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Selective Dopamine Receptor Stimulation Differentially Affects [^{3}H]Arachidonic Acid Incorporation, a Surrogate Marker for Phospholipase A_2-Mediated Neurotransmitter Signal Transduction, in a Rodent Model of Parkinson's Disease

TAKANORI HAYAKAWA, MICHAEL C. J. CHANG, STANLEY I. RAPOPORT, and NATHAN M. APPEL

Laboratory of Neurosciences, National Institute on Aging, National Institutes of Health, Bethesda, Maryland (T.H., M.C.J.C., S.I.R., N.M.A.); Department of Neurosurgery, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo, Japan (T.H.); and Division of Applied Pharmacology Research, Office of Testing and Research, Center for Drug Evaluation and Research, Food and Drug Administration, Laurel, Maryland (N.M.A.)

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ABSTRACT

Our laboratory has developed a technique whereby radiolabeled long-chain fatty acids are injected intravenously in awake rats to pulse-label brain lipids, mainly phospholipids, to measure regional brain lipid metabolism by autoradiography. The brain incorporation of [^{3}H]arachidonic acid ([^{3}H]AA), a polyunsaturated fatty acid, may reflect regional changes in neurotransmitter signal transduction using phospholipase A_2. Using this radiotracer, we examined the brain dopamine system in rats with a chronic unilateral 6-hydroxydopamine lesion of the substantia nigra pars compacta, a model of Parkinson's disease. Four weeks after lesioning, rats received either vehicle; SKF38393 or quinpirole (LY-171,555) (D_1- and D_2-dopamine-like agonists, respectively); or (+)-butaclamol (D_1/D_2 antagonist) followed by either vehicle, SKF38393, or quinpirole. They then were infused with [^{3}H]AA and their brains processed for autoradiography. SKF38393 increased [^{3}H]AA incorporation into the lesioned side compared with the intact side in the caudate putamen, somatosensory and motor cortices and subthalamic nucleus, but decreased incorporation in the ipsilateral ventrolateral thalamus. Quinpirole increased ipsilateral [^{3}H]AA incorporation in the caudate putamen and somatosensory and motor cortices, and decreased it in the ventrolateral thalamus. (+)-Butaclamol blocked this effect. The data suggest up-regulation in basal ganglia and cortical dopamine circuits mediated by phospholipase A_2 ipsilateral to the substantia nigra lesion.

It has been established that the regional cerebral metabolic rate for glucose ($rCMR_{glc}$) is a useful marker for regional brain activity and it can be accurately measured with quantitative autoradiography in animals using 2-[1-^{14}C]deoxy-D-glucose ([^{14}C]2DG) as a tracer (Sokoloff, 1977; Sokoloff et al., 1977). An analogous approach for examining regional brain lipid metabolism also has been developed. This "fatty acid technique" uses intravenously administered radiolabeled long-chain fatty acids to pulse-label brain lipids, mainly phospholipids, in awake animals (Robinson et al., 1992). In this manner one measures a regional "incorporation coefficient", k^*, of the radiolabeled plasma fatty acid into stable brain lipids using quantitative autoradiography.

Different fatty acid tracers pulse-label different stereospecific numbered (*sn*) positions in different phospholipids. In the case of labeled arachidonic acid (AA), an unsaturated (20:4, *n*-6) fatty acid, it is incorporated mainly into the *sn*-2 position of phosphatidylinositol and phosphatidylcholine (DeGeorge et al., 1989; Fonlupt et al., 1994) and it has been shown that this incorporation is affected by acute or chronic alterations of functional brain activity (DeGeorge et al., 1991; Nariai et al., 1991; Wakabayashi et al., 1994, 1995; Rabin et al., 1998). Moreover, this incorporation is independent of cerebral blood flow (Chang et al., 1997) and can be inhibited by the phospholipase A_2 (PLA_2) inhibitor manoalide (Jones et al., 1996; Grange et al., 1998). These data indicate that functional activity in brain circuits that contain receptors linked to PLA_2 activation can be revealed as patterns of regional radiolabeled AA incorporation. Dopamine receptor stimulation, in particular stimulating the D_2 subtype, results in increased AA release and this effect is seemingly in part PLA_2-dependent (Kanterman et al., 1991; Piomelli et al., 1991; Schinelli et al., 1994; Vial and Piomelli, 1995).

Parkinson's disease is a disabling neurological disorder that arises because of dysfunction of brain circuits involving

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ABBREVIATIONS: $rCMR_{glc}$, regional cerebral metabolic rates for glucose; 2DG, 2-deoxy-D-glucose; PLA_2, phospholipase A_2; 6-OHDA, 6-hydroxydopamine; AA, arachidonic acid; GABA, γ-aminobutyric acid.

the basal ganglia. Degeneration of dopaminergic neurons from the substantia nigra pars compacta results in dopamine deficiency that ultimately leads to the signs and symptoms of the disease (Ehringer and Hornykiewicz, 1960). Pharmacological therapeutic strategies to treat Parkinson's disease involve administering drugs that increase dopamine levels in dopaminergic synapses or reduce inhibition of dopaminergic neurons; the overall goal is to increase dopaminergic signaling in basal ganglia circuits (Narabayashi et al., 1993; Kohler and Paulson, 1995).

Parkinson's disease can be modeled in rats by injecting 6-hydroxydopamine (6-OHDA) unilaterally into the substantia nigra pars compacta to lesion meso-striatal dopamine neurons (Gerlach and Riederer, 1996). This model allows direct comparisons of experimental treatments on the ipsilateral (lesioned) and contralateral (intact) side of the brain in individual animals. Sagar and Snodgrass (1980) and Wooten and Collins (1981) studied $rCMR_{glc}$ in this model using [^{14}C]2DG autoradiography. They noted small regional asymmetries in brain metabolism between the lesioned and intact sides of rats receiving saline vehicle treatment. Subsequently, Hayakawa et al. (1998) used the fatty acid technique in the unilateral 6-OHDA lesion model. In contrast to the earlier studies using [^{14}C]2DG, they detected robust increases in [^{3}H]AA incorporation into basal ganglia structures ipsilateral to the lesion that included globus pallidus and caudate putamen, as well as affecting several anterior cerebral cortical regions.

Following the earlier 2DG studies, Trugman and Wooten (1987) examined the effects of the selective dopaminergic agonists SKF38393 (a D_1 receptor agonist) and quinpirole (LY-171,555; a D_2 receptor agonist) on $rCMR_{glc}$ in chronic unilaterally 6-OHDA-lesioned rats. They showed D_1 and D_2 agonist stimulated $rCMR_{glc}$ in a number of defined brain regions compared with vehicle treatment. They also demonstrated differential effects of D_1 and D_2 agonists on $rCMR_{glc}$ in the entopeduncular nucleus and substantia nigra pars compacta. In view that D_2 dopamine receptor stimulation appears to be linked to PLA_2 activation and arachidonic acid release (Kanterman et al., 1991; Piomelli et al., 1991; Schinelli et al., 1994; Vial and Piomelli, 1995) and of significant differences in [^{3}H]AA incorporation between the lesioned and intact sides in unilaterally substantia nigra-lesioned rats (Hayakawa et al., 1998), we decided to evaluate in this model the effects of selective dopamine D_1 and D_2 receptor stimulation by SKF38393 and quinpirole, respectively, on [^{3}H]AA incorporation. The results suggest general disinhibition of basal ganglia and cortical circuits containing neurotransmitters whose receptors' signal transduction mechanism is PLA_2 and that there are differences in responsiveness of these circuits to D_1 and D_2 receptor stimulation. An abstract of this work has been published (Hayakawa et al., 1997).

Materials and Methods

Animals. The studies were carried out with male Sprague-Dawley rats (Taconic Farms, Germantown, NY). They were maintained in a vivarium with controlled temperature, humidity, and light cycle (on 6:00 AM–6:00 PM) and had access ad libitum to standard pelleted laboratory chow and fresh tap water. The experiments were conducted in accordance with and approved by the National Institute of Child Health and Human Development Animal Care and Use Committee, Protocol #95-029 (Guide for the Care and Use of Laboratory Animals, National Institutes of Health Publication 86-23).

Drugs. [^{3}H]Arachidonic acid was purchased from Moravek Biochemicals (Brea, CA). *S*-(+)-Apomorphine HCl, 6-hydroxydopamine HBr, (+)-butaclamol HCl, (−)-quinpirole (LY-171,555), and *R*-(+)-SKF38393 were obtained from Research Biochemicals International (Natick, MA). Pentobarbital sodium was purchased from Richmond Veterinary Supply Co. (Richmond, VA). L-Ascorbic acid, desipramine HCl, HEPES, and fatty acid-free bovine serum albumin, propylene glycol, and Tween 80 were purchased from Sigma Chemical Co. (St. Louis, MO).

