.gov/ni-image/>). Regional brain incorporation coefficients, k^* (ml/s/g brain), were calculated using the following equation (Bhattacharjee et al., 2005; DeGeorge et al., 1991),

$$k^* = \frac{c_{brain}^*(20\text{min})}{\int_0^{20} c_{plasma}^* dt} \quad (1)$$

k^* is in units of ml/s/g wet weight (specific gravity of brain approximates 1); $c_{brain}^*(20\text{ min})$ nCi/g equals brain radioactivity at 20 min after onset of [$1\text{-}^{14}\text{C}$]AA infusion as determined by densitometry; c_{plasma}^* nCi/ml equals plasma AA radioactivity determined by scintillation counting; t min is time after onset of infusion.

Statistical Analysis

Data are presented as means $\pm$ S.D. The lesioned (left) versus intact (right) sides of the brain were compared using a two-tailed paired t test. The lesioned and intact brain sides of saline treated rats (control) were compared respectively with D-amphetamine (5.0 mg/kg) and quinpirole (1.0 ml/kg) treated rats using a one-way ANOVA with Bonferroni's *post-hoc* test. Statistical significance was taken as $p \leq 0.05$.

Results

Physiological Parameters

As shown in Table 1, there was no statistically significant effect of quinpirole compared with saline on body temperature. D-amphetamine significantly increased body temperature compared to its basal pre-injection level. This effect has been ascribed to hyperactivity and decreased heat loss associated with cutaneous vasoconstriction (Lin et al., 1980) and to a central effect of DA (Ulus et al., 1975).

Quinpirole increased arterial blood pressure compared to its basal level. The increase has been ascribed to elevated sympathetic activity and vasopressin release (Chen et al., 1988). D-

amphetamine also increased blood pressure and heart rate compared to respective basal values, consistent with prior reports (Florence et al., 2000). As reported previously (Bhattacharjee et al., 2006), D-amphetamine produced hyperactivity and stereotyped sniffing, chewing, licking, biting, and gnawing behaviors, which we did not attempt to quantify.

Quantitative autoradiography of k^* images under three experimental conditions

Figure 1 presents representative color-coded images of k^* for AA in coronal autoradiographs from rats administered saline, quinpirole or D-amphetamine. In the saline injected rat, higher values of k^* are evident qualitatively in regions on the lesioned compared to the intact side. In the rat given quinpirole, values of k^* are bilaterally higher compared with the respective values in the saline-injected rat, but more so on the lesioned than intact side. In the brain of the rat given D-amphetamine, k^* also is elevated bilaterally compared with respective values in the saline-injected rat, but less so on the lesioned than intact side. Asymmetries in each of the three conditions are most evident in frontal cortex, globus pallidus, and caudate-putamen.

Mean regional values of k^* from such studies are summarized and compared statistically in Table 2, whereas Figure 2 presents representative changes in representative affected region, Frontal Cortex layer IV. This figure shows that k^* for AA was higher in this region in the lesioned compared with intact side in saline-injected and quinpirole-injected rats, but lower in the D-amphetamine injected rats. In both quinpirole- and D-amphetamine injected rats, k^* was higher bilaterally in Frontal Cortex layer IV.

k^* in saline injected rats

Rats injected with saline had significantly *higher* values of k^* on the lesioned side compared to intact side in 13 of 62 brain regions (Table 2). Asymmetries were evident in frontal cortex I and IV, anterior cingulate cortex, olfactory tubercle, Islands of Calleja, entopeduncular nucleus, medial caudate-putamen, ventrolateral and ventromedial thalamus, substantia nigrapars compacta, and superior gray layer of superior colliculus, and cerebellar gray matter and white matter.

k^* in quinpirole injected rats

Quinpirole 1.0 mg/kg i.v. significantly increased k^* for AA in 58 brain regions on the intact (right) side compared to the right side in animals given saline, and in 59 brain regions on the lesioned (left) side compared to the left side in animals given saline. The increases were significant *higher* in 12 ipsilateral (left) compared with contralateral regions, consistent with increased ipsilateral responsiveness. Significant asymmetries occurred in prefrontal IV, frontal I and IV, motor IV and VI, somatosensory IV and V, ventral pallidum, entopeduncular nucleus, ventral and lateral caudate-putamen and substantia nigra pars reticulata.

k^* in D-amphetamine injected rats

D-amphetamine 5.0 mg/kg i.p. increased k^* in 29 brain regions on the intact (right) side compared to the corresponding right side in saline injected animals. It also increased k^* in 13 regions on the lesioned (left) side compared with the corresponding left side in saline-injected rats. In contrast to the effects following quinpirole, k^* was less on the lesioned compared with unlesioned side in 17 regions following D-amphetamine. These differences occurred in prefrontal cortex IV, primary olfactory cortex, frontal cortex I and IV, anterior cingulate, motor II–III, IV, V and VI, somatosensory IV and V, auditory IV and VI, olfactory tubercle, dorsal caudate-putamen, and hippocampus CA2 and CA3.

Discussion

By studying [$1\text{-}^{14}\text{C}$]AA incorporation into brain at rest and in response to quinpirole and D-amphetamine, we have dissected out signaling defects arising from changes in pre- and post-synaptic elements of DA synapses in a rat model of unilateral PD. Rats subjected to a unilateral lesion of the substantia nigra at 8.5 weeks of age, and studied while unanesthetized at 14 weeks of age, demonstrated significantly increased baseline (in response to saline) values of k^* , a marker of PLA_2 -mediated signaling *via* AA, in 13 brain regions on the lesioned compared with intact hemisphere. Quinpirole 1 mg/kg i.v., a D_2 -receptor agonist whose k^* responses can be blocked by pre-administration of the preferential D_2 -receptor antagonist, butaclamol (Bristow et al., 1998; Hayakawa et al., 2001), produced widespread bilateral statistically significant elevations in k^* for AA compared with saline. A paired t -test analysis showed that ipsilateral k^* was significantly *greater* than contralateral k^* in 12 regions following quinpirole. D-amphetamine (5.0 mg/kg i.p.) increased k^* on the intact and lesioned sides compared with saline values in 29 and 13 regions, respectively. In contrast to quinpirole, k^* in 17 regions on the lesioned side following D-amphetamine was significantly less than on the intact side. In each of the three paradigms, regions that were affected are known to have high densities of markers of DA synapses.

