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Knocking out the dopamine reuptake transporter (DAT) does not change the baseline brain arachidonic acid signal in the mouse

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Abstract

Background—Dopamine transporter (DAT) homozygous knockout (DAT^{-/-}) mice have a 10-fold higher extracellular DA concentration in the caudate-putamen and nucleus accumbens than do wildtype (DAT^{+/+}) mice, but show reduced presynaptic DA synthesis and fewer postsynaptic D₂ receptors. One aspect of neurotransmission involves DA binding to postsynaptic D₂-like receptors coupled to cytosolic phospholipase A₂ (cPLA₂), releasing second messenger arachidonic acid (AA) from synaptic membrane phospholipid. We hypothesized that tonic overactivation of D₂-like receptors in DAT^{-/-} mice due to elevated DA would not increase brain AA signaling, because of compensatory downregulation of postsynaptic signaling mechanisms.

Methods—[1-¹⁴C]AA was infused intravenously for 3 min in unanesthetized DAT^{+/+}, heterozygous (DAT^{+/-}) and DAT^{-/-} mice. AA incorporation coefficients k* and rates J_{in}, markers of AA metabolism and signaling, were imaged in 83 brain regions using quantitative autoradiography brain cPLA₂-IV activity also was measured.

Results—Neither k* nor J_{in} for AA in any brain region, or in brain cPLA₂-IV activity, differed significantly between DAT^{-/-}, DAT^{+/-} and DAT^{+/+} mice.

Conclusions—These results differ from reported increases in k* and J_{in} for AA, and brain cPLA₂ expression, in serotonin reuptake transporter (5-HTT) knockout mice, and suggest that postsynaptic dopaminergic neurotransmission mechanisms involving AA are downregulated despite elevated DA in DAT^{-/-} mice.

Keywords

arachidonic acid; phospholipase A₂; dopamine; dopamine transporter; knockout; mouse

INTRODUCTION

Dopaminergic (DA) D₂-like (D₂, D₃ and D₄) receptors in brain can be coupled *via* a Gα_o/i-protein to Ca²⁺-dependent cytosolic phospholipase (cPLA₂, EC 3.1.1.4), which when activated releases arachidonic acid (AA, 20:4n-6) from the stereospecifically numbered (*sn*)-2 position of synaptic membrane phospholipid [1, 2]. AA is an important second

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messenger in brain with multiple effects, and is a precursor of bioactive eicosanoids (e.g. prostaglandin E₂) [3].

Brain AA signaling and metabolism can be measured in unanesthetized rodents by infusing radiolabeled AA intravenously, integrating plasma radioactivity, and using quantitative autoradiography to determine regional brain radioactivity, which represents tracer AA incorporated in membrane phospholipid. A mathematical model is used to calculate AA incorporation coefficients and rates, k^* and J_{in} , respectively [3, 4]. Since the AA lost by metabolism cannot be synthesized *de novo* from 2-carbon fragments, or elongated significantly (< 1%) from its shorter chain polyunsaturated precursor, linoleic acid (18:2n-6) [5], k^* and J_{in} represent AA metabolic loss. The use of awake animals is critical for our studies, because brain fatty acid metabolism is inhibited by anesthesia [6].

Stimulating D₂-like receptors in unanesthetized rodents by acutely administering quinpirole (D₂-like receptor agonist), amphetamine (which reduces DA uptake by inducing loss of DAT activity, then increasing extracellular synaptic DA level [7]) or apomorphine (D₁/D₂ receptor agonist) produces a robust AA signal on neuroimaging [8–11]. The signal can be blocked by pre-administration of a D₂ receptor antagonist (e.g. butaclamol, raclopride) [8, 10], consistent with its mediation by D₂-like receptors [1]. Acute D₁-like receptor stimulation by SKF-38393 in unanesthetized rats does not produce an AA signal [8].

In contrast to the positive AA signal following acute amphetamine, chronic amphetamine followed by a 1-day washout decreases baseline AA incorporation into brain regions belonging to DA circuitry [12]. This difference suggests downregulated brain DA neurotransmission after chronic amphetamine, which is consistent with reduced striatal DA [13] and fewer striatal D₂ receptors in chronically treated rats [14].

DAT^{-/-} mice are another model in which D₂-like receptors are stimulated tonically throughout life, due to a 10-fold higher extracellular DA concentration in the caudateputamen and nucleus accumbens than in heterozygous DAT^{+/-} or wildtype DAT^{+/+} mice [15]. Compared with DAT^{+/+} and DAT^{+/-} mice, DAT^{-/-} mice are hyperactive and exhibit stereotypical behavior and persistent repetitive behavior in tests used to assess depressive behavior [16–18]. Although DAT^{-/-} mice commonly are described as “hyperdopaminergic”, many aspects of dopaminergic function in these mice reflect compensatory downregulation in response to the elevated extracellular DA level. The caudate-putamen and nucleus accumbens show reduced intracellular vesicular DA, reduced presynaptic DA synthesis and reuptake, and 50% fewer D₂ receptors compared with DAT^{+/+} mice [16, 19, 20].

