The rat rotenone model reproduces the abnormal pattern of central catecholamine metabolism found in Parkinson’s disease

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Abbreviations; AS=alpha-synuclein; ALDH=aldehyde dehydrogenase; AR=aldehyde/aldose reductase; DOPA=3,4-dihydroxyphenylalanine; DA=dopamine; DAT=cell membrane DA transporter; DBH=dopamine-beta-hydroxylase; DHPG=3,4-dihydroxyphenylglycol; DOPAL=3,4-dihydroxyphenylacetaldehyde; DOPAC=3,4-dihydroxyphenylacetic acid; DOPEGAL=3,4-dihydroxyphenylglycolaldehyde; MAO=monoamine oxidase; NE=norepinephrine; NET=cell membrane norepinephrine transporter; PD=Parkinson’s disease; TH=tyrosine hydroxylase
ABSTRACT

Background: Recent reports indicate that Parkinson’s disease (PD) involves specific functional abnormalities in residual neurons—decreased vesicular sequestration of cytoplasmic catecholamines via the vesicular monoamine transporter (VMAT) and decreased aldehyde dehydrogenase (ALDH) activity. This double hit builds up the autotoxic metabolite 3,4-dihydroxyphenylacetaldehyde (DOPAL), the focus of the catecholaldehyde hypothesis for the pathogenesis of PD. An animal model is needed that reproduces this abnormal catecholamine neurochemical pattern.

Methods: Adult rats received subcutaneous vehicle or rotenone (2 mg/kg/day via a minipump) for 10 days. Locomotor activity was recorded and striatal tissue sampled for catechol contents and catechol ratios that indicate the above abnormalities.

Results: Compared to vehicle, rotenone reduced locomotor activity (p=0.002), decreased tissue dopamine concentrations (p=0.00001), reduced indices of vesicular sequestration (3,4-dihydroxyphenylacetic acid (DOPAC)/dopamine) and ALDH activity (DOPAC/DOPAL) (p=0.0025, p=0.036), and increased DOPAL levels (p=0.04).

Conclusions: The rat rotenone model involves functional abnormalities in catecholaminergic neurons that replicate the pattern found in PD putamen. These include a vesicular storage defect, decreased ALDH activity, and DOPAL buildup. The rat rotenone model provides a suitable in vivo platform for studying the catecholaldehyde hypothesis.

Keywords: Rotenone; Parkinson's disease; DOPAL; vesicular uptake; aldehyde dehydrogenase; dopamine; catecholamine; norepinephrine; catechol; Lewy body diseases
INTRODUCTION

Parkinson’s disease (PD) is the second most common aging-related neurodegenerative disease. The movement disorder in PD is well known to result from profound striatal dopamine deficiency. The extent of catecholamine depletion in PD, however, is far greater than can be accounted for by loss of innervation alone (Goldstein et al., 2017). The discrepancy suggests that there are populations of dysfunctional catecholaminergic neurons that are “sick but not dead” and therefore might be salvageable (Goldstein et al., 2019). Bases for the selective vulnerability of dopaminergic neurons are incompletely understood. Studies have pointed to a double hit of reduced sequestration of cytoplasmic dopamine into vesicles via the vesicular monoamine transporter (VMAT) and reduced activity of aldehyde dehydrogenase (ALDH) (Figure 1), which together result in accumulation of the autotoxic catecholaldehyde 3,4-dihydroxyphenylacetaldehyde (DOPAL). DOPAL is the centerpiece of the catecholaldehyde hypothesis for the pathogenesis of PD (Masato et al., 2019). The catecholaldehyde not only is neurotoxic by itself but also interacts strongly with intracellular proteins including alpha-synuclein (Jinsmaa et al., 2020b, Burke et al., 2008), a key component in Lewy bodies and a focus of current research about PD pathogenetic mechanisms.