Stereotaxic Surgery. Rats weighing 233 ± 3 g (mean ± S.E.M.) were first administered desipramine HCl, 50 mg/kg i.p. (Breese and Traylor, 1971). They then were anesthetized with pentobarbital sodium, 50 mg/kg i.p., and placed in a stereotaxic frame (David Kopf, Tejunga, CA). Briefly, a 30-gauge stainless steel cannula connected to a microsyringe (Hamilton, Reno, NV) by PE20 polyethylene tubing (Clay Adams, Parsippany, NJ) was filled with freshly made 6-OHDA solution and positioned above the substantia nigra pars compacta at 1.6 mm lateral, −5.0 mm posterior, and −8.0 mm ventral to bregma (Paxinos and Watson, 1996) on the left side of the brain. One minute after cannula placement and 30 min after the desipramine injection, rats were infused with 8 μg of 6-OHDA in 4 μl of 0.02% ascorbic acid/normal saline (w/v) over 8 min using a Harvard Apparatus model 22 (Natick, MA) infusion pump. The cannula was left in place for 5 min after 6-OHDA infusion, and then slowly withdrawn. During surgery and for 1 h thereafter, body temperature was monitored via a rectal thermister probe and maintained using either a heating pad or radiant heat. To assess the efficacy of the lesion, rats were tested 10 and 17 days later for their response to an apomorphine challenge (Ungerstedt, 1971). They were injected with *S*-(+)-apomorphine HCl, 0.5 mg/kg i.p., and observed for 20 min. Only rats which had completed a minimum of 100 contralateral rotations in 20 min during both testing sessions were used in these studies.

Arterial and Venous Catheterizations. Four weeks ± 1 day after 6-OHDA lesioning, rats were prepared to receive i.v. infusion of treatment drugs and [^{3}H]AA tracer, and for collecting arterial blood samples (Appel et al., 1997). They weighed 360 ± 8 g at that time. Briefly, they were anesthetized with halothane (1–3% v/v in O_2) and PE50 polyethylene catheters (Clay Adams) filled with heparinized saline (100 IU/ml) were surgically implanted into a femoral artery and vein. The incision site was infiltrated with local anesthetic and closed with wound clips. Next, they were wrapped loosely in a fast-setting plaster cast, secured to a wooden block with their upper body free, and allowed to recover from anesthesia in a temperature-controlled and sound-dampened box for 4 to 5 h.

Drug and Tracer Infusions. After recovering from anesthesia, a baseline 125-μl blood sample was withdrawn through the arterial cannula. Thereafter, rats (eight per group) were administered either vehicle (77 mM NaCl/10% propylene glycol); *R*-(+)-SKF38393, 5 mg/kg; or (−)-quinpirole, 1 mg/kg, in a volume of 1 ml/kg through the venous cannula. In addition, eight rats each were administered (+)-butaclamol HCl, 0.3 mg/kg precisely 30 min before i.v. vehicle, SKF38393, or quinpirole. (+)-Butaclamol was dissolved in distilled water containing Tween 80, 3 drops/10 ml. Exactly 1 min after either vehicle, SKF38393, or quinpirole administration, rats were infused with 1.75 mCi/kg [5,6,8,9,11,12,14,15-^{3}H]AA (specific activity 160 Ci/mmol) in 2 ml of 5 mM HEPES buffer, pH 7.4, containing 50 mg/ml fatty acid-free bovine serum albumin through the venous cannula at 400 μl/min over a period of 5 min, using the Harvard pump. Timed 125-μl arterial blood samples were collected during and after the onset of infusion. Twenty minutes after the onset of infusion, rats were euthanized with 65 mg of pentobarbital sodium through the femoral vein catheter, and brains were removed and frozen at −50°C in 2-methylbutane for subsequent autoradiography. Plasma was separated from the arterial blood samples by centrifugation, lipids were extracted using the method of Folch et al. (1957), and radioactivity in the organic fraction was measured by liquid scintillation spectrometry.

Autoradiography and Histology. Frozen brains were sectioned at −20°C. Sets of three adjacent 20-μm sections were collected on 22 × 40 mm #1 glass coverslips at 140-μm intervals and dried. The coverslip-mounted sections and calibrated [^{3}H]methylmethacrylate autoradiographic standards (Amersham, Arlington Heights, IL) were exposed together with [^{3}H]Hyperfilm (Amersham) for approximately 12 weeks and then developed following the manufacturer's instructions. Remaining brain sections at the level of the substantia nigra were stained with cresyl violet to confirm the location of the 6-OHDA injection sites (Paxinos and Watson, 1996).

Quantitative Densitometry. Radioactivity in the different brain regions of interest was measured in sextuplicate by quantitative densitometry using the public domain image analysis program NIH Image (version 1.55), created by Wayne Rasband (National Institutes of Health, Bethesda, MD) installed on a Macintosh computer (Apple Computer; Cupertino, CA). Regional incorporation coefficients, k^*, for [^{3}H]AA were calculated using the formula:

$$k^* = \frac{c^*_{\text{brain}}(20\text{ min})}{\int_0^{20} c^*_{\text{plasma}}\mathrm{d}t}$$

k^* is in units of milliliters per second per gram. c^*_{brain} (20 min) is in units of nanocuries per gram and is brain radioactivity at 20 min as determined by densitometry. c^*_{plasma} is in units of nanocuries per milliliter and is the plasma fatty acid radioactivity determined by scintillation counting. t is in units of minutes and is the time after onset of [^{3}H]AA infusion.

Statistical Analysis. Data were evaluated using StatView and SuperANOVA software for the Macintosh (Abacus Concepts, Berkeley, CA) and are reported as mean ± S.E.M.

Results

We previously reported that 4 weeks after rats were unilaterally lesioned in their left substantia nigra pars compacta with 6-OHDA, and injected intravenously with saline (vehicle) there were significant increases in regional brain [^{3}H]AA incorporation on the side ipsilateral to the lesion, compared with the contralateral side (Hayakawa et al., 1998). These data appear under the heading "Vehicle" in both Tables 1 and 2. The basal ganglia-cortical circuit includes the substantia nigra, striatum, globus pallidus, and subthalamic nucleus, as well as input to the striatum originating from the cerebral cortex (Narabayashi et al., 1993). In vehicle-treated rats, when incorporation coefficients, k^*, on the lesioned and intact sides within subjects are compared using a paired t test, ipsilateral structures in the basal ganglia circuit show significantly increased [^{3}H]AA incorporation (Tables 1 and 2). The greatest effects in cerebral cortex occurred in somatosensory layers IV and V. In the basal ganglia, the most pronounced effect occurred in the globus pallidus. Increased incorporation also occurred in dorsal (adjacent to cingulum), lateral (adjacent to external capsule), and medial (adjacent to lateral ventricle) regions of caudate putamen, but not the ventral (adjacent to nucleus accumbens) region. There was no significant effect in nucleus accumbens, a "ventral" extension of the caudate putamen that is not considered to be part of the basal ganglia circuit. Other components of the circuit, the entopeduncular nucleus, subthalamic nucleus, and substantia nigra pars reticulata, also had increased [^{3}H]AA incorporation in the side ipsilateral to the lesion. Autoradiographs of patterns of [^{3}H]AA incorporation in vehicle-infused rats are depicted in the left-hand column of Fig. 1.

Effects of D_1 Dopamine Receptor Stimulation. Administering SKF38393 to stimulate D_1 receptors had profound effects in these chronically lesioned rats (Table 1; Figs. 1 and 2). The brain [^{3}H]AA incorporation coefficient k^* was significantly increased on the side ipsilateral to the lesion compared with the contralateral side. The pattern of this effect was comparable to the pattern in vehicle-infused controls in the basal ganglia circuit, however, with some exceptions. For the most part, the absolute magnitudes of increased [^{3}H]AA incorporation on the lesioned side were significantly greater in SKF38393-treated rats than in vehicle-treated rats in which [^{3}H]AA incorporation was also increased ipsilaterally (Table 1). The exceptions were the globus pallidus and subthalamic nucleus, where the magnitude of incorporation increased similar to the ipsilateral side in vehicle-treated controls. In addition, SKF38393 increased [^{3}H]AA incorporation in layers of motor cortex and somatosensory cortex compared with the ipsilateral side in vehicle controls.