In view of these adaptive neuroplastic changes, and of evidence that the baseline brain AA signal is decreased in rats given chronic amphetamine, which produces similar adaptive changes (see above), we hypothesized that the baseline AA signal would not be elevated in the DAT^{-/-} mouse, despite its 10-fold elevated extracellular brain DA concentration. To test this, we used our fatty acid method and model [4] to quantify regional brain incorporation coefficients k^* and rates J_{in} in unanesthetized DAT^{-/-}, DAT^{+/-} and DAT^{+/+} mice. We also measured brain cPLA₂-IV activity.

Methods

Chemicals

Radiolabeled [1-¹⁴C]AA in ethanol (53 mCi/mmol; 99.4% pure), purchased from Moravek Biomedicals (Brea, CA, USA), was evaporated and resuspended in HEPES buffer, pH 7.4, containing 50 mg/ml bovine serum albumin, as described [21, 22]. Tracer purity exceeded

99% by thin layer liquid chromatography and gas-liquid chromatography, and was measured after converting labeled AA to its methyl ester with 1% sulfuric acid in anhydrous methanol.

Animals

Experiments were conducted following the Guide for the Care and Use of Laboratory Animals (National Institute of Health Publication 86-23), under a protocol approved by the Animal Care and Use Committee of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development. Four- to 5-month-old male DAT^{+/-} (n = 8) and DAT^{-/-} (n = 7) mice and their DAT^{+/+} (n = 7) littermates, of a mixed C57BL/6J-129Sv genetic background [20], were maintained in an animal facility having constant temperature, humidity, and light cycle, with free access to water and food (Rodent NIH-07, Ziegler Bros, Gardens, PA, USA). The diet contained soybean, alfalfa, and fishmeal, and had 5% crude fat by weight. By gas-liquid chromatography, the diet contained (as % of total fatty acid) 30.6% saturated, 33.5% monounsaturated, 47.1% linoleic, 4.9% α -linolenic, 0.27% AA, 1.68% eicosapentaenoic, and 2.2% docosahexaenoic acid (Igarashi, M., unpublished data). The NIH diet has a low AA level as do other rodent chows (e.g., PicoLab® Rodent Diet 20 5053 and Purina 5001 chow, which contain 0.13% AA [23] and 0.39% [24], respectively, and 2018 Teklad Global 18% Protein, Harlan, which contains 0.03% AA) [25].

Surgical Procedures and Tracer Infusion

A mouse was anesthetized with halothane (2–3% v/v in O₂), and polyethylene PE 10 catheters were implanted into the right femoral artery and vein [21, 22]. After a 3–4 h recovery in a temperature-controlled and sound-dampened box, the mouse was infused for 3 min through the femoral vein with [1-¹⁴C]AA (45 μ l, 300 μ Ci/kg) at a rate of 15 μ l/min, using a Hamilton syringe and an infusion pump (Harvard Apparatus Model 22, Holliston, MA, USA). Ten (15–20 μ l) arterial blood samples were collected at fixed times during infusion (0, 0.25, 1.0, 1.5, 2.0, 2.8, 3.2, 5.0, 10, and 19 min). At 20 min, the mouse was killed by an overdose of Nembutal (50 mg/kg, i.v), then cardiac puncture was performed to rapidly collect 0.5 ml of blood and the mouse was decapitated. The brain was removed, quickly frozen in 2-methylbutane at –40°C, and stored at –80°C for later sectioning.

Chemical Analysis

Arterial blood samples were collected before, during and after [1-¹⁴C]AA infusion and were centrifuged (18,000 g for 30 s). Total lipids were extracted from 5 μ l plasma with chloroform:methanol (1 ml, 2:1, v/v) and KCl (0.5 ml, 0.1M), using a modified Folch procedure [26]. The lower organic phase (100 μ l) of the solution was used to measure the labeled unesterified AA concentration by scintillation spectrometry (Model 2200CA, Packard Instruments, Downers Grove, IL, USA). As reported, 95–98% of total plasma and brain radioactivity at 5 min was radiolabeled AA [22].

Concentrations of unesterified unlabeled fatty acids were determined in the arterial plasma (100–150 μ l) collected by cardiac puncture. Total lipids were extracted [26] and separated by thin layer chromatography on 60 silica gel plates (Whatman, Clifton, NJ USA), using the solvent system heptane:diethylether:glacial acetic acid (60:40:3, v/v/v). Unesterified fatty acids were scraped from the plate and methylated with 1% H₂SO₄ in anhydrous methanol (3 h at 70°C), then separated and quantified by gas-liquid chromatography using heptadecanoic acid (17:0) as an internal standard.