Post-mortem neurochemical analyses of putamen tissue from PD patients have supported the catecholaldehyde paradigm by documenting the double hit and DOPAL accumulation. The mean DOPAL/dopamine ratio in PD is about 5 times that in controls, and DOPAL buildup is determined by decreased vesicular sequestration and decreased ALDH activity (Goldstein et al., 2013). In humans, neuroimaging kinetic studies with 18F-DOPA positron emission tomographic scanning show increased washout of the tracer, reflecting reduced VMAT activity (Goldstein et al., 2008), and cerebrospinal fluid catechol analyses revealed decreased 3,4-dihydroxyphenylacetic acid levels without a concurrent decrease in 5-S-cysteinyldopamine (Goldstein et al., 2016), consistent with reduced ALDH activity. These findings provide in vivo support for the occurrence of the double hit in patients with PD.
If the deleterious neurochemical abnormalities resulting from the double hit could be modulated, this might slow the progression of the neurodegenerative process. Therefore, translational research based on the catecholaldehyde hypothesis requires an animal model that replicates the distinctive neurochemical pattern found in PD.

Parts of the double hit have been used in PD mouse genetic models of low VMAT activity (Miller et al., 2001, Bohnen et al., 2006, Miller et al., 1999, Okamura et al., 2010, Tong et al., 2011) or ALDH knockout mice (Wey et al., 2012, Galter et al., 2003, Grunblatt et al., 2010, Mandel et al., 2005, Molochnikov et al., 2012, Werner et al., 2008), but no animal model to date has been reported that demonstrates DOPAL accumulation concurrently with evidence for the double hit.

We chose the rotenone rat model for this purpose. Rotenone is a mitochondrial complex 1 inhibitor that in rat pheochromocytoma PC-12 cells produces both components of the double hit and increases endogenous DOPAL levels (Goldstein et al., 2015). Moreover, DOPAL contributes to rotenone-induced cytotoxicity in this model (Lamensdorf et al., 2000). Whether rotenone produces this pattern in vivo has been unknown. The rat-rotenone model is a well established and characterized animal model of PD (Betarbet et al., 2000, Cannon et al., 2009). Rotenone produces motor dysfunction without extensive destruction of dopaminergic neurons, as documented by detailed neuropathological data in this model (Cannon et al., 2009). The occurrence of neurobehavioral abnormalities that are not accounted for by extensive neuronal loss fits with the sick but not dead phenomenon (Goldstein et al., 2019).

The primary objective of this study was to evaluate the central catecholaminergic profile in the rat rotenone model. We also included representative locomotor tests to ascertain that the model replicates the PD-like locomotor abnormalities that have been reported previously. We did not perform neuropathologic analysis in the experimental design, since that has already been done in detail (Cannon et al., 2009).

In this study rats received rotenone or sham treatment via subcutaneous reservoir minipumps. Based on the concepts depicted in Figure 1 we hypothesized that rotenone would build up striatal DOPAL with respect to dopamine and decrease values for neurochemical indices of vesicular sequestration and ALDH activity. If there were decreased vesicular sequestration of cytoplasmic catecholamines, tissue dopamine and norepinephrine
would be depleted with respect to their deaminated metabolites DOPAL, 3,4-dihydroxyphenylglycol (DHPG), and 3,4-dihydroxyphenylacetic acid (DOPAC). If there were decreased ALDH activity, ratios of DOPAC/DOPAL would be decreased.

Regarding the locomotor assessments, we chose tests known to represent the movement abnormalities in this rat model. The tests we performed were used in the original studies that introduced the model (Betarbet et al., 2000, Cannon et al., 2009). The cylinder test (rearing test) and open field test tests are well established (Hua et al., 2002, Rial et al., 2014, Schaar et al., 2010, Su et al., 2018) and allow evaluation of the locomotor abnormalities in this model.

METHODS

The animal research procedures in this study were approved by the Animal Care Committee of the Chaim Sheba Medical Center, Tel Hashomer, Israel, where the animal experiments took place. The neurochemical assays were done in the laboratory of the Autonomic Medicine Section in the Division of Intramural Research of the National Institute of Neurological Disorders and Stroke at the National Institutes of Health in Bethesda, Maryland, USA.