We also compared relative ipsilateral-to-contralateral effects of SKF38393 on regional brain [^{3}H]AA incorporation to changes that occurred in corresponding regions of vehicle-treated rats (Fig. 2). The majority of brain regions on the side ipsilateral to the lesion had higher [^{3}H]AA incorporation levels following SKF38393 than after vehicle administration. The largest effects were in layers of motor cortex and the subthalamic nucleus with the greatest change occurring in the subthalamic nucleus. The effect of SKF38393 on [^{3}H]AA incorporation in the caudate putamen was slight. Relative [^{3}H]AA incorporation was decreased in ventral thalamus compared with vehicle-treated controls.

Effects of D_2 Dopamine Receptor Stimulation. Administering quinpirole to stimulate D_2 receptors also had profound effects in chronic unilaterally lesioned rats (Table 2; Figs. 1 and 3). In many brain regions, absolute [^{3}H]AA incorporation k^* in quinpirole-treated rats was significantly increased on both the lesioned and intact sides of the brain compared with vehicle-treated controls. Furthermore, absolute brain [^{3}H]AA incorporation was increased more on the side ipsilateral to the lesion compared with the contralateral side in these rats (Table 2). The pattern of the change was comparable to those observed after vehicle-infusion and in rats treated with SKF38393 in structures of the basal ganglia circuit. There were other notable differences in regional brain [^{3}H]AA incorporation between rats treated with quinpirole and rats that had received only vehicle, as with what was seen in lesioned rats treated with SKF38393 (Table 2; Figs. 1 and 3). Specifically, the absolute increases in regional [^{3}H]AA incorporation on the lesioned side were significantly greater in quinpirole-treated rats than in corresponding regions of vehicle-treated rats in which [^{3}H]AA incorporation was increased compared with the intact side (Table 2). D_2 dopamine receptor stimulation with quinpirole also increased [^{3}H]AA incorporation in layers of motor cortex and somatosensory cortex that were not affected by vehicle treatment. This outcome was similar to that seen in SKF38393-treated rats. Moreover, stimulating D_2 dopamine receptors with quinpirole increased absolute [^{3}H]AA incorporation in brain regions that were not affected by SKF38393. This was seen in distinct regions of the caudate putamen, thalamus, midbrain, and the substantia nigra pars reticulata.

We also examined the effects of quinpirole on regional

TABLE 1

Effects of D_1-like dopamine receptor stimulation on regional incorporation (k^*)[a] of [^{3}H]arachidonic acid in rats having a unilateral 6-OHDA lesion of the substantia nigra pars compacta

Brain Region	Side	Treatment			
		Vehicle k^*	SKF38393 k^*	Butaclamol k^*	SKF38393 + Butaclamol k^*
Motor cortex					
Layer I	Intact	6.34 ± 0.47[b]	7.38 ± 0.52	8.37 ± 0.72†	8.52 ± 0.37‡
	Lesioned	6.48 ± 0.53	8.39 ± 0.67**,†	8.90 ± 1.00†	9.52 ± 0.39**,‡
Layer II–III	Intact	6.30 ± 0.56	7.70 ± 0.62	8.44 ± 0.90†	8.88 ± 0.44‡
	Lesioned	6.47 ± 0.58*	8.62 ± 0.71**,†	8.88 ± 0.94**,†	9.79 ± 0.47**,‡
Layer IV	Intact	6.56 ± 0.55	8.07 ± 0.66	9.25 ± 1.11†	9.66 ± 0.55‡
	Lesioned	6.81 ± 0.65	9.22 ± 0.81**,†	9.71 ± 1.13**,†	10.59 ± 0.50**,‡
Layer V	Intact	6.09 ± 0.52	7.45 ± 0.60	8.22 ± 0.98	8.48 ± 0.37‡
	Lesioned	6.23 ± 0.53	8.29 ± 0.70**,†	8.55 ± 1.08	9.36 ± 0.36**,‡
Layer VI	Intact	5.17 ± 0.37	6.28 ± 0.50	6.58 ± 0.81	6.87 ± 0.29‡
	Lesioned	5.08 ± 0.33	6.82 ± 0.54**,†	6.83 ± 0.94	7.50 ± 0.21**,‡
Average motor cortex	Intact	5.81 ± 0.58	7.34 ± 0.74	8.24 ± 0.88†	8.25 ± 0.39‡
	Lesioned	6.04 ± 0.65	8.22 ± 0.85**,†	8.77 ± 1.02*,†	9.13 ± 0.38**,‡
Somatosensory cortex					
Layer I	Intact	6.84 ± 0.74	7.44 ± 0.47	9.29 ± 0.81†	9.20 ± 0.52‡
	Lesioned	7.08 ± 0.79*	7.83 ± 0.54*	9.12 ± 0.72†	9.62 ± 0.52**,‡
Layer II–III	Intact	7.06 ± 0.84	7.93 ± 0.55	9.43 ± 0.74†	9.57 ± 0.62‡
	Lesioned	7.35 ± 0.96	8.60 ± 0.65**	9.79 ± 0.83*,†	10.17 ± 0.63**,‡
Layer IV	Intact	7.59 ± 0.90	8.41 ± 0.61	10.48 ± 0.99†	10.32 ± 0.62‡
	Lesioned	7.99 ± 1.02*	9.23 ± 0.74**	10.66 ± 1.01†	11.01 ± 0.65**,‡
Layer V	Intact	6.62 ± 0.70	7.34 ± 0.47	9.00 ± 0.93†	8.93 ± 0.49‡
	Lesioned	6.84 ± 0.74*	8.07 ± 0.54**	9.22 ± 0.94†	9.62 ± 0.50**,‡
Layer VI	Intact	5.33 ± 0.50	6.04 ± 0.32	7.16 ± 0.79	7.14 ± 0.43‡
	Lesioned	5.49 ± 0.49	6.59 ± 0.40**,†	7.18 ± 0.87	7.67 ± 0.38**,‡
Average somatosensory cortex	Intact	6.83 ± 0.92	7.59 ± 0.59	9.67 ± 0.90†	9.03 ± 0.58‡
	Lesioned	6.96 ± 0.94	7.93 ± 0.62*	9.67 ± 0.93†	9.74 ± 0.50**,‡
Nucleus accumbens	Intact	5.54 ± 0.70	5.97 ± 0.61	7.38 ± 0.62†	7.56 ± 0.36‡
	Lesioned	5.53 ± 0.69	6.06 ± 0.62*	7.26 ± 0.65†	7.42 ± 0.34‡
Anterior cingulate cortex	Intact	6.68 ± 0.77	7.63 ± 0.87	8.73 ± 0.84†	9.08 ± 0.45‡
	Lesioned	6.65 ± 0.81	7.96 ± 0.91**	8.93 ± 0.93†	9.47 ± 0.53*,‡
Caudate putamen-dorsal	Intact	5.13 ± 0.76	5.60 ± 0.52	6.45 ± 0.44†	6.47 ± 0.32‡
	Lesioned	5.34 ± 0.78*	6.08 ± 0.62*	6.95 ± 0.70	7.03 ± 0.40**,‡
Caudate putamen-ventral	Intact	5.37 ± 0.72	5.66 ± 0.48	7.47 ± 0.45‡	6.51 ± 0.44†
	Lesioned	5.57 ± 0.73	5.73 ± 0.52	7.86 ± 0.51*,‡	7.03 ± 0.50*,†
Caudate putamen-lateral	Intact	5.27 ± 0.76	5.94 ± 0.49	7.07 ± 0.47‡	6.63 ± 0.36‡
	Lesioned	5.57 ± 0.85*	6.17 ± 0.49**	7.41 ± 0.61†	7.24 ± 0.44**,‡
Caudate putamen-medial	Intact	5.43 ± 0.85	5.76 ± 0.50	6.96 ± 0.42‡	6.59 ± 0.35†
	Lesioned	5.61 ± 0.88*	5.90 ± 0.50	7.08 ± 0.58†	6.93 ± 0.41**,†
Globus pallidus	Intact	3.73 ± 0.38	3.79 ± 0.28	5.20 ± 0.28‡	4.44 ± 0.31
	Lesioned	4.06 ± 0.42**	4.16 ± 0.29**	5.53 ± 0.38‡	5.09 ± 0.30**,†
Entopeduncular nucleus	Intact	3.78 ± 0.48	3.56 ± 0.34	4.60 ± 0.49	4.21 ± 0.52
	Lesioned	3.98 ± 0.53*	4.03 ± 0.41**	4.81 ± 0.48**	4.62 ± 0.53**
Subthalamic nucleus	Intact	6.54 ± 0.71	6.92 ± 0.66	8.59 ± 0.74†	8.26 ± 0.43‡
	Lesioned	6.99 ± 0.86	8.44 ± 0.81**	9.00 ± 0.91	9.47 ± 0.63**,‡
Substantia nigra pars reticulata	Intact	4.32 ± 0.42	4.68 ± 0.44	5.84 ± 0.53†	5.70 ± 0.40†
	Lesioned	4.83 ± 0.43**	5.74 ± 0.56**	6.37 ± 0.50**,†	6.93 ± 0.58**,‡
Parafascicular nucleus	Intact	5.80 ± 0.95	5.93 ± 0.31	7.72 ± 1.06	7.68 ± 0.39‡
	Lesioned	6.45 ± 1.10*	6.99 ± 0.48**	8.24 ± 1.04**	8.42 ± 0.54*,‡
Ventroposterior thalamus	Intact	6.80 ± 0.83	6.66 ± 0.68	8.63 ± 0.93	8.60 ± 0.53†
	Lesioned	7.05 ± 0.89	6.94 ± 0.76	8.98 ± 1.00*	8.67 ± 0.58†
Ventromedial thalamus	Intact	5.96 ± 0.73	6.92 ± 0.73	8.58 ± 0.90†	7.55 ± 0.47†
	Lesioned	6.19 ± 0.80*	6.97 ± 0.75	9.04 ± 0.99**,†	7.92 ± 0.51*,†
Ventrolateral thalamus	Intact	6.05 ± 0.70	6.36 ± 0.53	8.79 ± 0.64‡	7.76 ± 0.55†
	Lesioned	6.40 ± 0.77*	6.31 ± 0.56	9.11 ± 0.68‡	8.06 ± 0.64†
Lateral habenular nucleus	Intact	7.79 ± 1.02	8.28 ± 0.74	10.78 ± 1.05†	9.65 ± 0.47‡
	Lesioned	7.83 ± 1.03	8.14 ± 0.68	10.77 ± 1.19†	9.51 ± 0.44‡
Pretectal area	Intact	6.14 ± 0.89	5.80 ± 0.38	7.44 ± 0.82	7.34 ± 0.47†
	Lesioned	6.19 ± 0.92	6.12 ± 0.38	7.93 ± 0.72*,†	8.09 ± 0.52**,‡
Deep layers of superior colliculus	Intact	5.09 ± 0.66	5.41 ± 0.39	6.83 ± 0.64†	6.54 ± 0.35‡
	Lesioned	5.12 ± 0.62	5.51 ± 0.47	7.05 ± 0.68†	6.48 ± 0.44†
Lateral midbrain reticular formation	Intact	3.81 ± 0.34	3.53 ± 0.15	5.51 ± 0.44‡	4.88 ± 0.19‡
	Lesioned	3.80 ± 0.35	3.70 ± 0.13*	5.73 ± 0.53‡	5.23 ± 0.30‡
Pedunculopontine nucleus	Intact	3.64 ± 0.33	3.83 ± 0.32	5.20 ± 0.49†	4.78 ± 0.27†
	Lesioned	3.86 ± 0.36*	3.90 ± 0.32	5.47 ± 0.53*,†	5.18 ± 0.35*,†