Quantitative Autoradiography and Calculations

Frozen brains were cut in serial 20- μ m thick coronal sections on a cryostat at –20°C, then placed for 4 weeks with calibrated [¹⁴C]methylmethacrylate standards (Amersham,

Arlington Heights, IL, USA) on X-ray film (Ektascan C/RA film, Eastman Kodak, Rochester, NY, USA). Radioactivity (nCi/g wet weight brain) in 83 identified regions [27] was measured bilaterally six times by quantitative densitometry, using the public domain NIH Image program 1.62. Regional brain AA incorporation coefficients from plasma into brain phospholipids, k^* (ml/s/g brain), were calculated as [4]:

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

where $c_{brain}^*(20 \text{ min})$ nCi/g is brain radioactivity 20 min after the onset of infusion; c_{plasma}^* nCi/ml is plasma unesterified AA radioactivity, and t is time after beginning infusion. The integral of plasma radioactivity ("input function" in denominator) was determined in each experiment by trapezoidal integration, and divided into brain radioactivity to calculate k^* for each experiment.

Regional rates of incorporation of unesterified AA from plasma into brain phospholipid, J_{in} (nmol/s/g), were calculated as:

$$J_{in} = k^* c_{plasma} \quad (\text{Eq. 2})$$

where c_{plasma} is the plasma concentration (nmol/ml) of unlabeled unesterified AA.

Brain cPLA₂-IV Activity

In a separate experiment, mice were anesthetized with Nembutal (50 mg/kg i.p.) and decapitated. Brains were removed quickly and frozen in 2-methylbutane at -40°C on dry ice, then stored at -80°C . Half-brains were homogenized using a Tenbroeck tissue grinder on ice (Kontes Glass Co., Vineland, NJ, USA) in 2 vol of cold buffer containing 10 mM HEPES, pH 7.5, 1 mM EDTA, 0.34 M sucrose and protease inhibitor cocktail tablet (Complete, Roche, Mannheim, Germany). Homogenates were centrifuged at 14,000 g for 20 min, then at 100,000 g for 1 h at 4°C (Beckman L8-M Ultracentrifuge, Fullerton, CA USA). Supernatants corresponding to the cytosolic fractions were assayed for cPLA₂-IV activity as described [28]. Protein concentrations of the cytosolic fraction were determined by the Bradford method [29].

Statistical Analysis

Data were analyzed using GraphPad Prism version 4.0b (GraphPad Software, San Diego, CA, USA) and are reported as mean $\pm$ SD. A one-way analysis of variance (ANOVA), followed by Dunnett's *post-hoc* comparisons, was used to compare mean body weight, arterial input function, cPLA₂-IV activity, plasma unesterified fatty acid concentrations, and k^* and J_{in} among the DAT genotypes. Statistical significance was taken as $p < 0.05$.

Results

Body Weight and Arterial Plasma Input Function

Mean body weight was significantly lower ($p < 0.05$; $n = 7$) in DAT^{-/-} than in DAT^{+/+} mice (22.3 ± 3.4 g and 28.9 ± 2.5 g, respectively) as previously reported [16]. Body weight equaled 29.1 ± 4.7 g ($n = 8$) in the DAT^{+/-} mice. A one-way ANOVA on the plasma input function (denominator of Eq. 1, integral of radioactivity in plasma organic fraction) revealed no statistically significant difference among the three genotypes ($p = 0.38$). Plasma input functions [(nCi $\times$ s)/ml] $\pm$ SD equaled: DAT^{+/+} ($n = 7$), 107096 ± 12896 ; DAT^{+/-} ($n = 8$), 99859 ± 27835 ; and DAT^{-/-} ($n = 7$), 134305 ± 80360 .

Plasma Concentrations of Unlabeled Unesterified Fatty Acids

As summarized in Table 1, there was no statistically significant difference in the mean concentration of any measured plasma unesterified fatty acid, including AA, among the DAT^{+/+}, DAT^{+/-} and DAT^{-/-} mice.

Regional Brain AA Incorporation Coefficients, k^*

Figure 1 presents color-coded coronal autoradiographs of brain slices from DAT^{+/+}, DAT^{+/-} and DAT^{-/-} mice, illustrating regional incorporation coefficients k^* for AA, obtained using Eq. 1. Qualitatively, the figure shows no regional difference among the three groups. Using such data, mean values of k^* were calculated for each of the 83 brain regions. A one-way ANOVA showed no statistically significant effect of either the partial or complete DAT deletion on mean baseline values of k^* for AA in any region. Because no significant difference was found, in Table 2, we present data only for representative regions most relevant to dopaminergic circuitry [30, 31], as well as for white matter (corpus callosum), whose values for k^* were less than for the gray matter regions.

Regional Incorporation Rates of Unlabeled Unesterified AA from Plasma into Brain

Rates of incorporation J_{in} of unlabeled unesterified AA from plasma into brain phospholipids were calculated by multiplying regional values of k^* by the unlabeled unesterified plasma AA concentration in each experiment (Eq. 2) (data not shown). Since the mean plasma unesterified AA concentration did not differ significantly among the three genotypes (Table 1), differences in J_{in} corresponded to differences in respective values of k^* (Table 2) and also did not differ significantly for any region. In the DAT^{+/+} mice, J_{in} (nmol/s/g) ranged from 46.8×10^{-4} in the corpus callosum to 247.1×10^{-4} in the choroid plexus. In the DAT^{-/-} mice, J_{in} ranged from 54.3×10^{-4} nmol/s/g to 231.6×10^{-4} nmol/s/g in the respective regions.