Animals: Sprague-Dawley male rats (200 ± 20 g, 10 weeks old) were obtained from Harlan Laboratories (Jerusalem, Israel). The rats acclimated for at least 3 days before surgery (minipump implantation) and experiments. Postoperatively the rats were housed separately in cages in an animal care facility at 22 °C with a 14-hour light (6:00–20:00) and 10-hour dark (20:00–6:00) cycle, with free access to food and water.

Materials: Dimethyl sulfoxide (DMSO) and polyethylene glycol (PEG) were mixed in a 1:1 ratio and used as the vehicle for rotenone. Rotenone was emulsified in a 5.6 mg/mL of the DMSO/PEG mixture and injected into an Alzet minipump until the pump was full. For vehicle-treated rats, the 1:1 DMSO/PEG mixture alone was used. Pumps were prepared to release rotenone at a rate of 2 mg/kg/day. This dose was selected after a pilot experiment to determine the optimal dosage regimen.
**Study protocol:** For minipump insertion each rat was anesthetized using 3% isoflurane and placed in a small animal stereotaxic frame. An Alzet minipump was implanted subcutaneously at the dorsum of the neck. In this model the parkinsonian behavioral phenotype is evident by several days of treatment (Cannon et al., 2009); therefore, the duration of the study was 10 days. Rats were stratified into 2 groups: vehicle-treated (Group 1, N=8), rotenone-treated (Group 2, N=11). In Group 1 the minipump contained only the vehicle, in Groups 2 and 3 the minipump contained rotenone emulsified in the vehicle. On day 10 the animals were euthanized by 3% isoflurane overdose. Tissue harvesting began immediately after sacrifice. The brain was removed and dissected, and the striatum was explored and the central portion was sampled. Approximately 3 x 3 x 3 mm tissue was taken and immediately placed on ice, transferred to a plastic cryotube, frozen in liquid nitrogen, and stored at -80 °C or in dry ice until assayed.

**Motor function testing:** Motor function tests were carried out on days 0, 1, and 9. For measuring rearing behavior and spontaneous exploration a modified cylinder test (Hua et al., 2002, Schaar et al., 2010) was used and rats were placed individually in a rectangular plastic box (30 x 34 x 16 cm length x width x height). Each rat was allowed free exploration within the apparatus while being videotaped for 2 minutes. Total rearing number was the number of times the animal was leaning on its hind legs and dethatching its forelegs. Change in activity time was the amount of time in seconds that the animal was moving at baseline minus the total amount of time the animal was moving on day 1 or 9.

**Neurochemical assays:** The neurochemical assays were done by personnel who were blinded as to the treatment group until the assay results were recorded. Freshly thawed tissue was homogenized in 20% 0.2 M phosphoric acid:80% 0.2 M acetic acid in a fume hood. Aliquots of the supernate were assayed for catechol contents by high performance liquid chromatography as described previously (Goldstein et al., 2013). Briefly, the supernate subjected to alumina extraction followed by reverse phase, ion pairing liquid chromatography with series electrochemical detection. There were 3 electrodes in series, with the first set at an oxidizing potential and the third at a reducing potential. Signals from the third potential were recorded, providing a
measure of reversibly oxidized species. Norepinephrine, dopamine, DOPAC, DHPG, DOPAL, and DOPA were assayed simultaneously. Tissue concentrations were expressed in pmol/mg wet weight.

**Statistics:** Motor function data were analyzed using factorial analyses of variance with Tukey’s post-hoc test. For comparing results of biochemical analyses between the groups, independent-means t-tests were conducted on log-transformed data. Data were tabulated as mean values ± SEM. Statistical significance was defined by a p-value < 0.05.

**RESULTS**

**Motor function measures:** Rotenone exerted clear effects on the locomotor indices as follows. At baseline and on day 1 there were no differences in the number of rearings/2 minutes between the vehicle- and rotenone-treated groups (baseline: 7.3±1.1 for vehicle, 9.7±0.8 for rotenone, p=0.17; day 1: 6.2±1.3, and 7.6±1.1, p=0.66), whereas on day 9 the rotenone-treated group reared significantly fewer times than did the vehicle-treated group (1.8±0.5 vs. 5.2±1.0; p=0.003) (Figure 2). The groups did not differ in the change in activity time on day 1 (-6.6±4.4 vs. -9.1±7.0, p=0.92), whereas on day 9 the rotenone-treated group had significantly reduced activity compared to the vehicle-treated group (-62.3±8.8 and -3.0±8.8 s, p=0.002) (Figure 3).