The higher baseline ipsilateral values of k^* (following saline) confirm our prior report (Hayakawa et al., 2001). They likely represent upregulated or disinhibited baseline AA signaling in ipsilateral extrapyramidal and related circuitry, due to increased sensitivity to dopamine in relation to increased levels of D_2 -receptors and of activity of cPLA_2 , to which D_2 -receptors are coupled (HJ Lee et al., Unpublished results) (Cadet & Zhu, 1992; Chalon et al., 1999; Ichise et al., 1999; Nikolaus et al., 2003). The increased baseline values of k^* also could have reflected increased AA signaling *via* other cPLA_2 -coupled neuroreceptors, including NMDA and cholinergic receptors (Marti et al., 1999; Robinson et al., 2001).

D-amphetamine increased k^* in 29 regions on the intact (right) side compared to saline control, and in 13 regions on the lesioned (left) side. A lesser effect on the lesioned side was supported by paired t -test comparisons that lesser values of k^* in 17 regions on the lesioned compared with intact side. In addition to increasing k^* in brain regions normally rich in D_2 -receptors, 5.0 mg/kg D-amphetamine increased k^* on the intact side in the habenular nucleus, ventroposterior thalamic nucleus, parafascicular nucleus, geniculate and pretectal nuclei, and substantia nigra, which normally contain few D_2 -receptors or low levels D_2 -receptor mRNA (Levant et al., 1992; Levey et al., 1993; Meador-Woodruff, 1994). A lower dose of D-amphetamine, 2.5 mg/kg i.p., does not significantly increase k^* in any of these regions in unlesioned rats (Bhattacharjee et al., 2006).

D-amphetamine increases synaptic DA by reversing the direction of the DAT and stimulating DA release from pre-synaptic dopaminergic nerve terminals (Creese & Iversen, 1975; Jones et al., 1998). It also can increase extracellular serotonin and epinephrine, which will stimulate serotonergic $5\text{-HT}_{2A/2C}$ and β_2 -adrenergic receptors, respectively (Jones et al., 1998; Kuczenski & Segal, 1989; Segal et al., 1980). These receptors, like D_2 -receptors, can be coupled to cPLA_2 to release AA from membrane phospholipid (Berg et al., 1998; Garcia & Kim, 1997; Kurrasch-Orbaugh et al., 2003; Qu et al., 2003). In unlesioned rats, the AA signal initiated by 2.5 mg/kg i.p. D-amphetamine is entirely D_2 -receptor mediated, as it can be blocked by pre-administration of the selective D_2 -receptor antagonist, raclopride (Bhattacharjee et al., 2006).

Severe pre-synaptic losses likely accounted for reduced responsiveness to D-amphetamine in regions of the lesioned compared with intact hemisphere. D-amphetamine requires intact DA terminals to exert its effects on the pre-synaptic DAT and to increase synaptic DA. Marked

loss of these terminals with a concomitant reduction of intrasynaptic DA has been reported in 6-OHDA lesioned rats as well as in PD patients (Chalon et al., 1999; Ichise et al., 1999; Ribeiro et al., 2002; Zuch et al., 2000). Immunoreactivities of tyrosine hydroxylase and DAT, markers of pre-synaptic DA terminals, also are lost. However, one might expect that less severe pre-synaptic dropout comparable to early stages of PD (Deumens et al., 2002), accompanied by upregulated post-synaptic D₂-receptor densities and cPLA₂ activity, might result in increased ipsilateral *k** responses to D-amphetamine.

Turning in response to a dopaminergic drug is thought to reflect asymmetry of DA receptor stimulation in the basal ganglia, with the animal turning away from the more sensitive side. Consistent with our higher ipsilateral increments in *k** following quinpirole, chronically lesioned rats show contralateral rotation (they turn away from the lesion) following quinpirole or apomorphine, a D₂/D₁-receptor agonist (Arnt & Hyttel, 1985; Ungerstedt, 1971; Wooten & Collins, 1983). On the other hand, consistent with the decreased ipsilateral increments in *k** following D-amphetamine, lesioned rats given this drug show rotation towards the side of the lesion (Wooten & Collins, 1983).

The regional cerebral metabolic rate for glucose, rCMR_{glc}, thought to represent energy consumed following depolarization of fine pre-synaptic axonal terminals (Sokoloff, 1999), is elevated in ipsilateral more than contralateral globus pallidus, lateral habenula, parafascicular and subthalamic nuclei and superior colliculus in 6-OHDA lesioned rats administered quinpirole (Morelli et al., 1993; Trugman & Wooten, 1987), as were our *k** responses (Table 2). D-amphetamine increased rCMR_{glc} less on the lesioned than intact side in one study (Wooten & Collins, 1983), as we found with our *k** responses, but results in other studies involving rCMR_{glc} are inconsistent (Palacios & Wiederhold, 1985; Trugman & Wooten, 1987).

This study shows that we can use our fatty acid imaging method to characterize three functional effects of a substantia nigra lesion, each related to signal transduction involving AA at the DA synapse. The first is an upregulated baseline AA signal in ipsilateral basal ganglia and frontal cortex, likely reflecting increased post-synaptic D₂ receptor densities and cPLA₂ activity in these regions. The second is increased responsiveness to quinpirole, attributable to the same causes. The third is reduced responsiveness to D-amphetamine, while likely reflects severe loss of pre-synaptic terminals containing DAT in this animal model. These three effects thus identify defects in both pre- and post-synaptic integrity of the DA synapse in this model.

Comparable changes in pre- and post-synaptic elements of the DA synapse including reduced Ca²⁺-dependent PLA₂ activity occur in PD (Heiss & Hilker, 2004; Hornykiewicz, 1982; Ichise et al., 1999; Ross et al., 2001). Thus, it would be of interest to use our AA imaging method for early diagnosis, evaluating the course of synaptic changes and their response to therapy, in patients with PD and other disorders involving DA (Solanto, 2002; Stone & Pilowsky, 2006). This could be accomplished with positron emission tomography (PET) following the intravenous injection of the positron-emitting tracer [1-¹¹C]AA (Chang et al., 1997; Giovacchini et al., 2002; Giovacchini et al., 2004; Esposito, 2007 #6108). If successful, image AA signaling could be added to other PET imaging techniques used to study PD (Antonini et al., 1995; Berding et al., 2001; Heiss & Hilker, 2004; Rinne et al., 1999).

Acknowledgements

This research was supported by the Intramural Research Program of the National Institute on Aging. We thank the NIH Fellows Editorial Board for editorial assistance.