Whole-brain cPLA₂-IV Activity

As illustrated in Figure 2, whole brain cPLA₂-IV activity did not differ significantly among the three DAT genotypes.

Discussion

A 10-fold higher synaptic DA concentration in DAT^{-/-} compared with DAT^{+/+} or DAT^{+/-} mice, which would tonically stimulate D₂-like receptors coupled to cPLA₂ activation and AA hydrolysis from synaptic membrane phospholipid [1, 2, 8–11], did not produce any statistically significant changes in these measures. Thus, the lifetime deletion of the DAT gene did not significantly affect baseline values of k^* or J_{in} , markers of AA consumption and signaling, compared with values in wildtype mice, nor did it affect brain cPLA₂-IV activity. There also was no significant difference in the AA signal or cPLA₂-IV activity between DAT^{+/-} and DAT^{+/+} mice. These results suggest that baseline brain AA signaling involving cPLA₂ is maintained despite the marked lifelong perturbation in extracellular DA produced by DAT deletion, which would be expected to increase DA binding to D₂-like receptors.

Thus, there likely is a compensatory downregulation of signaling at different levels of postsynaptic neurotransmission involving D₂-like receptors, cPLA₂ and AA in DAT^{-/-} mice. Supporting this interpretation, densities of postsynaptic D₂ receptors and of the synaptic scaffolding protein PSD-95, a regulator of DA-mediated synaptic plasticity, are reduced by 50% in the striatum of DAT^{-/-} compared with DAT^{+/+} mice [16, 32]. However, DAT^{-/-} mice demonstrate an elevated high-affinity state (D₂^{High}) receptor density (80–90%) in the striatum [33] and increased D₃ mRNA in the caudate-putamen and nucleus

accumbens [34]. Despite the enhanced extracellular DA levels [15, 17], there is evidence for downregulation of presynaptic dopaminergic function, including reductions in tissue DA [17], in DA autoreceptor function [35], and in the tyrosine hydroxylase level [36]. DAT^{-/-} mice also show less electrically-evoked DA release in the striatum [16, 17].

The lack of change in baseline AA incorporation in DAT^{-/-} mice is consistent with the lack of change in cPLA₂-IV activity, which can be coupled to D₂-like receptors in the intact brain. Coupling has been evidenced by the concurrent elevation of the baseline AA signal and cPLA₂-IV activity in a rat model of Parkinson disease [37, 38], in rats chronically given fluoxetine, which increases the concentration of serotonin (5-HT) in the synaptic cleft [39], and in cyclooxygenase-2-deficient mice [21].

Extracellular concentrations of 5-HT [15] and acetylcholine [40] and basal glutamatergic N-methyl-D aspartate transmission [41] are unchanged in DAT^{-/-} mice, which suggest that alterations in these neurotransmitters did not influence their brain AA signal. DAT^{-/-} mice show changes in behavioral and neurochemical responses to fluoxetine, which increases their brain DA [15, 42], in contrast to the absence of either of these effects in wildtype mice. These changes may involve alterations in 5-HT_{2A} receptors, as a 5-HT_{2A} antagonist reverses some of them [43]. If this represents an enhancement of 5-HT_{2A}-mediated AA signaling in these mice, the observations in the present experiments result from a shift in signaling between dopaminergic and serotonergic mechanisms, the net result being no effect on overall signaling.

The impetus to these experiments was previous observations of decreased baseline k* for AA, following tonic D₂-receptor activation in rats given chronic amphetamine, which increases extrasynaptic DA [12]. However, chronic amphetamine also can reduce extracellular DA concentration after withdrawal [44], although not under all conditions [45]. Chronic amphetamine also reduces D₂ receptor densities [14] without altering the AA signal in response to quinpirole [12]. Together, the data in the present study and following chronic amphetamine suggest that postsynaptic DA signaling mechanisms, involving AA are dampened in response to chronic D₂-like receptor stimulation by DA. The effects likely depend on the specific conditions, chronicity, and duration. In DAT^{-/-} mice where neuroadaptation has been demonstrated at presynaptic and postsynaptic levels, there is no significant change in baseline AA signaling.

The baseline parameters of AA signaling (k* for AA, input function) observed in DAT^{+/+} mice are comparable to reported values in wildtype mice fed the same diet [21, 22]. We did not examine AA responses to the D₂-like receptor agonist quinpirole, since competition between administered quinpirole and the 10-fold elevated synaptic DA for D₂-like receptor occupancy (D₂-like receptors are already occupied by endogenous DA; both compounds are full agonists, and have equal affinities for D₂-like receptors [46, 47]) would limit comparisons between DAT^{+/+} and DAT^{-/-} mice and the interpretability of results [48]. Indeed, one way to interpret these data is that there is a substantial downregulation of postsynaptic AA signaling in DAT^{-/-} mice in response to the lifelong elevation in extracellular DA. This downregulation may be relevant to clinical conditions in which changes are thought to occur in dopaminergic systems, such as addiction [49], attention-deficit hyperactivity disorder [50], and obsessive-compulsive disorder [51].