**Neurochemical measures:** Rotenone evoked several clear effects on tissue levels of catechols. Compared to the vehicle-treated group the rotenone-treated group had an 82% reduction in tissue dopamine levels (p=0.0001), whereas its deaminated metabolites were increased – 1.69 fold for DOPAL (p=0.04) and 1.34 fold for DHPG (p=0.04) (Table 1). The precursor of DA, DOPA, increased by 2.68 fold (p=0.0005). The groups did not differ in tissue norepinephrine or DOPAC (Table 1). The mean DA/DOPA ratio, an index of LAAAD, was also decreased in the rotenone-treated group (P=0.0005) (Table 2). Compared to the vehicle-treated group, in the rotenone-treated group indices of VMAT activity were all significantly decreased: the mean ratio of DA to the sum of its deaminated metabolites (P=0.0003), DA/DOPAL ratio (p=0.0025), DA/DOPAC ratio (p=0.035),
DISCUSSION

According to the catecholaldehyde hypothesis a double hit of reduced vesicular sequestration of cytoplasmic dopamine and reduced ALDH activity results in the accumulation of endogenous DOPAL, with multiple deleterious downstream effects. Tissue neurochemistry and neuroimaging data from patients with PD show a distinctive catecholaminergic pattern that fits with the catecholaldehyde hypothesis. Here we report that systemic administration of rotenone to rats depletes dopamine levels and decreases values for indices of vesicular sequestration and ALDH activity while increasing DOPAL levels. This is the first report of an animal model that does so. The body of evidence about DOPAL toxicity is based on in vitro studies—hence the usefulness of an in vivo model that reproduces DOPAL accumulation as well as the double hit.

DOPAL is gaining attention as a factor in neuronal toxicity in general and in particular through its interactions with alpha-synuclein, a central theme in PD pathogenesis (Masato et al., 2019). In vitro experiments show that DOPAL oligomerizes, aggregates, and forms quinone-protein adducts with alpha-synuclein, likely converting the protein to toxic forms. DOPAL directly produces toxic effects on mitochondrial functions, and DOPAL-induced alpha-synuclein oligomers impede synaptic vesicular functions (Plotegher et al., 2017), potentially setting the stage for pathogenic vicious cycles.

It is important for this emerging field of neurochemistry-oriented research to establish an in vivo model that demonstrates both the catecholaminergic and neurobehavioral phenotypic features of the disease. There are several animal models for PD. Genetics-based approaches include generation of transgenic animals and altered gene expression via viral vector are used to highlight genetic aspects of PD. Some models are based on catecholaminergic neurotoxicity evoked by chemical compounds such as MPTP and 6-hydroxydopamine, which produce both dopamine depletion and parkinsonian locomotor abnormalities. Some models induce profound dopamine depletion by direct destruction of the striatum. The rotenone model presented here, however, is particularly suitable for applying the catecholaldehyde hypothesis, according to which the dopamine
depletion and neurobehavioral phenotype are related to abnormal catecholamine metabolism in extant neurons. This model is not associated with extensive neuronal destruction.

It is well established that systemic rotenone administration evokes an “energy crisis” that depletes brain dopamine. The issue at hand is the link between these abnormalities. Rotenone produces metabolic stress in all cells of the body. How does it produce a relatively specific syndrome that involves dopamine depletion and consequently a parkinsonian movement disorder? According to the catecholaldehyde hypothesis, DOPAL is the missing link. Mitochondrial complex 1 inhibition induced by rotenone decreases availability of NAD+, which is a required co-factor for detoxifying DOPAL. The energy crisis in dopaminergic neurons also impedes ATP-dependent vesicular uptake of catecholamines, meaning that the fate of cytoplasmic dopamine is shifted toward DOPAL formation. In this report we provide key evidence supporting such a link—that DOPAL is indeed built up in the rat rotenone model. Since this model features the same distinctive central catecholaminergic pattern that is found post-mortem in Parkinson’s disease, the model is suitable for testing the catecholaldehyde hypothesis.