Reference

- Antonini A, Vontobel P, Psylla M, Gunther I, Maguire PR, Missimer J, Leenders KL. Complementary positron emission tomographic studies of the striatal dopaminergic system in Parkinson's disease. *Arch Neurol* 1995;52(12):1183–1190. [PubMed: 7492293]
- Arnt J, Hyttel J. Differential involvement of dopamine D-1 and D-2 receptors in the circling behaviour induced by apomorphine, SK & F 38393, pergolide and LY 171555 in 6-hydroxydopamine-lesioned rats. *Psychopharmacology (Berl)* 1985;85(3):346–352. [PubMed: 2860689]
- Basselin M, Chang L, Bell JM, Rapoport SI. Chronic lithium chloride administration to unanesthetized rats attenuates brain dopamine D₂-like receptor-initiated signaling via arachidonic acid. *Neuropsychopharmacology* 2005;30(6):1064–1075. [PubMed: 15812572]
- Berding G, Odin P, Brooks DJ, Nikkhah G, Matthies C, Peschel T, Shing M, Kolbe H, van Den Hoff J, Fricke H, Dengler R, Samii M, Knapp WH. Resting regional cerebral glucose metabolism in advanced Parkinson's disease studied in the off and on conditions with [(18)F]FDG-PET. *Mov Disord* 2001;16(6):1014–1022. [PubMed: 11748732]
- Berg KA, Maayani S, Goldfarb J, Clarke WP. Pleiotropic behavior of 5-HT_{2A} and 5-HT_{2C} receptor agonists. *Ann N Y Acad Sci* 1998;861:104–110. [PubMed: 9928246]
- Bhattacharjee AK, Chang L, Lee HJ, Bazinet RP, Seemann R, Rapoport SI. D₂ but not D₁ dopamine receptor stimulation augments brain signaling involving arachidonic acid in unanesthetized rats. *Psychopharmacology (Berl)* 2005;180(4):735–742. [PubMed: 16163535]
- Bhattacharjee AK, Chang L, White L, Bazinet RP, Rapoport SI. D-Amphetamine stimulates D₂ dopamine receptor-mediated brain signaling involving arachidonic acid in unanesthetized rats. *J Cereb Blood Flow Metab* 2006;26(11):1378–1388. [PubMed: 16511499]
- Bristow LJ, Cook GP, Patel S, Curtis N, Mawer I, Kulagowski JJ. Discriminative stimulus properties of the putative dopamine D₃ receptor agonist, (+)-PD 128907: role of presynaptic dopamine D₂ autoreceptors. *Neuropharmacology* 1998;37:793–802. [PubMed: 9707293]
- Cadet JL, Zhu SM. The intrastriatal 6-hydroxydopamine model of hemiparkinsonism: quantitative receptor autoradiographic evidence of correlation between circling behavior and presynaptic as well as postsynaptic nigrostriatal markers in the rat. *Brain Res* 1992;595(2):316–326. [PubMed: 1467973]
- Cai G, Wang HY, Friedman E. Increased dopamine receptor signaling and dopamine receptor-G protein coupling in denervated striatum. *J Pharmacol Exp Ther* 2002;302(3):1105–1112. [PubMed: 12183669]
- Chalon S, Emond P, Bodard S, Vilar MP, Thiercelin C, Besnard JC, Guilloteau D. Time course of changes in striatal dopamine transporters and D₂ receptors with specific iodinated markers in a rat model of Parkinson's disease. *Synapse* 1999;31(2):134–139. [PubMed: 10024010]
- Chang MC, Arai T, Freed LM, Wakabayashi S, Channing MA, Dunn BB, Der MG, Bell JM, Sasaki T, Herscovitch P, Eckelman WC, Rapoport SI. Brain incorporation of [1–11C]arachidonate in normocapnic and hypercapnic monkeys, measured with positron emission tomography. *Brain Res* 1997;755(1):74–83. [PubMed: 9163542]
- Chen YF, Jin HK, Paul R, Nagahama S. Blunted pressor responsiveness to quinpirole, a specific dopamine D₂ receptor agonist, in conscious deoxycorticosterone acetate/NaCl hypertensive rats is related to atrial natriuretic peptide release. *J Pharmacol Exp Ther* 1988;246(2):485–492. [PubMed: 2969976]
- Clark JD, Lin LL, Kriz RW, Ramesha CS, Sultzman LA, Lin AY, Milona N, Knopf JL. A novel arachidonic acid-selective cytosolic PLA₂ contains a Ca⁽²⁺⁾-dependent translocation domain with homology to PKC and GAP. *Cell* 1991;65(6):1043–1051. [PubMed: 1904318]
- Corvol JC, Muriel MP, Valjent E, Feger J, Hanoun N, Girault JA, Hirsch EC, Herve D. Persistent increase in olfactory type G-protein alpha subunit levels may underlie D₁ receptor functional hypersensitivity in Parkinson disease. *J Neurosci* 2004;24(31):7007–7014. [PubMed: 15295036]
- Creese I, Iversen SD. The pharmacological and anatomical substrates of the amphetamine response in the rat. *Brain Res* 1975;83(3):419–436. [PubMed: 234270]
- DeGeorge JJ, Nariai T, Yamazaki S, Williams WM, Rapoport SI. Arecoline-stimulated brain incorporation of intravenously administered fatty acids in unanesthetized rats. *J Neurochem* 1991;56(1):352–355. [PubMed: 1824784]