In contrast to the unchanged baseline AA signal and cPLA₂-IV activity in DAT^{-/-} mice, baseline k* and J_{in} for AA and cPLA₂ activity are elevated in serotonin reuptake transporter (5-HTT)^{-/-} and 5-HTT^{+/-} compared with 5-HTT^{+/+} mice [22], consistent with the effects of fluoxetine noted above, and a 3- to 6-fold higher extracellular striatal 5-HT level in these mice [52]. The 5-HTT deficient mouse represents a positive control for the current study of

the DAT deficient mouse. Neuroadaptations of serotonergic systems in 5-HTT deficient mice (see [53] for review) include changes in presynaptic 5-HT function, but 5-HT_{2A/2C} receptors are unchanged except for a reduction of striatal 5-HT_{2A} [54]. Thus, postsynaptic AA signaling involving cPLA₂-coupled 5-HT_{2A/2C} receptors is not downregulated, but AA signaling coupled to D₂-like receptors is relatively downregulated (taking into account increased extracellular neurotransmitter release), in response to a tonic elevation of 5-HT vs. DA [22, 54, 55].

In summary, unanesthetized DAT^{-/-} mice showed no significant difference in regional values of baseline k^* and J_{in} for AA, measures of brain AA signaling and metabolism, or in brain cPLA₂-IV activity, compared with DAT^{+/-} or DAT^{+/+} mice, despite their 10-fold higher extracellular striatal DA level. The absence of differences may reflect dampening of postsynaptic DA signaling mechanisms, similar to that found after chronic amphetamine administration, but in this case in the context of elevated presynaptic DA function. These results contrast with upregulated k^* or J_{in} for AA and cPLA₂ activity in 5-HTT^{-/-} deficient mice having elevated brain 5-HT levels, and show that our *in vivo* AA imaging method can distinguish differences in neuroplastic responses to chronic changes in brain neurotransmitter levels. Similar differences might be tested for in humans having abnormalities in DAT or 5-HTT expression, when using our fatty acid model with positron emission tomography [56].

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Abbreviations

AA	arachidonic acid
cPLA₂	Ca ²⁺ -dependent cytosolic phospholipase A ₂
DA	dopamine
DAT	dopamine reuptake transporter
5-HT	serotonin
5-HTT	serotonin reuptake transporter

References

1. Vial D, Piomelli D. Dopamine D₂ receptors potentiate arachidonate release via activation of cytosolic, arachidonic-specific phospholipase A₂. *J Neurochem.* 1995; 64:2765–2772. [PubMed: 7760057]
2. Ong WY, Sandhya TL, Horrocks LA, Farooqui AA. Distribution of cytoplasmic phospholipase A₂ in the normal rat brain. *J Hirnforsch.* 1999; 39:391–400. [PubMed: 10536872]
3. Rapoport SI. Arachidonic acid and the brain. *J Nutr.* 2008; 138:2515–2520. [PubMed: 19022981]
4. 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; 17:187–214. [PubMed: 1467810]
5. Demar JC Jr, Ma K, Chang L, Bell JM, Rapoport SI. alpha-Linolenic acid does not contribute appreciably to docosahexaenoic acid within brain phospholipids of adult rats fed a diet enriched in docosahexaenoic acid. *J Neurochem.* 2005; 94:1063–1076. [PubMed: 16092947]