[a] $k^* = (\text{ml s}^{-1}\ \text{g}^{-1}) \times 10^4$.

[b] Values are mean ± S.E.M. of k^*; each region of interest was measured in sextuplicate in each of eight rats.

$^{*}p < 0.05$, $^{**}p < 0.01$ versus intact side by paired t test.

$^{\dagger}p < 0.05$, $^{\ddagger}p < 0.01$ versus vehicle by unpaired t test.

TABLE 2

Effects of D_2-like dopamine receptor stimulation on regional incorporation (k^*)[a] of [^{3}H]arachidonic acid in rats having a unilateral 6-OHDA lesion of the substantia nigra pars compacta

Brain Region	Side	Treatment			
		Vehicle k^*	Quinpirole	Butaclamol	Quinpirole + Butaclamol k^*
Motor cortex					
Layer I	Intact	6.34 ± 0.47[b]	9.37 ± 0.74‡	8.37 ± 0.72†	9.68 ± 0.60‡
	Lesioned	6.48 ± 0.53	10.47 ± 0.76**,‡	8.90 ± 1.00†	9.77 ± 0.60‡
Layer II–III	Intact	6.30 ± 0.56	9.25 ± 0.71‡	8.44 ± 0.90†	9.65 ± 0.59‡
	Lesioned	6.47 ± 0.58*	10.49 ± 0.76**,‡	8.88 ± 0.94**,†	9.87 ± 0.54‡
Layer IV	Intact	6.56 ± 0.55	10.02 ± 0.76‡	9.25 ± 1.11†	10.30 ± 0.62‡
	Lesioned	6.81 ± 0.65	11.46 ± 0.82**,‡	9.71 ± 1.13**,†	10.59 ± 0.55‡
Layer V	Intact	6.09 ± 0.52	8.97 ± 0.65‡	8.22 ± 0.98	9.06 ± 0.55‡
	Lesioned	6.23 ± 0.53	10.25 ± 0.68**,‡	8.55 ± 1.08	9.50 ± 0.57**,‡
Layer VI	Intact	5.17 ± 0.37	7.44 ± 0.55‡	6.58 ± 0.81	7.30 ± 0.40‡
	Lesioned	5.08 ± 0.33	8.42 ± 0.60**,‡	6.83 ± 0.94	7.43 ± 0.46‡
Average motor cortex	Intact	5.81 ± 0.58	9.09 ± 0.89‡	8.24 ± 0.88†	9.25 ± 0.57‡
	Lesioned	6.04 ± 0.65	10.31 ± 0.85**,‡	8.77 ± 1.02*,†	9.52 ± 0.56‡
Somatosensory cortex					
Layer I	Intact	6.84 ± 0.74	9.19 ± 0.80†	9.29 ± 0.81†	9.99 ± 0.52‡
	Lesioned	7.08 ± 0.79*	10.08 ± 0.69**,‡	9.12 ± 0.72†	10.53 ± 0.69*,‡
Layer II–III	Intact	7.06 ± 0.84	9.62 ± 0.87†	9.43 ± 0.74†	10.74 ± 0.63‡
	Lesioned	7.35 ± 0.96	10.40 ± 0.83**,‡	9.79 ± 0.83*,†	11.19 ± 0.73*,‡
Layer IV	Intact	7.59 ± 0.90	10.64 ± 0.86‡	10.48 ± 0.99†	11.86 ± 0.80‡
	Lesioned	7.99 ± 1.02*	11.71 ± 0.88**,‡	10.66 ± 1.01†	12.31 ± 0.78**,‡
Layer V	Intact	6.62 ± 0.70	9.07 ± 0.68‡	9.00 ± 0.93†	9.91 ± 0.65‡
	Lesioned	6.84 ± 0.74*	10.15 ± 0.67**,‡	9.22 ± 0.94†	10.21 ± 0.67*,‡
Layer VI	Intact	5.33 ± 0.50	7.19 ± 0.56†	7.16 ± 0.79	7.62 ± 0.51‡
	Lesioned	5.49 ± 0.49	8.10 ± 0.47**,‡	7.18 ± 0.87	7.77 ± 0.49‡
Average somatosensory cortex	Intact	6.83 ± 0.92	9.79 ± 0.88†	9.67 ± 0.90†	10.25 ± 0.61‡
	Lesioned	6.96 ± 0.94	10.62 ± 0.82**,‡	9.67 ± 0.93†	10.52 ± 0.61**,‡
Nucleus accumbens	Intact	5.54 ± 0.70	7.47 ± 0.64†	7.38 ± 0.62†	8.45 ± 0.56†
	Lesioned	5.53 ± 0.69	7.65 ± 0.65†	7.26 ± 0.65†	8.49 ± 0.60‡
Anterior cingulate cortex	Intact	6.68 ± 0.77	9.90 ± 0.77‡	8.73 ± 0.84†	10.07 ± 0.51‡
	Lesioned	6.65 ± 0.81	10.62 ± 0.87**,‡	8.93 ± 0.93†	10.33 ± 0.55*,‡
Caudate putamen-dorsal	Intact	5.13 ± 0.76	6.43 ± 0.57	6.45 ± 0.44†	7.46 ± 0.51‡
	Lesioned	5.34 ± 0.78*	7.56 ± 0.66**,†	6.95 ± 0.70	8.07 ± 0.46**,‡
Caudate putamen-ventral	Intact	5.37 ± 0.72	6.90 ± 0.63†	7.47 ± 0.45‡	8.40 ± 0.85‡
	Lesioned	5.57 ± 0.73	7.78 ± 0.74**,†	7.86 ± 0.51*,‡	8.60 ± 0.90†
Caudate putamen-lateral	Intact	5.27 ± 0.76	6.94 ± 0.59†	7.07 ± 0.47‡	7.78 ± 0.61‡
	Lesioned	5.57 ± 0.85*	8.39 ± 0.73**,‡	7.41 ± 0.61†	8.24 ± 0.58**,‡
Caudate putamen-medial	Intact	5.43 ± 0.85	6.64 ± 0.62	6.96 ± 0.42‡	8.02 ± 0.61‡
	Lesioned	5.61 ± 0.88*	7.41 ± 0.70**,†	7.08 ± 0.58†	8.18 ± 0.64‡
Globus pallidus	Intact	3.73 ± 0.38	4.37 ± 0.39	5.20 ± 0.28‡	5.65 ± 0.60†
	Lesioned	4.06 ± 0.42**	4.84 ± 0.41**	5.53 ± 0.38‡	6.17 ± 0.67*,†
Entopeduncular nucleus	Intact	3.78 ± 0.48	3.90 ± 0.43	4.60 ± 0.49	4.38 ± 0.26
	Lesioned	3.98 ± 0.53*	4.12 ± 0.40*	4.81 ± 0.48**	4.61 ± 0.29
Subthalamic nucleus	Intact	6.54 ± 0.71	9.41 ± 0.92†	8.59 ± 0.74†	9.39 ± 0.70‡
	Lesioned	6.99 ± 0.86	9.84 ± 0.91*,†	9.00 ± 0.91	9.89 ± 0.71**,‡
Substantia nigra pars reticulata	Intact	4.32 ± 0.42	5.88 ± 0.47†	5.84 ± 0.53†	6.36 ± 0.79†