- Deumens R, Blokland A, Prickaerts J. Modeling Parkinson's disease in rats: an evaluation of 6-OHDA lesions of the nigrostriatal pathway. *Exp Neurol* 2002;175(2):303–317. [PubMed: 12061862]
- Florence G, Bonnier R, Plagnes D, Pierard C, Satabin P, Peres M, Lagarde D. Effect of modafinil on cerebral blood flow of anaesthetised rats. Comparison with amphetamine. *Exp Brain Res* 2000;135(4):552–556. [PubMed: 11156319]
- Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* 1957;226(1):497–509. [PubMed: 13428781]
- Garcia MC, Kim HY. Mobilization of arachidonate and docosaehaenoate by stimulation of the 5-HT_{2A} receptor in rat C6 glioma cells. *Brain Res* 1997;768(1–2):43–48. [PubMed: 9369299]
- Gerlach M, Riederer P. Animal models of Parkinson's disease: an empirical comparison with the phenomenology of the disease in man. *J Neural Transm* 1996;103(8–9):987–1041. [PubMed: 9013391]
- Giovacchini G, Chang MC, Channing MA, Toczek M, Mason A, Bokde AL, Connolly C, Vuong BK, Ma Y, Der MG, Doudet DJ, Herscovitch P, Eckelman WC, Rapoport SI, Carson RE. Brain incorporation of [¹¹C]arachidonic acid in young healthy humans measured with positron emission tomography. *J Cereb Blood Flow Metab* 2002;22(12):1453–1462. [PubMed: 12468890]
- Giovacchini G, Lerner A, Toczek MT, Fraser C, Ma K, DeMar JC, Herscovitch P, Eckelman WC, Rapoport SI, Carson RE. Brain incorporation of ¹¹C-arachidonic acid, blood volume, and blood flow in healthy aging: a study with partial-volume correction. *J Nucl Med* 2004;45(9):1471–1479. [PubMed: 15347713]
- Hayakawa T, Chang MC, Rapoport SI, Appel NM. Selective dopamine receptor stimulation differentially affects [³H]arachidonic acid incorporation, a surrogate marker for phospholipase A₂-mediated neurotransmitter signal transduction, in a rodent model of Parkinson's disease. *J Pharmacol Exp Ther* 2001;296(3):1074–1084. [PubMed: 11181943]
- Heiss WD, Hilker R. The sensitivity of 18-fluorodopa positron emission tomography and magnetic resonance imaging in Parkinson's disease. *Eur J Neurol* 2004;11(1):5–12. [PubMed: 14692881]
- Hornykiewicz O. Imbalance of brain monoamines and clinical disorders. *Prog Brain Res* 1982;55:419–429. [PubMed: 7163495]
- Ichise M, Kim YJ, Ballinger JR, Vines D, Erami SS, Tanaka F, Lang AE. SPECT imaging of pre- and postsynaptic dopaminergic alterations in L-dopa-untreated PD. *Neurology* 1999;52(6):1206–1214. [PubMed: 10214745]
- Jones SR, Gainetdinov RR, Wightman RM, Caron MG. Mechanisms of amphetamine action revealed in mice lacking the dopamine transporter. *J Neurosci* 1998;18(6):1979–1986. [PubMed: 9482784]
- Kimes AS, Sweeney D, Rapoport SI. Brain palmitate incorporation in awake and anesthetized rats. *Brain Res* 1985;341(1):164–170. [PubMed: 4041785]
- Kohler C, Hall H, Ogren SO, Gawell L. Specific in vitro and in vivo binding of ³H-raclopride. A potent substituted benzamide drug with high affinity for dopamine D-2 receptors in the rat brain. *Biochem Pharmacol* 1985;34(13):2251–2259. [PubMed: 4015674]
- Kuczenski R, Segal D. Concomitant characterization of behavioral and striatal neurotransmitter response to amphetamine using in vivo microdialysis. *J Neurosci* 1989;9(6):2051–2065. [PubMed: 2566664]
- Kurrasch-Orbaugh DM, Parrish JC, Watts VJ, Nichols DE. A complex signaling cascade links the serotonin_{2A} receptor to phospholipase A₂ activation: the involvement of MAP kinases. *J Neurochem* 2003;86(4):980–991. [PubMed: 12887695]
- Levant B, Grigoriadis DE, DeSouza EB. Characterization of [³H]quinpirole binding to D₂-like dopamine receptors in rat brain. *J Pharmacol Exp Ther* 1992;262:929–935. [PubMed: 1356154]
- Levey AI, Hersch SM, Rye DB, Sunahara RK, Niznik HB, Kitt CA, Price DL, Maggio R, Brann MR, Ciliax BJ. Localization of D₁ and D₂ dopamine receptors in brain with subtype-specific antibodies. *Proc. Natl. Acad. Sci. U S A* 1993;90:8861–8865.
- Lin MT, Chandra A, Chern YF, Tsay BL. Effects of intracerebroventricular injection of d-amphetamine on metabolic, respiratory, and vasomotor activities and body temperatures in the rat. *Can J Physiol Pharmacol* 1980;58(8):903–908. [PubMed: 7225928]
- Marti M, Sbrenna S, Fuxe K, Bianchi C, Beani L, Morari M. In vitro evidence for increased facilitation of striatal acetylcholine release via pre- and postsynaptic NMDA receptors in hemiparkinsonian rats. *J Neurochem* 1999;72(2):875–878. [PubMed: 9930765]