6. Contreras MA, Chang MC, Kirkby D, L, Bell JM, Rapoport SI. Reduced palmitate turnover in brain phospholipids of pentobarbital-anesthetized rats. *Neurochem Res.* 1999; 24:833–841. [PubMed: 10403622]
7. Saunders C, Ferrer JV, Shi L, Chen J, Merrill G, Lamb ME, Leeb-Lundberg LM, Carvelli L, Javitch JA, Galli A. Amphetamine-induced loss of human dopamine transporter activity: an internalization-dependent and cocaine-sensitive mechanism. *Proc Natl Acad Sci USA.* 2000; 97:6850–6855. [PubMed: 10823899]
8. 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; 180:735–742. [PubMed: 16163535]
9. 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; 26:1378–1388. [PubMed: 16511499]
10. Bhattacharjee AK, Chang L, White L, et al. Imaging apomorphine stimulation of brain arachidonic acid signaling via D₂-like receptors in unanesthetized rats. *Psychopharmacology (Berl).* 2008; 197:557–566. [PubMed: 18274730]
11. 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; 30:1064–1075. [PubMed: 15812572]
12. Bhattacharjee AK, Chang L, Chen M, White L, Bell JM, Bazinet RP, Rapoport SI. Chronic d-amphetamine depresses an imaging marker of arachidonic acid metabolism in rat brain. *Int J Neuropsychopharmacol.* 2008; 11:1–13.
13. Weiss F, Imperato A, Casu MA, Mascia MS, Gessa GL. Opposite effects of stress on dopamine release in the limbic system of drug-naïve and chronically amphetamine-treated rats. *Eur J Pharmacol.* 1997; 337:219–222. [PubMed: 9430417]
14. Robertson HA. Cerebral decortication reverses the effect of amphetamine on striatal D₂ dopamine binding site density. *Neurosci Lett.* 1986; 72:325–329. [PubMed: 2950342]
15. Shen HW, Hagino Y, Kobayashi H, Shinohara-Tanaka K, Ikeda K, Yamamoto H, Yamamoto T, Lesch KP, Murphy DL, Hall FS, Uhl GR, Sora I. Regional differences in extracellular dopamine and serotonin assessed by in vivo microdialysis in mice lacking dopamine and/or serotonin transporters. *Neuropsychopharmacology.* 2004; 29:1790–1799. [PubMed: 15226739]
16. Giros B, Jaber M, Jones SR, Wightman RM, Caron MG. Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter. *Nature.* 1996; 379:606–612. [PubMed: 8628395]
17. Jones SR, Gainetdinov RR, Jaber M, Giros B, Wightman RM, Caron MG. Profound neuronal plasticity in response to inactivation of the dopamine transporter. *Proc Natl Acad Sci USA.* 1998; 95:4029–4034. [PubMed: 9520487]
18. Perona MT, Waters S, Hall FS, Sora I, Lesch KP, Murphy DL, Caron M, Uhl GR. Animal models of depression in dopamine, serotonin, and norepinephrine transporter knockout mice: prominent effects of dopamine transporter deletions. *Behav Pharmacol.* 2008; 19:566–574. [PubMed: 18690111]
19. Gainetdinov RR, Jones SR, Caron MG. Functional hyperdopaminergia in dopamine transporter knock-out mice. *Biol Psychiatry.* 1999; 46:303–311. [PubMed: 10435196]
20. Sora I, Wichems C, Takahashi N, Li XF, Zeng Z, Revay R, Lesch KP, Murphy DL, Uhl GR. Cocaine reward models: conditioned place preference can be established in dopamine- and in serotonin-transporter knockout mice. *Proc Natl Acad Sci USA.* 1998; 95:7699–7704. [PubMed: 9636213]
21. Basselin M, Villacreses NE, Langenbach R, Ma K, Bell JM, Rapoport SI. Resting and arecoline-stimulated brain metabolism and signaling involving arachidonic acid are altered in the cyclooxygenase-2 knockout mice. *J Neurochem.* 2006; 96:669–679. [PubMed: 16405503]
22. Basselin M, Fox M, Chang L, Bell JM, Greenstein D, Chen M, Murphy DL, Rapoport SI. Imaging elevated brain arachidonic acid signaling in unanesthetized serotonin transporter (5-HTT)-deficient mice. *Neuropsychopharmacology.* 2009; 34:1695–1709. [PubMed: 19145225]

23. Basselin M, Rosa AO, Ramadan E, Cheon Y, Chang L, Chen M, Greenstein D, Wohltmann M, Turk J, Rapoport SI. Imaging decreased brain docosahexaenoic acid metabolism and signaling in iPLA₂β (VIA)-deficient mice. *J Lipid Res.* 2010; 51:3166–3173. [PubMed: 20686114]
24. Basselin M, Kim HW, Chen M, Ma K, Rapoport SI, Murphy RC, Farias SE. Lithium modifies brain arachidonic and docosahexaenoic metabolism in rat lipopolysaccharide model of neuroinflammation. *J Lipid Res.* 2010; 51:1049–1056. [PubMed: 20040630]
25. Basselin M, Ramadan E, Igarashi M, Chang L, Chen M, Kraft AD, Harry GJ, Rapoport SI. Imaging upregulated brain arachidonic acid metabolism in HIV-1 transgenic rats. *J Cereb Blood Flow Metab.* 2011; 31:486–493. [PubMed: 20664612]
26. 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; 226:497–509. [PubMed: 13428781]
27. Franklin, KBJ.; Paxinos, G. The mouse brain in stereotaxic coordinates. San Diego: Academic Press, Inc; 1997.
28. Yang HC, Mosior M, Johnson CA, Chang L, Chen M, Kraft AD, Harry GJ, Rapoport SI. Group-specific assays that distinguish between the four major types of mammalian phospholipase A₂. *Anal Biochem.* 1999; 269:278–288. [PubMed: 10221999]
29. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem.* 1976; 72:248–254. [PubMed: 942051]
30. 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 USA.* 1993; 90:8861–8865. [PubMed: 8415621]
31. Alexander GE, Crutcher MD, Delong MR. Basal ganglia-thalamocortical circuits: parallel substrates for motor, oculomotor, "prefrontal" and "limbic" functions. *Prog Brain Res.* 1990; 85:119–146. [PubMed: 2094891]
32. Yao WD, Gainetdinov RR, Arbuckle MI, Sotnikova TD, Cyr M, Beaulieu JM, Torres GE, Grant SG, Caron MG. Identification of PSD-95 as a regulator of dopamine-mediated synaptic and behavioral plasticity. *Neuron.* 2004; 41:625–638. [PubMed: 14980210]
33. Seeman P, Hall FS, Uhl G. Increased dopamine D₂High receptors in knockouts of the dopamine transporter and the vesicular monoamine transporter may contribute to spontaneous hyperactivity and dopamine supersensitivity. *Synapse.* 2007; 61:573–576. [PubMed: 17447256]
34. Fauchey V, Jaber M, Caron MG, Bloch B, Le Moine C. Differential regulation of the dopamine D₁, D₂ and D₃ receptor gene expression and changes in the phenotype of the striatal neurons in mice lacking the dopamine transporter. *Eur J Neurosci.* 2000; 2:19–26. [PubMed: 10651856]
35. Jones SR, Gainetdinov RR, Hu XT, Cooper DC, Wightman RM, White FJ, Caron MG. Loss of autoreceptor functions in mice lacking the dopamine transporter. *Nat Neurosci.* 1999; 2:649–655. [PubMed: 10404198]
36. Gainetdinov RR, Wetsel WC, Jones SR, Levin ED, Jaber M, Caron MG. Role of serotonin in the paradoxical calming effect of psychostimulants on hyperactivity. *Science.* 1999; 283:397–401. [PubMed: 9888856]
37. 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; 296:1074–1084. [PubMed: 11181943]
38. Bhattacharjee AK, Meister LM, Chang L, Bazinet RP, White L, Rapoport SI. In vivo imaging of disturbed pre- and post-synaptic dopaminergic signaling via arachidonic acid in a rat model of Parkinson's disease. *Neuroimage.* 2007; 37:1112–1121. [PubMed: 17681816]
39. Lee HJ, Rao JS, Ertley RN, Chang L, Rapoport SI, Bazinet RP. Chronic fluoxetine increases cytosolic phospholipase A₂ activity and arachidonic acid turnover in brain phospholipids of the unanesthetized rat. *Psychopharmacology (Berl).* 2007; 190:103–115. [PubMed: 17093977]
40. Parikh V, Apparsundaram S, Kozak R, Richards JB, Sarter M. Reduced expression and capacity of the striatal high-affinity choline transporter in hyperdopaminergic mice. *Neuroscience.* 2006; 141:379–389. [PubMed: 16675138]