	Lesioned	4.83 ± 0.43**	6.82 ± 0.68**,†	6.37 ± 0.50**,†	7.61 ± 0.92**,†
Parafascicular nucleus	Intact	5.80 ± 0.95	8.48 ± 0.91†	7.72 ± 1.06	8.35 ± 0.71‡
	Lesioned	6.45 ± 1.10*	9.26 ± 0.92**,†	8.24 ± 1.04**	8.79 ± 0.72**,†
Ventroposterior thalamus	Intact	6.80 ± 0.83	8.97 ± 0.85†	8.63 ± 0.93	9.12 ± 0.72†
	Lesioned	7.05 ± 0.89	9.49 ± 0.79*,†	8.98 ± 1.00*	9.20 ± 0.78†
Ventromedial thalamus	Intact	5.96 ± 0.73	7.54 ± 0.62†	8.58 ± 0.90†	8.63 ± 0.52‡
	Lesioned	6.19 ± 0.80*	7.65 ± 0.66	9.04 ± 0.99**,†	8.85 ± 0.51‡
Ventrolateral thalamus	Intact	6.05 ± 0.70	7.52 ± 0.70	8.79 ± 0.64‡	8.72 ± 0.45‡
	Lesioned	6.40 ± 0.77*	7.65 ± 0.70	9.11 ± 0.68‡	8.75 ± 0.48‡
Lateral habenular nucleus	Intact	7.79 ± 1.02	10.07 ± 0.95†	10.78 ± 1.05†	10.42 ± 0.74‡
	Lesioned	7.83 ± 1.03	10.08 ± 0.97	10.77 ± 1.19†	10.44 ± 0.76†
Pretectal area	Intact	6.14 ± 0.89	7.84 ± 0.56†	7.44 ± 0.82	8.09 ± 0.69†
	Lesioned	6.19 ± 0.92	8.05 ± 0.51‡	7.93 ± 0.72*,†	8.97 ± 0.85*,†
Deep layers of superior colliculus	Intact	5.09 ± 0.66	6.79 ± 0.41‡	6.83 ± 0.64†	7.32 ± 0.75†
	Lesioned	5.12 ± 0.62	6.83 ± 0.49†	7.05 ± 0.68†	7.53 ± 0.77†
Lateral midbrain reticular formation	Intact	3.81 ± 0.34	5.24 ± 0.35‡	5.51 ± 0.44‡	5.69 ± 0.76†
	Lesioned	3.80 ± 0.35	5.31 ± 0.28‡	5.73 ± 0.53‡	6.00 ± 0.74†
Pedunculopontine nucleus	Intact	3.64 ± 0.33	4.77 ± 0.18‡	5.20 ± 0.49†	5.50 ± 0.79
	Lesioned	3.86 ± 0.36*	5.11 ± 0.18**,‡	5.47 ± 0.53*,†	5.79 ± 0.79†

[a] $k^* = (\text{ml s}^{-1}\ \text{g}^{-1}) \times 10^4$.
[b] Values are mean ± S.E.M. of k^*; each region of interest was measured in sextuplicate in each of eight rats.
* $p < 0.05$, ** $p < 0.01$ versus intact side by paired t test.
† $p < 0.05$, ‡ $p < 0.01$ versus vehicle by unpaired t test.

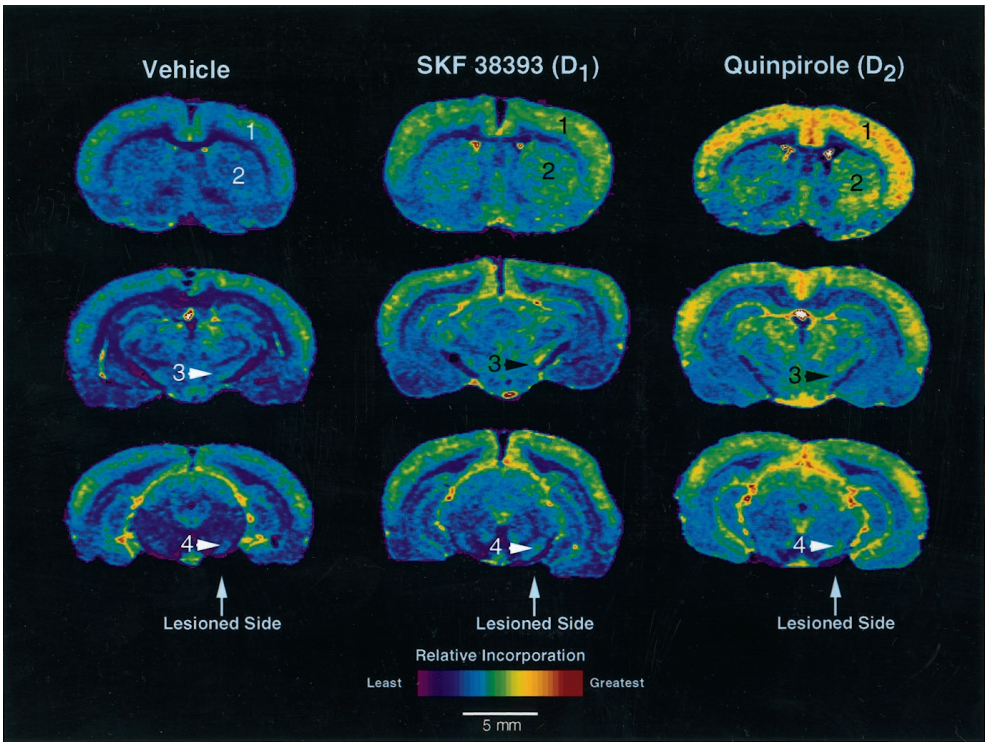

Fig. 1. Effects of dopamine receptor stimulation on regional of [^{3}H]arachidonic acid incorporation in rats having a unilateral 6-OHDA lesion in the substantia nigra pars compacta. Rats were treated as described under *Materials and Methods*. These are pseudocolored digital images of the actual autoradiographs. Warm tones (red-yellow) depict greatest amounts of [^{3}H]arachidonic acid incorporation. Note regions of increased incorporation on the side ipsilateral to the lesion (arrows) compared with the intact side in the cerebral cortex (1), caudate putamen (2), subthalamic nucleus (3), and substantia nigra pars reticulata (4). Scale bar, 5 mm.

brain [^{3}H]AA incorporation relative to changes that occurred in corresponding regions following vehicle (Fig. 3). The majority of brain regions on the side ipsilateral to the lesion had higher levels of [^{3}H]AA incorporation following quinpirole than following vehicle administration. As with SKF38393, the biggest effects of quinpirole were measured in defined layers of motor cortex. The magnitude of the relative regional responses to quinpirole appeared to be greater than with SKF38393. There were some noteworthy differences in regional brain [^{3}H]AA incorporation in quinpirole-treated rats compared with SKF38393-treated rats. In quinpirole-treated rats, [^{3}H]AA incorporation was significantly and robustly increased throughout the caudate putamen, in contrast to SKF38393-treated rats in which a significant effect was detected only in dorsal caudate putamen. Quinpirole did not significantly affect [^{3}H]AA incorporation in the subthalamic nucleus, in contrast to the robust increase in [^{3}H]AA incorporation caused by SKF38393 in this region. As was the case for SKF38393-treated rats, relative [^{3}H]AA incorporation was decreased in ventral thalamus of quinpirole-treated rats compared with vehicle-treated controls.