- Meador-Woodruff JH. Update on dopamine receptors. *Ann Clin Psychiatry* 1994;6(2):79–90. [PubMed: 7804392]
- Morelli M, Pontieri FE, Linfante I, Orzi F, Di Chiara G. Local cerebral glucose utilization after D1 receptor stimulation in 6-OHDA lesioned rats: effect of sensitization (priming) with a dopaminergic agonist. *Synapse* 1993;13(3):264–269. [PubMed: 8098880]
- Nikolaus S, Larisch R, Beu M, Forutan F, Vosberg H, Muller-Gartner HW. Bilateral increase in striatal dopamine D2 receptor density in the 6-hydroxydopamine-lesioned rat: a serial in vivo investigation with small animal PET. *Eur J Nucl Med Mol Imaging* 2003;30(3):390–395. [PubMed: 12634967]
- Nilsson CL, Hellstrand M, Ekman A, Eriksson E. Direct dopamine D²-receptor-mediated modulation of arachidonic acid release in transfected CHO cells without the concomitant administration of a Ca²⁺-mobilizing agent. *Br J Pharmacol* 1998;124:1651–1658. [PubMed: 9756380]
- Ohata M, Takei H, Fredericks WR, Rapoport SI. Effects of immobilization stress on cerebral blood flow and cerebrovascular permeability in spontaneously hypertensive rats. *J Cereb Blood Flow Metab* 1982;2(3):373–379. [PubMed: 7096462]
- Ong WY, Sandhya TL, Horrocks LA, Farooqui AA. Distribution of cytoplasmic phospholipase A2 in the normal rat brain. *J Hirnforsch* 1999;39(3):391–400. [PubMed: 10536872]
- Palacios JM, Wiederhold KH. Dopamine D2 receptor agents, but not dopamine D1, modify brain glucose metabolism. *Brain Res* 1985;327(1–2):390–394. [PubMed: 3157422]
- Pardue S, Rapoport SI, Bosetti F. Co-localization of cytosolic phospholipase A₂ and cyclooxygenase-2 in Rhesus monkey cerebellum. *Brain Res Mol Brain Res* 2003;116:106–114. [PubMed: 12941466]
- Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. 2. Academic Press; New York: 1987.
- Qu Y, Chang L, Klaff J, Balbo A, Rapoport SI. Imaging brain phospholipase A2 activation in awake rats in response to the 5-HT_{2A/2C} agonist (+/-)2,5-dimethoxy-4-iodophenyl-2-aminopropane (DOI). *Neuropsychopharmacology* 2003;28:244–252. [PubMed: 12589377]
- Rapoport SI. In vivo approaches to quantifying and imaging brain arachidonic and docosahexaenoic acid metabolism. *J Pediatr* 2003;143(4 Suppl):S26–34. [PubMed: 14597911]
- Ribeiro MJ, Vidailhet M, Loc'h C, Dupel C, Nguyen JP, Ponchant M, Dolle F, Peschanski M, Hantraye P, Cesaro P, Samson Y, Remy P. Dopaminergic function and dopamine transporter binding assessed with positron emission tomography in Parkinson disease. *Arch Neurol* 2002;59(4):580–586. [PubMed: 11939892]
- Rinne JO, Ruottinen H, Bergman J, Haaparanta M, Sonninen P, Solin O. Usefulness of a dopamine transporter PET ligand [(18)F]beta-CFT in assessing disability in Parkinson's disease. *J Neurol Neurosurg Psychiatry* 1999;67(6):737–741. [PubMed: 10567489]
- Robinson PJ, Noronha J, DeGeorge JJ, Freed LM, Nariai T, Rapoport SI. A quantitative method for measuring regional in vivo fatty-acid incorporation into and turnover within brain phospholipids: review and critical analysis. *Brain Res Brain Res Rev* 1992;17(3):187–214. [PubMed: 1467810]
- Robinson S, Krentz L, Moore C, Meshul CK. Blockade of NMDA receptors by MK-801 reverses the changes in striatal glutamate immunolabeling in 6-OHDA-lesioned rats. *Synapse* 2001;42(1):54–61. [PubMed: 11668591]
- Ross BM, Mamalias N, Moszczynska A, Rajput AH, Kish SJ. Elevated activity of phospholipid biosynthetic enzymes in substantia nigra of patients with Parkinson's disease. *Neuroscience* 2001;102(4):899–904. [PubMed: 11182251]
- Segal DS, Weinberger SB, Cahill J, McCunney SJ. Multiple daily amphetamine administration: behavioral and neurochemical alterations. *Science* 1980;207(4433):905–907. [PubMed: 7188815]
- Shinotoh H, Inoue O, Hirayama K, Aotsuka A, Asahina M, Suhara T, Yamazaki T, Tateno Y. Dopamine D1 receptors in Parkinson's disease and striatonigral degeneration: a positron emission tomography study. *J Neurol Neurosurg Psychiatry* 1993;56(5):467–472. [PubMed: 8505636]
- Sokoloff L. Energetics of functional activation in neural tissues. *Neurochem Res* 1999;24(2):321–329. [PubMed: 9972882]
- Sokoloff L, Reivich M, Kennedy C, Des Rosiers MH, Patlak CS, Pettigrew KD, Sakurada O, Shinohara M. The [14C]deoxyglucose method for the measurement of local cerebral glucose utilization: theory, procedure, and normal values in the conscious and anesthetized albino rat. *J Neurochem* 1977;28(5):897–916. [PubMed: 864466]

- Solanto MV. Dopamine dysfunction in AD/HD: integrating clinical and basic neuroscience research. *Behav Brain Res* 2002;130(1–2):65–71. [PubMed: 11864719]
- Soncrant TT, Holloway HW, Stipetic M, Rapoport SI. Cerebral glucose utilization in rats is not altered by hindlimb restraint or by femoral artery and vein cannulation. *J Cereb Blood Flow Metab* 1988;8(5):720–726. [PubMed: 3417799]
- Stone JM, Pilowsky LS. Antipsychotic drug action: targets for drug discovery with neurochemical imaging. *Expert Rev Neurother* 2006;6(1):57–64. [PubMed: 16466312]
- Trugman JM, Wooten GF. Selective D1 and D2 dopamine agonists differentially alter basal ganglia glucose utilization in rats with unilateral 6-hydroxydopamine substantia nigra lesions. *J Neurosci* 1987;7(9):2927–2935. [PubMed: 3114439]
- Turjanski N, Lees AJ, Brooks DJ. In vivo studies on striatal dopamine D1 and D2 site binding in L-dopa-treated Parkinson's disease patients with and without dyskinesias. *Neurology* 1997;49(3):717–723. [PubMed: 9305330]
- Ulus IH, Kiran BK, Ozkurt S. Involvement of central dopamine in the hyperthermia in rats produced by d-amphetamine. *Pharmacology* 1975;13(4):309–316. [PubMed: 1187755]
- Ungerstedt U. Postsynaptic supersensitivity after 6-hydroxy-dopamine induced degeneration of the nigro-striatal dopamine system. *Acta Physiol Scand Suppl* 1971;367:69–93. [PubMed: 4332693]
- Vial D, Piomelli D. Dopamine D₂ receptors potentiate arachidonate release via activation of cytosolic, arachidonate-specific phospholipase A₂. *J Neurochem* 1995;64:2765–2772. [PubMed: 7760057]
- Wooten GF, Collins RC. Effects of dopaminergic stimulation on functional brain metabolism in rats with unilateral substantia nigra lesions. *Brain Res* 1983;263(2):267–275. [PubMed: 6839175]
- Yuan H, Sarre S, Ebinger G, Michotte Y. Histological, behavioural and neurochemical evaluation of medial forebrain bundle and striatal 6-OHDA lesions as rat models of Parkinson's disease. *J Neurosci Methods* 2005;144(1):35–45. [PubMed: 15848237]
- Zuch CL, Nordstroem VK, Briedrick LA, Hoernig GR, Granholm AC, Bickford PC. Time course of degenerative alterations in nigral dopaminergic neurons following a 6-hydroxydopamine lesion. *J Comp Neurol* 2000;427(3):440–454. [PubMed: 11054705]

Abbreviations

PD	Parkinson disease
DA	dopamine
DAT	dopamine transporter
AA	arachidonic acid
PLA₂	phospholipase A ₂
cPLA₂	cytosolic PLA ₂
6-OHDA	6-hydroxydopamine
rCMR_{glc}	regional cerebral metabolic rate for glucose

Figure 1.

Coronal autoradiographs of effects of D-amphetamine (5.0 mg/kg i.p.) and of quinpirole (1.0 mg/kg i.v.) on regional incorporation coefficients k^* for arachidonic acid in unanesthetized unilaterally 6-OHDA lesioned rats. Values for k^* are color-coded. Quinpirole increased k^* on both sides of the brain compared with saline injected rats, but k^* values were higher on the lesioned side compared with the unlesioned intact side. D-amphetamine increased k^* on both sides of the brain compared with saline injected rats, but to a lesser extent on the lesioned compared with the unlesioned intact side. Brain slices displayed are +3.2 mm, then -0.3 mm, -5.8 mm, -10.3 mm from the bregma (Paxinos & Watson, 1987). Abbreviations: I, intact and L, lesioned side of brain; PFr ctx, prefrontal cortex; Mot ctx, motor cortex; Som ctx, somatosensory cortex; CPU, caudate-putamen; Vis ctx, visual cortex; MGN, medial geniculate nucleus; Aud ctx, auditory cortex.