41. Morice E, Billard JM, Denis C, Mathieu F, Betancur C, Epelbaum J, Giros B, Nosten-Bertrand M. Parallel loss of hippocampal LTD and cognitive flexibility in a genetic model of hyperdopaminergia. *Neuropsychopharmacology*. 2007; 32:2108–2116. [PubMed: 17342172]
42. Hall FS, Li XF, Sora I, Xu F, Caron MG, Lesch KP, Murphy DL, Uhl GR. Cocaine mechanisms: enhanced cocaine, fluoxetine and nisoxetine place preferences following monoamine transporter deletions. *Neuroscience*. 2002; 115:153–161. [PubMed: 12401330]
43. Barr AM, Lehmann-Masten V, Paulus M, Gainetdinov RR, Caron MG, Geyer MA. The selective serotonin-2A receptor antagonist M100907 reverses behavioral deficits in dopamine transporter knockout mice. *Neuropsychopharmacology*. 2004; 29:221–228. [PubMed: 14603268]
44. Rossetti ZL, Hmaidan Y, Gessa GL. Marked inhibition of mesolimbic dopamine release: a common feature of ethanol, morphine, cocaine and amphetamine abstinence in rats. *Eur J Pharmacol*. 1992; 221:227–234. [PubMed: 1426002]
45. Paulson PE, Robinson TE. Amphetamine-induced time-dependent sensitization of dopamine neurotransmission in the dorsal and ventral striatum: a microdialysis study in behaving rats. *Synapse*. 1995; 19:56–65. [PubMed: 7709344]
46. List SJ, Seeman P. [³H]dopamine labeling of D₃ dopaminergic sites in human, rat, and calf brain. *J Neurochem*. 1982; 39:1363–1373. [PubMed: 7119802]
47. Seeman P, Schaus JM. Dopamine receptors labelled by [³H]quinpirole. *Eur J Pharmacol*. 1991; 203:105–109. [PubMed: 1686763]
48. Seeman P, Tallerico T, Ko F, Tenn C, Kapur S. Amphetamine-sensitized animals show a marked increase in dopamine D₂ high receptors occupied by endogenous dopamine, even in the absence of acute challenges. *Synapse*. 2002; 46:235–239. [PubMed: 12373738]
49. Jones SR, Gainetdinov RR, Wightman RM, Caron MG. Mechanisms of amphetamine action revealed in mice lacking the dopamine transporter. *J Neurosci*. 1998; 18:1979–1986. [PubMed: 9482784]
50. Hesse S, Ballaschke O, Barthel H, Sabri O. Dopamine transporter imaging in adult patients with attention-deficit/hyperactivity disorder. *Psychiatry Res*. 2009; 171:120–128. [PubMed: 19176281]
51. Kim CH, Cheon KA, Koo MS, Ryu YH, Lee JD, Chang JW, Lee HS. Dopamine transporter density in the basal ganglia in obsessive-compulsive disorder, measured with [¹²³I]IPT SPECT before and after treatment with serotonin reuptake inhibitors. *Neuropsychobiology*. 2007; 55:156–162. [PubMed: 17657168]
52. Mathews TA, Fedele DE, Coppelli FM, Avila AM, Murphy DL, Andrews AM. Gene dose-dependent alterations in extraneuronal serotonin but not dopamine in mice with reduced serotonin transporter expression. *J Neurosci Methods*. 2004; 140:169–181. [PubMed: 15589347]
53. Sora I, Li B, Igari M, Hall FS, Ikeda K. Transgenic mice in the study of drug addiction and the effects of psychostimulant drugs. *Ann N Y Acad Sci*. 2010; 1187:218–246. [PubMed: 20201856]
54. Li Q, Wichems CH, Ma L, Van de Kar LD, Garcia F, Murphy DL. Brain region-specific alterations of 5-HT_{2A} and 5-HT_{2C} receptors in serotonin transporter knockout mice. *J Neurochem*. 2003; 84:1256–1265. [PubMed: 12614326]
55. Qu Y, Chang L, Klaff J, Balbo A, Rapoport SI. Imaging brain phospholipase A₂ activation in awake rat in response to 5-HT_{2A/2C} agonist, (+)-2,5-dimethoxy-4-iodophenyl-2-aminopropane (DOI). *Neuropsychopharmacology*. 2003; 28:244–252. [PubMed: 12589377]
56. 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; 45:1471–1479. [PubMed: 15347713]