Our study assessed rotenone effects on processes and enzymes by calculating tissue product/substrate ratios. The assessment of ALDH is straightforward, since the direct substrate and immediate product are in the same cellular compartment, the cytoplasm. The assessment of VMAT2 is more complex because of multiple metabolites and different intra-neuronal compartments. We used several ratios for assessing the effect of rotenone on VMAT2 in both dopaminergic and noradrenergic pathways, and all the indices agreed in pointing to rotenone-induced inhibition of VMAT2.

There are alternative methods to assess VMAT2 and ALDH activity that are more direct than measuring catechol ratios. We chose the same methodology that we used with human tissue: the substrate/product ratios provide indirect measures of these processes. At this point we thought it important to replicate the abnormal pattern of tissue catechols that is found post-mortem in PD. A limitation of our approach is that tissue catechol ratios provide only indirect indices of intra-neuronal processes such as vesicular uptake. A variety of methods are available for examining VMAT2 function, such as uptake and retention of $^3$H-DA. It should be noted that these do not easily separate attenuated active uptake from augmented passive vesicular leakage as determinants.
of decreased vesicular catecholamine stores. Recent evidence indicates that both types of functional abnormalities are found in catecholaminergic neurons in Lewy body diseases (Goldstein et al., 2019).

The finding of increased tissue DOPA levels can be explained by LAAAD inhibition, which is part of the pattern of metabolic abnormalities found in post-mortem studies (Goldstein et al., 2017). Addressing these complex patterns seems to require a systems biological approach. We recently reported that in patients with Lewy body diseases computational modeling reveals multiple functional abnormalities in catecholaminergic neurons (Goldstein et al., 2019). The data about LAAAD and vesicular sequestration in the present study are in line with the results reported in patients with Lewy body diseases.

In a previous study using the PC12 cellular model we found that the suppressive effect of rotenone on ALDH is mediated by decreased availability of the co-factor NAD+, as a result of rotenone’s inhibition of complex 1 (Goldstein et al., 2015). As for the effect on VMAT2, Sai et al. showed that rotenone downregulates expression of VMAT2 in the PC12 model (Sai et al., 2008). The effect of rotenone on VMAT2 was also evaluated in the SH-SY5Y cellular model by Watabe and Nakaki, who demonstrated a direct effect of rotenone on the transporter (Watabe and Nakaki, 2008). Moreover, animal studies applying genetic manipulations expected to increase DOPAL in dopaminergic neurons show features resembling those in PD (Chiu et al., 2015, Kao et al., 2020, Landrock et al., 2018, Wey et al., 2012). Here we report that rotenone not only increases DOPAL levels but also produces other catecholaminergic abnormalities that seem relevant to PD pathogenesis; hence the value of this model. With regard to neuropathology, a report by Cannon et al. provided histological evidence of dysfunctional dopaminergic terminals in the rat rotenone model, without severe neuronal loss (Cannon et al., 2009). Our neurochemical data fit well with their histological findings.