Effects of Dopamine Receptor Antagonism. Groups of rats were treated with (+)-butaclamol alone or pretreated with (+)-butaclamol in combination with either SKF38393 or quinpirole to ensure that effects seen following treatment with the dopamine agonists did, in fact, result from dopamine receptor stimulation. (+)-Butaclamol treatment alone increased absolute [^{3}H]AA incorporation in many brain regions, especially in somatosensory and motor regions of cerebral cortex. In the majority of brain regions affected by (+)-butaclamol, however, there was no significant difference between [^{3}H]AA incorporation on the lesioned side compared with the intact side. This is in sharp contrast to effects on regional [^{3}H]AA incorporation by the D_1 and D_2 dopamine agonists SKF38393 and quinpirole, respectively.

Butaclamol pretreatment did not inhibit the stimulatory effects of SKF38393 on regional brain [^{3}H]AA incorporation when examined either as absolute or relative effects of [^{3}H]AA incorporation. In fact, effects of (+)-butaclamol alone on regional absolute [^{3}H]AA incorporation were not significantly different than effects of (+)-butaclamol and SKF38393 in combination in corresponding brain regions (Table 1). For the most part, analyzing the data in terms of relative regional changes between intact and lesioned sides did not affect the outcome (Fig. 2). In contrast, (+)-butaclamol pretreatment effectively inhibited the stimulatory effects of the D_2 dopamine agonist quinpirole on relative [^{3}H]AA incorporation (Fig. 3).

Discussion

We have demonstrated widespread, yet specific, alterations in [^{3}H]AA incorporation in response to dopamine receptor activation in rats with a chronic unilateral 6-OHDA lesion of the substantia nigra pars compacta, an animal model of Parkinson's disease (Gerlach and Riederer, 1996). These dopamine agonist-induced effects on incorporation were robust, located in "circuit-relevant" brain loci, and revealed differential physiological responsivity of the brain to dopamine D_1 and D_2 receptor activation. Thus, a cardinal conclusion of these studies is the exquisite sensitivity of the fatty acid technique to reveal differential functional activation of brain circuits in this model.

SKF38393 and quinpirole are, respectively, selective dopamine D_1 and D_2 receptor agonists (Setler et al., 1978; Seeman et al., 1986; Andersen and Jansen, 1990; Levant et al., 1992). Both SKF38393 and quinpirole have been radiolabeled and their autoradiographic binding patterns correlate well with those of other well characterized D_1 and D_2 ligands (Dawson et al., 1986; Dubois et al., 1986; Charuchinda et al., 1987;

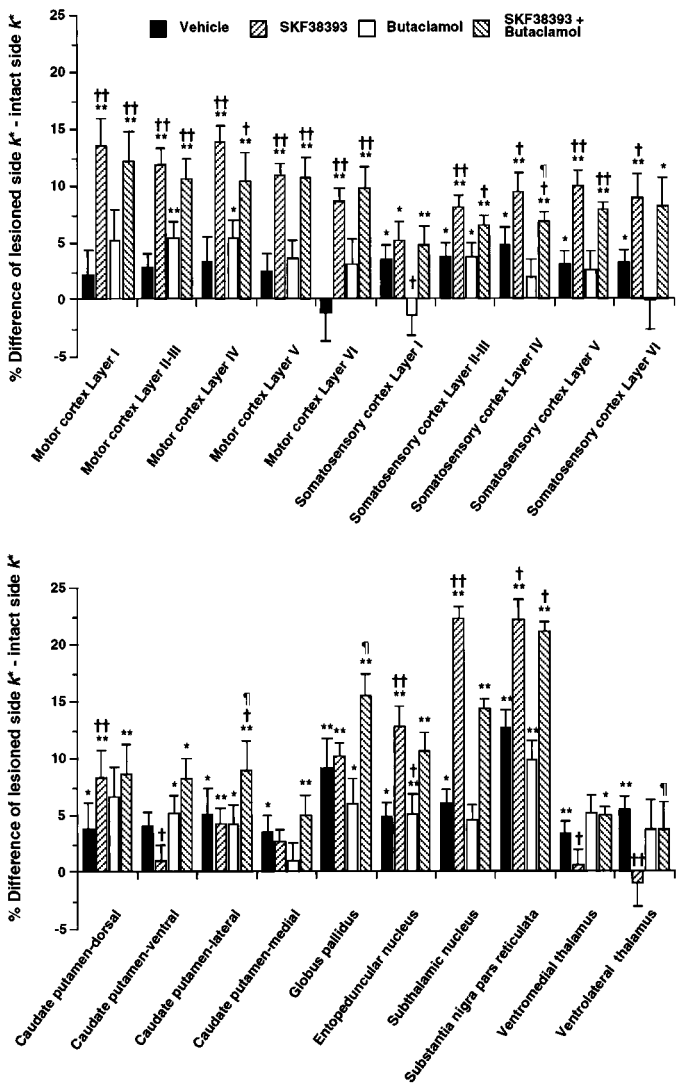

Fig. 2. Effects of D_1-like dopamine receptor stimulation on regional incorporation k^* [(ml s^{-1} g^{-1}) $\times$ 10^4] of [^{3}H]arachidonic acid in rats having a unilateral 6-OHDA lesion in the substantia nigra pars compacta. Rats were treated as described under *Materials and Methods* and each region of interest was measured in sextuplicate in each of eight rats. Values are mean ± S.E.M. of the percentage difference between the lesioned side k^* and the intact side k^*. $^*p < 0.05$ and $^{**}p < 0.01$ compared with zero difference by using an unpaired t test. $^{\dagger}p < 0.05$ and $^{\dagger\dagger}p < 0.01$ compared with the vehicle-treatment group using one-way ANOVA and Duncan's multiple range test. $^{\P}p < 0.05$ and $^{\P\P}p < 0.01$ compared with the SKF38393 treatment group using one-way ANOVA and Duncan's multiple range test.