Figure 2.

k^* for arachidonic acid in frontal cortex layer IV of unanesthetized unilaterally 6-OHDA lesioned rats. Quinpirole compared with saline increased k^* on both lesioned and intact sides, whereas D-amphetamine compared with saline increased k^* only on the intact side. Ψ , significant difference from mean on corresponding side in saline-injected rats, $p \leq 0.05$. * $p \leq 0.05$, ** $p \leq 0.01$, significant difference between mean on lesioned compared with intact side using paired t test.

Effect of acute administration of saline, quinpirole, and D-amphetamine on physiological parameters in unanesthetized unilaterally 6-OHDA lesioned rats.

	Before		After	
	(Basal)		Saline	Amph (5.0 mg/kg)
Physiological parameters:				
Rectal temperature (°C)	36.4 ± 0.6		36.4 ± 0.4	38.4 ± 0.8***
Heart rate (beats/min)	447 ± 39		420 ± 54	512 ± 19***
Arterial blood pressure (mmHg)	118.8 ± 18.1		121.3 ± 34.7	138.3 ± 22.8*

Data was analyzed from 7 to 11 rats and reported as the mean ± S.D.

* $p < 0.05$,
** $p < 0.01$,
*** $p < 0.001$ vs. before any injection (basal).

Table 2

Effect of Quinpirole 1.0 mg/kg i.v. and D-amphetamine 5.0 mg/kg i.p. on regional unidirectional incorporation coefficients, $k^*,^2$ of [$1\text{-}^{14}\text{C}$]AA in unanesthetized unilaterally 6-OHDA lesioned rats

		Treatment		
Brain Regions	Side	Saline (<i>n</i> = 11)	Quinpirolo (<i>n</i> = 7)	D-amphetamine (<i>n</i> = 7)
Telencephalon				
Prefrontal cortex -I	Lesioned	4.27 ± 0.86	6.91 ± 1.56 ^ψ	4.86 ± 1.20
	Intact	3.94 ± 0.93	6.48 ± 1.68 ^ψ	5.05 ± 1.21
Prefrontal cortex -IV	Lesioned	4.99 ± 1.36	8.09 ± 1.14 ^{*ψ}	5.44 ± 1.11 ^{* *}
	Intact	4.61 ± 0.91	7.29 ± 1.46 ^ψ	5.79 ± 1.05
Primary olfactory cortex	Lesioned	4.04 ± 1.15	6.14 ± 0.75 ^ψ	4.65 ± 1.18 [*]
	Intact	3.85 ± 0.80	6.05 ± 1.14 ^ψ	4.80 ± 1.09
Frontal cortex Layer I	Lesioned	4.44 ± 0.88 [*]	7.58 ± 1.46 ^{* *ψ}	4.98 ± 1.23 [*]
	Intact	4.10 ± 0.88	7.04 ± 1.54 ^ψ	5.52 ± 1.43
Layer IV	Lesioned	5.29 ± 1.20 [*]	9.49 ± 1.43 ^{* *ψ}	6.10 ± 0.91 [*]
	Intact	4.78 ± 0.82	8.22 ± 1.49 ^ψ	6.75 ± 1.32 ^ψ
Pyriform cortex	Lesioned	3.52 ± 0.76	5.01 ± 1.10 ^ψ	4.41 ± 0.80
	Intact	3.51 ± 0.80	5.05 ± 1.03 ^ψ	4.45 ± 0.80
Anterior cingulate cortex	Lesioned	5.31 ± 0.90 ^{* *}	8.45 ± 1.21 ^ψ	6.65 ± 1.44 [*]
	Intact	4.99 ± 0.93	8.21 ± 1.13 ^ψ	6.86 ± 1.45 ^ψ
Motor cortex Layer I	Lesioned	4.63 ± 0.91	7.56 ± 1.14 ^ψ	5.13 ± 1.28
	Intact	4.24 ± 1.01	7.22 ± 0.94 ^ψ	5.90 ± 0.89 ^ψ
Layer II–III	Lesioned	4.71 ± 0.78	7.77 ± 1.20 ^ψ	5.57 ± 0.96 [*]
	Intact	4.52 ± 0.99	7.75 ± .071 ^ψ	6.23 ± 1.10 ^ψ
Layer IV	Lesioned	5.29 ± 0.97	9.17 ± 0.77 ^{*ψ}	6.20 ± 0.92 [*]
	Intact	5.15 ± 1.06	8.50 ± 0.86 ^ψ	7.05 ± 1.19 ^ψ
Layer V	Lesioned	4.70 ± 0.97	7.69 ± 0.80 ^ψ	5.53 ± 0.82 ^{* *}
	Intact	4.56 ± 0.93	7.39 ± 0.95 ^ψ	6.02 ± 0.92 ^ψ
Layer VI	Lesioned	4.30 ± 0.96	6.94 ± 1.05 ^{*ψ}	4.95 ± 0.84 [*]
	Intact	4.02 ± 0.84	6.54 ± 1.20 ^ψ	5.39 ± 0.87 ^ψ
Somatosensory cortex Layer I	Lesioned	4.64 ± 0.96	7.48 ± 1.18 ^ψ	5.48 ± 1.02
	Intact	4.50 ± 1.06	7.14 ± 0.94 ^ψ	5.56 ± 0.81
Layer II–III	Lesioned	4.85 ± 0.97	7.77 ± 0.80 ^ψ	5.63 ± 0.89
	Intact	4.86 ± 0.90	7.47 ± 0.94 ^ψ	6.17 ± 1.10 ^ψ
Layer IV	Lesioned	5.40 ± 1.12	9.33 ± 0.65 ^{* *ψ}	6.62 ± 1.21 ^{* *}
	Intact	5.21 ± 1.04	8.39 ± 0.92 ^ψ	7.11 ± 1.19 ^ψ
Layer V	Lesioned	4.66 ± 0.94	8.10 ± 0.91 ^{*ψ}	5.61 ± 0.85 [*]
	Intact	4.75 ± 0.91	7.51 ± 0.76 ^ψ	5.98 ± 0.91 ^ψ
Layer VI	Lesioned	4.21 ± 0.78	6.64 ± 0.94 ^ψ	4.91 ± 0.95
	Intact	4.26 ± 0.73	6.37 ± 0.88 ^ψ	5.13 ± 0.79
Auditory cortex Layer I	Lesioned	4.77 ± 1.56	8.54 ± 1.84 ^ψ	6.19 ± 1.17
	Intact	4.37 ± 1.31	8.22 ± 1.65 ^ψ	6.49 ± 1.12 ^ψ
Layer IV	Lesioned	5.73 ± 1.70	10.12 ± 1.47 ^ψ	7.53 ± 1.57 [*]
	Intact	5.48 ± 1.40	10.05 ± 1.59 ^ψ	7.86 ± 1.71 ^ψ
Layer VI	Lesioned	4.45 ± 1.15	7.49 ± 1.67 ^ψ	5.31 ± 0.87 [*]
	Intact	4.14 ± 1.21	7.65 ± 1.73 ^ψ	5.99 ± 1.06 ^ψ
Visual cortex Layer I	Lesioned	4.56 ± 1.11	6.94 ± 1.46 ^ψ	5.67 ± 0.77
	Intact	4.32 ± 1.27	7.35 ± 1.84 ^ψ	5.81 ± 0.66
Layer IV	Lesioned	5.02 ± 1.38	8.31 ± 1.43 ^ψ	6.57 ± 0.98
	Intact	4.95 ± 1.39	8.64 ± 1.93 ^ψ	7.03 ± 1.25 ^ψ
Layer VI	Lesioned	4.51 ± 1.34	7.30 ± 1.37 ^ψ	5.54 ± 0.77
	Intact	4.36 ± 1.16	7.71 ± 2.11 ^ψ	5.65 ± 0.95
Olfactory tubercle	Lesioned	5.06 ± 1.40 ^{* *}	8.78 ± 1.24 ^ψ	5.79 ± 1.40 ^{* *}
	Intact	4.51 ± 1.52	8.45 ± 1.18 ^ψ	6.25 ± 1.46
Islands of Calleja	Lesioned	3.92 ± 0.98 [*]	5.94 ± 1.36 ^ψ	4.52 ± 0.96
	Intact	3.51 ± 1.08	6.09 ± 1.35 ^ψ	4.60 ± 1.05
Accumbens nucleus	Lesioned	5.22 ± 1.34	8.19 ± 1.73 ^ψ	5.56 ± 0.88
	Intact	4.91 ± 1.09	7.36 ± 1.48 ^ψ	5.51 ± 0.93
Ventral pallidum	Lesioned	3.77 ± 0.81	5.50 ± 1.09 ^{*ψ}	4.80 ± 0.87