DA, DOPAL, and DOPAC are substrates for catechol-O-methyltransferase (COMT); however, catecholaminergic neurons have little of any COMT activity. This is one reason that measuring tissue DA/DOPAC ratios has long been an accepted method for assessing the turnover of endogenous DA. The tissue turnover of catecholamines, in turn, depends mainly on the balance of vesicular uptake vs. vesicular leakage (Eisenhofer et al., 2004). It is reasonable to infer that the abnormalities reported here in tissue catechol ratios can be accounted for by decreased vesicular storage. To our knowledge there is no pure test of vesicular uptake
that can be applied in vivo. This is because of (1) the concurrent high rate of vesicular leakage, (2) the series arrangement of cellular uptake and then vesicular uptake of exogenous tracer-labelled catecholamines, and (3) the likelihood of substantial protein reactivity of oxidized catecholamines. In particular, because of (1) and (2), one cannot draw inferences about vesicular uptake based on assessing the kinetics of tissue $^3$H-DA. Even after taking cellular uptake into account, it would be difficult if not impossible to separate decreased vesicular uptake from increased vesicular permeability in explaining decreased tissue content of $^3$H-DA. Analyzing these processes separately would seem to require highly non-physiologic experiments on isolated vesicles. Regarding (3), when tissue levels of $^3$H-catecholamines have been tracked after administration into animals, a substantial proportion of the $^3$H is not accounted for by free levels of all the known catecholamines and their metabolites (Chang et al., 1990). We speculate that $^3$H-DOPAL formed in the neuronal cytoplasm oxidizes spontaneously to $^3$H-DOPAL-quinone, which in turn binds covalently with (“quinonizes”) numerous intracellular proteins (Jinsmaa et al., 2018a, Zhu et al., 2004, Jinsmaa et al., 2020a).

The product of aldose reductase acting on DOPAL is 3,4-dihydroxyphenylethanol (DOPET), which we included in our assay. As expected, the amount of DOPET detected was small compared to 3,4-dihydroxyphenylacetic acid (DOPAC), which is the product of ALDH acting on DOPAL. Directly assaying ALDH activity would not provide the information sought, since the assay would involve supplying NAD cofactor for the assay. If rotenone decreased ALDH activity indirectly, via decreased NAD$^+$ production as a result of complex 1 inhibition, which is likely to be the case (Goldstein et al., 2015), ALDH activity measured in a test tube experiment would not identify the decrease in enzyme activity as a result of rotenone.

Along with the neurochemical data, the study's primary objective, we conducted the neurobehavioral assessments in order to link the neurochemical results with motor dysfunction in this model. We used motor tests known to represent the movement abnormalities in the model. Indeed the two main tests were chosen from the original studies that introduced the rotenone model (Betarbet et al., 2000, Cannon et al., 2009) where full description of the locomotor abnormalities induced by rotenone were presented. Our results confirmed reduced motor mobility and activity with rotenone treatment.
We recognize that the locomotor tests employed were not comprehensive but were adequate to demonstrate that the rotenone-treated animals in this study had the same locomotor abnormalities as have been reported previously. The cylinder test (rearing test) and open field test are well established (Hua et al., 2002, Rial et al., 2014, Schaar et al., 2010, Su et al., 2018) and verified the locomotor abnormalities known in this model.

**Perspective:** This model should prove valuable in further neurochemistry-oriented research about testing and applying the catecholaldehyde hypothesis for the pathogenesis and treatment of PD. Several directions can be taken. The rotenone model provides a platform for further studies on in vivo mechanisms by which DOPAL interacts with alpha-synuclein and other intracellular proteins (Jinsmaa et al., 2018b) to threaten neuronal homeostasis. The catecholamine functional abnormalities reported here render the model suitable for testing interventional strategies that might salvage dysfunctional catecholaminergic neurons. This understanding might lead to novel disease-modifying treatments such as enhancing VMAT or ALDH activity (Lohr and Miller, 2014, Xiong et al., 2016, Chiu et al., 2015) or combined strategies for which this model is useful. We believe that in the future, combination pharmacotherapy will prove to be effective for neurodegenerative diseases, just as in other areas of medicine.

In conclusion, the rat rotenone model exhibits a set of functional abnormalities in catecholaminergic neurons that reproduces the pattern found in PD putamen. These include a vesicular storage defect, decreased ALDH activity, and DOPAL buildup. The catecholaminergic functional abnormalities reported here render the rat rotenone model a suitable *in vivo* platform for studying the catecholaldehyde paradigm for the neurodegenerative process in PD.

**STATEMENTS**

All authors read and approved the final manuscript.
Ethics approval: The animal research procedures in this study complied with the guidelines of and were approved by the Animal Care Committee of the Chaim Sheba Medical Center, Tel Hashomer, Israel.

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The research reported here was supported in part by the Division of Intramural Research, NINDS, NIH. No NIH grant was involved.