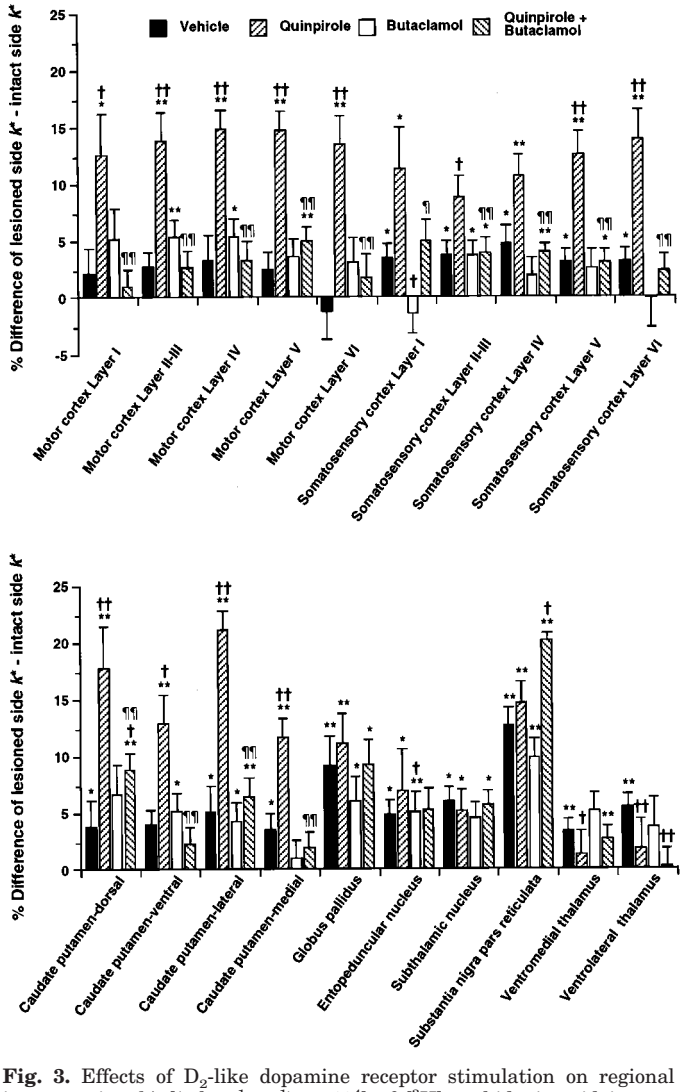

Fig. 3. Effects of D_2-like dopamine receptor stimulation on regional incorporation k^* [(ml s^{-1} g^{-1}) $\times$ 10^4] of [^{3}H]arachidonic acid in rats having a unilateral 6-OHDA lesion in the substantia nigra pars compacta. Rats were treated as described under *Materials and Methods* and each region of interest was measured in sextuplicate in each of eight rats. Values are mean ± S.E.M. of the percentage difference between the lesioned side k^* and the intact side k^*. $^*p < 0.05$ and $^{**}p < 0.01$ compared with zero difference by using an unpaired t test. $^{\dagger}p < 0.05$ and $^{\dagger\dagger}p < 0.01$ compared with the vehicle-treatment group using one-way ANOVA and Duncan's multiple range test. $^{\P}p < 0.05$ and $^{\P\P}p < 0.01$ compared with the quinpirole treatment group using one-way ANOVA and Duncan's multiple range test.

Wamsley et al., 1989; Levant et al., 1993), although [^{3}H]quinpirole also labels dopamine D_3 receptors (Gehlert et al., 1992; Levant et al., 1993). The patterns of SKF38393- and quinpirole-stimulated [^{3}H]AA incorporation in rats with unilateral 6-OHDA substantia nigra lesions correlated with regions known to avidly bind [^{3}H]SKF38393 and [^{3}H]quinpirole as reported by others in intact rats (caudate putamen, thalamus, globus pallidus, entopeduncular nucleus). In addition, [^{3}H]AA incorporation responses to SKF38393 and quinpirole on the side ipsilateral to the 6-OHDA lesion were increased relative to the contralateral side in a manner consistent with reported supersensitive effects in this model on other dopaminergic physiological parameters (for review, see Schwarting and Huston, 1996). In contrast, significant SKF38393- and quinpirole-stimulated [^{3}H]AA incorporation occurred in layers of somatosensory and motor cortex, brain regions where SKF38393 and quinpirole binding are relatively low. This result points to an essential difference between the fatty acid technique and receptor autoradiography, that is, the ability of pulse-labeling brain lipids with [^{3}H]AA to detect changes between resting and activated brain circuits involving PLA_2 signaling as opposed to up-regulated local receptor binding. In the 6-OHDA-lesioned rat, changes on the side ipsilateral to the lesion likely reflect the summation of altered PLA_2 activity in the basal ganglia circuit of other neurotransmitter-coded afferents such as those containing dynorphin, enkephalin, substance P, glutamic acid, and γ-aminobutyric acid (GABA) (Gerfen et al., 1991) in addition to those containing dopamine. Substance P, glutamic acid,

and GABA receptors may be coupled to PLA_2 activity. Changes could also reflect downstream effects on cholinergic or serotonergic neurons following stimulation of dopamine receptors. Indeed, it has been shown that PLA_2 is involved in mediating cholinergic and serotonergic activity (Felder et al., 1990; Claustre et al., 1991; DeGeorge et al., 1991). The opposite effect was also seen, that is, a lack of effect of either SKF38393 or quinpirole to stimulate [^{3}H]AA incorporation in the ventral caudate putamen and nucleus accumbens, brain regions in which levels of [^{3}H]SKF38393 and [^{3}H]quinpirole binding are high (Dubois et al., 1986; Levant et al., 1993). In contrast to the basal ganglia, those nuclei receive their dopaminergic afferents predominantly from the ventral tegmentum (Björklund and Lindvall, 1984) and thus may not develop supersensitivity to dopamine agonists. These results further demonstrate the specificity of the fatty acid incorporation technique.

We examined the effects of SKF38393 and quinpirole after (+)-butaclamol treatment to determine that effects of dopamine agonists on [^{3}H]AA incorporation were, in fact, due to dopamine receptor activation rather than nonspecific. (+)-Butaclamol antagonized the effects of quinpirole on [^{3}H]AA incorporation but not SKF38393. This result was unexpected because (+)-butaclamol is a potent nonspecific dopamine receptor antagonist (Miller et al., 1975; Seeman, 1981; Boyson et al., 1986). There are a number of possible explanations for the inability of (+)-butaclamol to antagonize SKF38393 in our study. The simplest interpretation is that the dose of SKF38393 was too high compared with that of (+)-butaclamol to obtain complete inhibition of the effects on [^{3}H]AA incorporation. The molar ratio of SKF38393 to (+)-butaclamol was ~18:1 in this study, whereas the molar ratio of quinpirole to (+)-butaclamol was ~5:1. Another simple explanation may be the pharmacokinetics of SKF38393 compared with quinpirole and (+)-butaclamol. SKF38393 and quinpirole were administered intravenously compared with (+)-butaclamol that was administered intraperitoneally. Similarly, the timing of the (+)-butaclamol pretreatment may have affected its effectiveness. A more intriguing explanation comes from evidence that (+)-butaclamol can act as an inverse agonist in cultured HEK-293 cells transfected with and expressing functional D_1 receptors (Tiberi and Caron, 1994). Perhaps this was the case in our unilateral substantia nigra 6-OHDA-lesioned rats in which D_1 receptors have been rendered supersensitive to SKF38393.

Although both SKF38393 and quinpirole fatty acid stimulated PLA_2 activity on the side ipsilateral to the 6-OHDA substantia nigra lesion, as evidenced by increased [^{3}H]AA incorporation, the [^{3}H]AA response for SKF38393 was different than that for quinpirole. In general, [^{3}H]AA incorporation in response to SKF38393 was less robust than to quinpirole. The simplest explanation for the different magnitudes of [^{3}H]AA responses to SKF38393 and quinpirole is that D_1 and D_2 receptor binding does not change similarly in response to 6-OHDA denervation. Most studies report sharply increased D_2 receptor binding in denervated neostriatum, whereas the effect on D_1 receptors is either small, decreased, or none (for review, see Schwarting and Huston, 1996). The different responses can be explained by the fact that D_2 dopamine receptors appear to be coupled to PLA_2 activation and arachidonic acid release, whereas D_1 receptors do not (Kanterman et al., 1991; Piomelli et al., 1991; Schinelli et al., 1994; Vial and Piomelli, 1995). Moreover, it has recently been shown that SKF38393 inhibits arachidonic acid release in rat striatum primary cultures, whereas quinpirole enhances it (Schinelli et al., 1994). Thus, the increased magnitude of [^{3}H]AA incorporation in caudate putamen in response to quinpirole compared with SKF38393 might be due to summing direct quinpirole effects on D_2 receptors as well as indirect consequences of the supersensitive basal ganglia circuit in contrast to SKF38393 having only indirect effects on [^{3}H]AA incorporation in caudate putamen.

There was a striking difference between the [^{3}H]AA responses to quinpirole and SKF38393 in the subthalamic nucleus. In this nucleus, D_1 receptor activation with SKF38393 potently stimulated [^{3}H]AA incorporation but quinpirole was without effect. Interestingly, there is only weak D_1 binding in human subthalamic nucleus (Augood et al., 2000). Increased activity in the subthalamic nucleus has been implicated in contributing to motor abnormalities secondary to Parkinson's disease (Goetz et al., 1993; Tseng et al., 2000). In a rat model of Parkinson's disease, lesion of the subthalamic nucleus reverses Parkinsonian-like symptoms, whereas in human patients suffering from Parkinson's disease the stimulation of this nucleus can improve akinesia (Bergman et al., 1990; Ceballos-Baumann et al., 1999; Krack et al., 1999). The fatty acid technique has been adapted for positron emission tomography using [1-^{11}C]arachidonic acid (Chang et al., 1997). In view of the present data, positron emission tomography studies using the fatty acid technique with labeled arachidonic acid could elucidate the "state" of the subthalamic nucleus in particular and of the basal ganglia in general in Parkinson's disease, and in this way help to monitor effects of surgical interventions or drug therapy.