Brain Regions	Side	Treatment		
		Saline (n = 11)	Quinpirole (n = 7)	D-amphetamine (n = 7)
Globus pallidus	Intact	3.53 ± 0.93	5.77 ± 1.08 ^Ψ	4.74 ± 1.00
	Lesioned	3.98 ± 0.75	6.23 ± 1.02 ^Ψ	5.14 ± 0.79 ^Ψ
	Intact	3.60 ± 1.02	6.34 ± 0.94 ^Ψ	5.32 ± 1.03 ^Ψ
Entopeduncular nucleus	Lesioned	3.10 ± 0.93 [*]	4.65 ± 1.06 ^{*Ψ}	3.82 ± 1.00
	Intact	2.88 ± 1.04	4.34 ± 1.12 ^Ψ	3.82 ± 1.11
Caudate putamen Dorsal	Lesioned	4.46 ± 0.90	7.36 ± 1.08 ^Ψ	5.31 ± 0.64 ^{* *}
	Intact	4.21 ± 0.68	6.84 ± 0.89 ^Ψ	6.09 ± 0.69 ^Ψ
Ventral	Lesioned	4.76 ± 1.03	7.79 ± 0.94 ^{* * *}	5.59 ± 0.80
	Intact	4.49 ± 0.96	6.44 ± 1.01 ^Ψ	5.27 ± 0.77
Lateral	Lesioned	4.58 ± 0.91	8.40 ± 1.08 ^{* * *}	5.39 ± 0.74
	Intact	4.38 ± 0.84	7.49 ± 1.07 ^Ψ	5.66 ± 0.87 ^Ψ
Medial	Lesioned	4.46 ± 0.78 [*]	7.06 ± 1.09 ^Ψ	5.16 ± 0.74
	Intact	4.07 ± 0.78	6.76 ± 1.28 ^Ψ	5.23 ± 0.81
Hippocampus CA1	Lesioned	3.23 ± 0.84	4.19 ± 0.84	4.15 ± 1.18
	Intact	3.21 ± 0.79	4.16 ± 0.82	4.16 ± 1.25
CA2	Lesioned	3.39 ± 0.81	4.48 ± 0.81	4.37 ± 1.16 [*]
	Intact	3.43 ± 0.89	4.53 ± 0.69	4.48 ± 1.26
CA3	Lesioned	3.51 ± 0.81	4.65 ± 0.76 ^Ψ	4.46 ± 1.11 [*]
	Intact	3.46 ± 0.85	4.64 ± 0.74 ^Ψ	4.58 ± 1.11
Dentate Gyrus	Lesioned	3.95 ± 0.72	5.10 ± 0.62 ^Ψ	4.91 ± 0.98
	Intact	4.04 ± 0.82	4.98 ± 0.76	4.98 ± 0.99
Diencephalon				
Habenular nu lateral	Lesioned	5.51 ± 1.01	9.41 ± 1.53 ^Ψ	7.74 ± 1.24 ^Ψ
	Intact	5.87 ± 1.28	9.51 ± 1.00 ^Ψ	7.66 ± 1.47 ^Ψ
medial	Lesioned	5.04 ± 1.05	7.23 ± 1.03 ^Ψ	6.31 ± 0.85 ^Ψ
	Intact	5.20 ± 1.06	7.54 ± 0.91 ^Ψ	6.46 ± 1.09
Dorsal lat. geniculate nu	Lesioned	5.08 ± 0.98	7.45 ± 0.85 ^Ψ	6.92 ± 0.87 ^Ψ
	Intact	5.13 ± 1.29	7.50 ± 0.70 ^Ψ	6.97 ± 0.97 ^Ψ
Medial geniculate nu	Lesioned	5.62 ± 1.54	9.85 ± 1.71 ^Ψ	7.69 ± 1.46 ^Ψ
	Intact	5.48 ± 1.57	9.67 ± 1.62 ^Ψ	7.97 ± 1.77 ^Ψ
Ant. pretectal nu	Lesioned	4.87 ± 0.98	7.62 ± 0.75 ^Ψ	6.92 ± 0.99 ^Ψ
	Intact	4.82 ± 0.96	7.28 ± 0.74 ^Ψ	6.92 ± 0.98 ^Ψ
Thalamus				
Ventroposterior lat. nu	Lesioned	4.62 ± 0.84	6.90 ± 0.75 ^Ψ	6.58 ± 0.85 ^Ψ
	Intact	4.61 ± 0.92	6.76 ± 0.90 ^Ψ	6.37 ± 0.74 ^Ψ
Ventroposterior med. nu	Lesioned	4.37 ± 0.77	6.46 ± 0.83 ^Ψ	6.15 ± 0.63 ^Ψ
	Intact	4.69 ± 0.88	6.19 ± 0.69 ^Ψ	5.91 ± 0.76 ^Ψ
Ventrolateral	Lesioned	6.67 ± 1.65 ^{* *}	10.10 ± 0.72 ^Ψ	7.97 ± 1.22
	Intact	6.16 ± 1.35	10.21 ± 1.71 ^Ψ	7.67 ± 1.57
Ventromedial	Lesioned	6.29 ± 1.65 [*]	9.24 ± 0.71 ^Ψ	7.44 ± 1.04
	Intact	5.88 ± 1.32	9.02 ± 1.09 ^Ψ	7.24 ± 1.15
Parafascicular nu	Lesioned	4.81 ± 0.92	7.29 ± 0.66 ^Ψ	6.76 ± 0.86 ^Ψ
	Intact	4.63 ± 0.88	7.07 ± 0.62 ^Ψ	6.61 ± 0.77 ^Ψ
Subthalamic nucleus	Lesioned	5.15 ± 1.19	8.44 ± 0.57 ^Ψ	6.67 ± 0.97 ^Ψ
	Intact	5.35 ± 1.36	8.36 ± 0.46 ^Ψ	6.52 ± 0.80
Mesencephalon				
Interpeduncular nucleus	Lesioned	6.76 ± 1.85	11.75 ± 1.37 ^Ψ	8.56 ± 2.35
	Intact	6.36 ± 1.73	11.61 ± 1.66 ^Ψ	8.56 ± 2.37
Substantia nigra pars reticulata	Lesioned	4.58 ± 1.15	7.82 ± 1.51 ^{* *Ψ}	5.85 ± 1.06
	Intact	4.28 ± 0.97	7.08 ± 1.42 ^Ψ	6.02 ± 0.77 ^Ψ
pars compacta	Lesioned	4.61 ± 1.18 [*]	6.82 ± 1.63 ^Ψ	5.41 ± 0.97
	Intact	3.98 ± 0.90	6.82 ± 1.57 ^Ψ	5.43 ± 1.09 ^Ψ
Ventral tegmental area	Lesioned	3.94 ± 0.96	5.69 ± 1.38 ^Ψ	4.31 ± 1.54
	Intact	3.53 ± 0.87	5.72 ± 1.22 ^Ψ	4.54 ± 1.64
Colliculus Superior	Lesioned	5.27 ± 1.40	8.41 ± 1.84 ^Ψ	6.97 ± 1.30
	Intact	5.03 ± 1.44	8.29 ± 1.86 ^Ψ	6.79 ± 1.19
Inferior	Lesioned	6.94 ± 1.92	12.03 ± 1.78 ^Ψ	9.04 ± 2.78
	Intact	6.84 ± 1.81	12.10 ± 2.16 ^Ψ	9.25 ± 3.18
Sup gray layers of sup. colliculus	Lesioned	4.87 ± 0.95 [*]	7.97 ± 1.29 ^Ψ	6.89 ± 1.26 ^Ψ
	Intact	4.43 ± 0.70	7.48 ± 1.11 ^Ψ	7.04 ± 1.34 ^Ψ