Figure 1. Coronal autoradiographs of brain showing regional AA incorporation coefficients k^* in mice of different genotypes
 Values of k^* (ml/s/g brain $\times 10^{-4}$) are on a color scale from 6 (blue) to 20 (orange). Acg, anterior cingulate cortex; Cb, cerebellar gray matter; CPu, caudate putamen; Hipp, hippocampus; Mot, motor cortex.

Figure 2. Brain cPLA₂-IV activity in relation to DAT genotype

Data are mean $\pm$ SD (n = 5–7). No significant genotype effect was observed in the one-way ANOVA.

Table 1

Plasma unesterified fatty acid concentrations from DAT^{+/+}, DAT^{+/-} and DAT^{-/-} mice.

Fatty acids	DAT ^{+/+} (n = 7)	DAT ^{+/-} (n = 8)	DAT ^{-/-} (n = 7)
nmol/ml plasma			
Palmitate (16:0)	234.4 ± 40.9	182.6 ± 33.6	190.7 ± 46.1
Palmitoleate (16:1n-9)	26.7 ± 5.2	26.4 ± 6.5	24.8 ± 12.7
Stearate (18:0)	49.4 ± 14.8	41.8 ± 11.8	40.5 ± 11.6
Oleate (18:1n-9)	143.7 ± 38.7	146.9 ± 33.6	129.3 ± 36.3
Linoleate (18:2n-6)	207.6 ± 70.2	221.2 ± 63.1	182.8 ± 60.6
α-Linolenate (18:3n-3)	19.8 ± 7.8	17.9 ± 6.2	16.7 ± 6.7
Arachidonate (20:4n-6)	5.3 ± 1.4	6.8 ± 2.4	5.9 ± 1.7
Docosahexaenoate (22:6n-3)	30.7 ± 10.3	25.7 ± 11.3	24.7 ± 12.9

Values are mean ± SD.

Table 2

Selected regional brain arachidonic acid incorporation coefficients k^* in unanesthetized DAT^{+/+}, DAT^{+/-} and DAT^{-/-} mice

	DAT ^{+/+} (n = 7)	DAT ^{+/-} (n = 8)	DAT ^{-/-} (n = 7)
Brain Region	k* = (ml/s/g) × 10 ⁻⁴		
Gray matter			
Prefrontal cortex layer I	11.55 ± 1.39	13.48 ± 2.80	13.11 ± 4.29
Prefrontal cortex layer IV	12.58 ± 1.53	15.37 ± 3.56	15.11 ± 5.46
Frontal cortex (10)			
Layer I	11.72 ± 2.29	13.64 ± 3.80	13.31 ± 4.06
Layer IV	13.82 ± 2.64	15.08 ± 3.86	15.56 ± 4.96
Globus pallidus	11.62 ± 1.81	12.20 ± 2.76	12.09 ± 4.05
Nucleus Accumbens	12.54 ± 2.50	12.23 ± 3.21	13.11 ± 4.07
Ventral Striatum	12.20 ± 2.43	12.06 ± 3.39	12.71 ± 3.81
Posterior Striatum	12.54 ± 2.70	12.55 ± 3.39	13.35 ± 4.47
Subthalamic nucleus	16.54 ± 2.83	16.50 ± 3.68	17.03 ± 4.40
Substantia nigra	13.63 ± 2.54	12.47 ± 3.03	13.90 ± 3.42
Ventral tegmental area	13.22 ± 2.85	13.90 ± 3.88	13.48 ± 4.17
White matter			
Corpus callosum	8.85 ± 1.61	8.96 ± 2.36	9.21 ± 2.41

Values are mean ± SD.