A number of earlier studies examined effects of dopamine receptor agonists on brain energy metabolism in chronic unilateral substantia nigra 6-OHDA-lesioned rats using [^{14}C]2DG autoradiography. Initial studies examined effects of nonspecific dopaminergic agonists such as L-dopa, apomorphine, and amphetamine (Wooten and Collins, 1983; Trugman and Wooten, 1986). L-Dopa and apomorphine increased $rCMR_{glc}$ in structures on the side ipsilateral to the lesion, whereas the dopamine releaser amphetamine affected structures on the contralateral side. However, the effects of these drugs, as estimated by measurements of $rCMR_{glc}$, were relatively small and limited to entopeduncular nucleus, substantia nigra pars reticulata, defined portions of neostriatum, and somatosensory cortex. Subsequent studies applied pharmacological strategies to distinguish dopamine D_1 versus D_2 receptor effects and, in general, revealed increased $rCMR_{glc}$ on the side ipsilateral to the lesion; however, the results were inconsistent. Indeed, Palacios and Wiederhold (1985) reported stimulatory effects with the D_2 agonist LY-141,865, but documented no changes with SKF38393. In contrast, Trugman and Wooten (1987) and Trugman et al. (1989) reported increased $rCMR_{glc}$ in response to both D_2 and D_1 drugs, including SKF38393. One might account for this difference by the fact that in the latter studies, as well as in the present study, drugs were administered intravenously and immediately before radiotracer infusion, whereas in the former study drugs were administered intraperitoneally and 30 min before radiotracer.

Our studies build upon the earlier $rCMR_{glc}$ results. They provide a larger and more metabolically specific window for

viewing the extent to which the brain (basal ganglia circuit) is affected by the loss of nigro-striatal dopaminergic input. As presently conceived, the loss of striatal dopamine in Parkinson's disease results in hyperactivity by inhibitory basal ganglia output nuclei. This occurs as a consequence of combined effects on two brain pathways that ultimately converge on the substantia nigra/entopeduncular nucleus complex. In the so-called direct pathway, inhibitory GABAergic striatonigral and striatoentopeduncular neurons are controlled by stimulatory D_1 receptors. In the so-called indirect pathway, inhibitory striatopallidal GABAergic neurons are under the control of inhibitory D_2 receptors. Those neurons project inhibitory GABAergic afferents to the subthalamic nucleus that projects excitatory glutamatergic afferents to the substantia nigra/entopeduncular nucleus complex. The result is increased output from the substantia nigra/entopeduncular nucleus complex due to decreased activity in the inhibitory indirect pathway and increased output from the subthalamic nucleus (Tseng et al., 2000). The substantia nigra/entopeduncular nucleus complex sends inhibitory projections to the thalamus that in turn has excitatory afferents to the motor cortex that projects back onto the striatum (caudate putamen) as well as to the brain stem and spinal cord (Narabayashi et al., 1993). This relation is illustrated in Fig. 4.

Figure 4 also depicts the perturbation of the basal ganglia circuit with respect to [^{3}H]AA incorporation in response to lesioning the substantia nigra pars compacta with 6-OHDA and the [^{3}H]AA responses of the perturbed circuit to dopa-

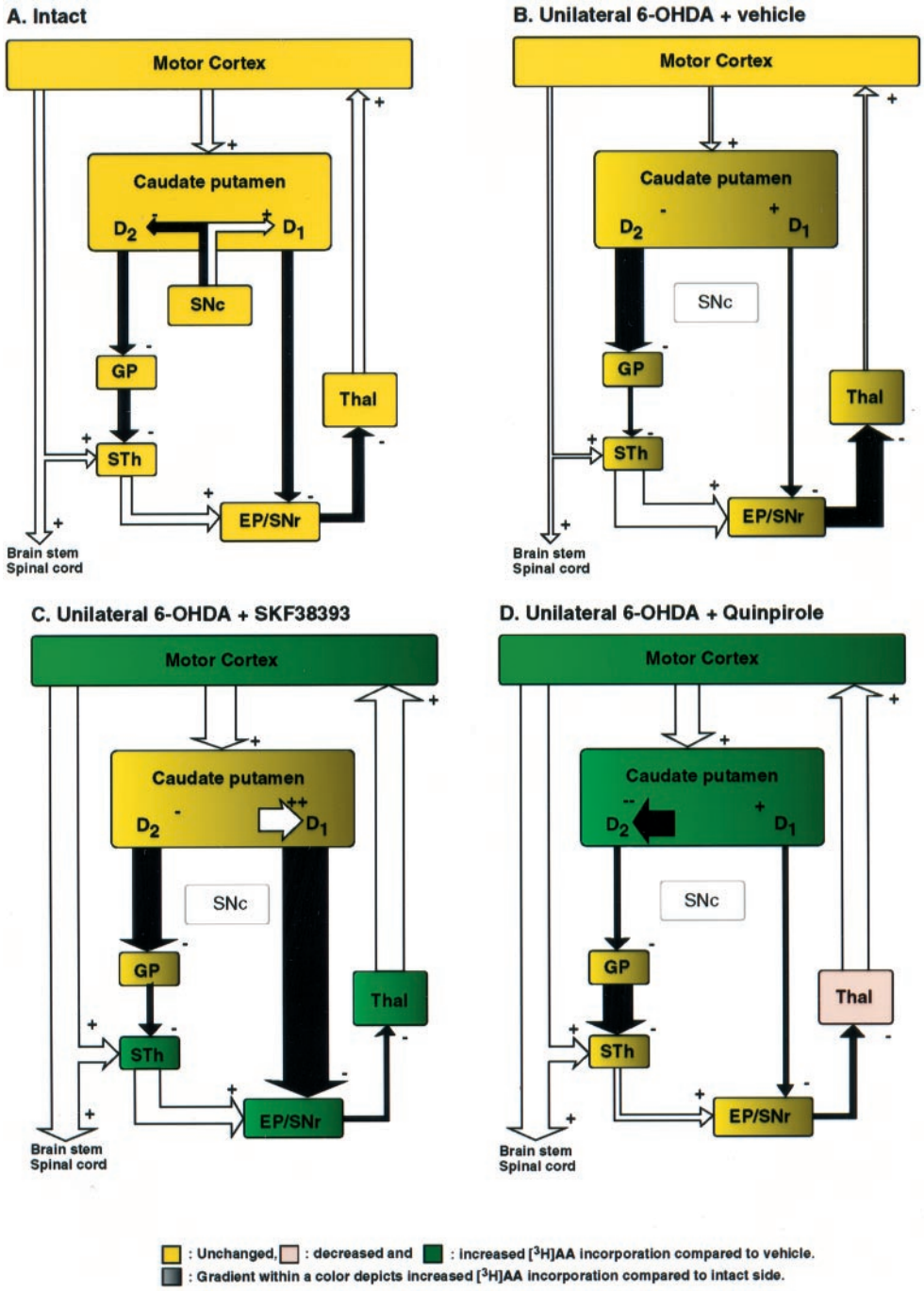

Fig. 4. States of brain metabolic activity within the thalamocortical circuit of the basal ganglia as revealed using [^{3}H]arachidonic acid incorporation. This figure is adapted from a figure appearing in Goetz et al. (1993). The present figure depicts stimulation of neurotransmitter receptors positively linked to phospholipase A_2 activity and, by extension, to functional brain activity. Open arrows depict excitatory pathways, solid arrows depict inhibitory pathways, and arrow width represents the direction of altered magnitude of functional activity within that pathway. Abbreviations: D_1, dopamine D_1-like receptors; D_2, dopamine D_2-like receptors; EP, entopeduncular nucleus; GP, globus pallidus; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; STh, subthalamic nucleus; Thal, thalamus.

mine D_1 and D_2 receptor stimulation. The diagrams not only serve to illustrate the extent to which the brain is affected by a localized lesion but also illustrate the extent to which affected circuitry is mediated by phospholipase A_2-coupled neurotransmitter receptors. The present results suggest that novel neurochemical pathways, in addition to dopamine receptors, could be exploited as putative targets to alleviate symptoms of Parkinson's disease. The fatty acid incorporation technique appears to be useful to investigate further direct and indirect effects of Parkinson's disease as well as other neurodegenerative pathologies, and may thus aid in developing new surgical and/or pharmacological therapies to relieve the neurological symptoms of these diseases.

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Send reprint requests to: Nathan M. Appel, Ph.D., Division of Treatment Research and Development, National Institute on Drug Abuse, National Institutes of Health, 6001 Executive Blvd., Room 4123, MSC 9551, Bethesda, MD 20892-9551. E-mail: an69k@nih.gov