Brain Regions	Side	Treatment		
		Saline (<i>n</i> = 11)	Quinpirole (<i>n</i> = 7)	D-amphetamine (<i>n</i> = 7)
Deep layers of sup. colliculus	Lesioned	4.65 ± 0.88	7.75 ± 1.47 ^ψ	6.53 ± 1.07 ^ψ
	Intact	4.34 ± 0.85	7.55 ± 1.36 ^ψ	6.74 ± 1.24 ^ψ
Pedunculopontine tegmental nucleus	Lesioned	3.76 ± 0.68	6.27 ± 1.02 ^ψ	4.95 ± 1.49
	Intact	3.71 ± 0.72	6.04 ± 0.98 ^ψ	4.83 ± 1.51
Rhombencephalon				
Flocculus	Lesioned	4.81 ± 1.25	7.13 ± 1.00 ^ψ	6.60 ± 1.54 ^ψ
	Intact	4.55 ± 1.07	7.15 ± 1.22 ^ψ	6.78 ± 1.81 ^ψ
Cerebellar gray matter	Lesioned	4.33 ± 0.60 [*]	6.57 ± 1.24 ^ψ	5.26 ± 1.39
	Intact	4.09 ± 0.64	6.71 ± 1.29 ^ψ	5.26 ± 1.41
Molecular layer of cerebellar gray matter	Lesioned	5.94 ± 1.43	9.90 ± 2.70 ^ψ	8.02 ± 2.12
	Intact	5.88 ± 1.17	9.89 ± 2.42 ^ψ	8.29 ± 2.51
White matter				
Corpus callosum	Lesioned	2.69 ± 1.23	4.31 ± 1.26 ^ψ	3.88 ± 1.07
	Intact	2.70 ± 1.13	4.27 ± 1.18 ^ψ	3.98 ± 1.08
Cerebellar white matter	Lesioned	2.94 ± 0.91 [*]	3.92 ± 1.01	3.58 ± 1.67
	Intact	2.61 ± 0.98	3.97 ± 0.83	3.55 ± 1.74

$k^* = (\text{ml/s/g}) \times 10^{-4}$. Values are mean ± S.D. of k^* . Each region of interest was measured in sextuplicate in each rat. Within the same animal, statistical analysis was done using paired *t* test to compare lesioned versus intact side.

^{*} $p < 0.05$,

^{**} $p < 0.01$,

^{***} $p < 0.001$ versus other side of the brain.

Rats were acutely administered either D-amphetamine 5.0 mg/kg i.p. or quinpirole 1.0 mg/kg i.v., and compared to control (saline) using One way ANOVA with Bonferroni *post-hoc* test.

^ψ $p < 0.05$ vs. Control (saline).

Abbreviations: nu, nucleus; lat, lateral; med, medial.