Declaration of interests: none

Availability of data and materials: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request

Authors' contributions: RL took part in planning the experiment, conducted the experiment, assisted in analyzing the data, and contributed to writing the manuscript. RH advised on the behavioral studies, analyzed the behavioral data, and contributed to writing the manuscript. PS assayed the brain tissue and provided the neurochemical data. ZZ assisted in collecting and preparing brain tissue from the rats. AL assisted in the preparation of materials for the study and collecting brain tissue from the rats. DSG designed the experiment, analyzed and interpreted the neurochemical data, and contributed to writing the manuscript. YS designed the experiment, supervised its execution, analyzed and interpreted data, and contributed to writing the manuscript.

Acknowledgment: This research was supported (in part) by the Intramural Research Program of the NIH, NINDS.

REFERENCES


Figures

**Figure 1:** Concept diagram showing enzymatic steps in the synthesis, vesicular storage, release, reuptake, and metabolism of dopamine (DA) and norepinephrine (NE).

The six endogenous catechols in white rectangles were measured simultaneously. Dopamine (DA) is synthesized in the neuronal cytoplasmic via tyrosine hydroxylase (TH) acting on tyrosine to form 3,4-dihydroxyphenylalanine (DOPA) and then L-aromatic-amino-acid decarboxylase (LAAAD) acting on DOPA. Most of cytoplasmic DA is taken up into vesicles via the vesicular monoamine transporter (VMAT), but a minority undergoes enzymatic oxidation catalyzed by monoamine oxidase (MAO) to form 3,4-dihydroxyphenylacetaldehyde (DOPAL). DOPAL is metabolized by aldehyde dehydrogenase (ALDH) to form 3,4-dihydroxyphenylacetic acid (DOPAC), which exits the cell. DA in the vesicles undergoes enzymatic hydroxylation by DA-beta-hydroxylase (DBH) to form NE. Catecholamines released into the extracellular fluid is taken back up into the cytoplasm via the cell membrane DA transporter (DAT) or NE transporter (NET). NE in the cytoplasm can undergo vesicular uptake or MAO-catalyzed oxidative deamination to form 3,4-dihydroxyphenylglycolaldehyde (DOPEGAL), which is reduced by aldehyde/aldose reductase (AR) to form 3,4-dihydroxyphenylglycol (DHPG). DHPG rapidly exits the neuron. Font sizes correspond roughly to tissue concentrations of the analytes in rat striatum.
Figure 2: Rearing behavior (counts/2 minutes) comparison between day 1 and 9 in the rotenone (N=8)- and vehicle (N=11)-treated groups. Individual representation of rats (dotted lines) with mean values of the groups represented as solid lines. There is a significant difference (t-test) in rearing count between days in the rotenone (p=0.0015) but not in the vehicle (p=0.41) group.

Figure 3: Activity difference (activity in seconds on Day 0 minus activity on Day 1 and Day 9) in the rotenone (N=8)- and vehicle (N=11)-treated groups. Individual representation of rats (dotted lines) with mean values of the groups represented as solid lines. There is a significant difference (t-test) in activity between days in the rotenone (p=0.0001) but not in the vehicle group (p=0.44).
Table 1: Brain tissue catechols (pmol/mg) (means ± SEM) in rats treated with vehicle (VEH) or rotenone (ROT). P values are for independent-means t-tests comparing 2 treatments.

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<th>VEH</th>
<th>ROT</th>
<th>P</th>
</tr>
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<tr>
<td>DOPA</td>
<td>0.047 ± 0.003</td>
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<td>DA</td>
<td>0.85 ± 0.07</td>
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<td>NE</td>
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<td>0.090 ± 0.011</td>
<td>0.04</td>
</tr>
<tr>
<td>DHPG</td>
<td>0.22 ± 0.04</td>
<td>0.51 ± 0.11</td>
<td>0.04</td>
</tr>
<tr>
<td>DOPAC</td>
<td>0.25 ± 0.04</td>
<td>0.17 ± 0.05</td>
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</table>

See Concept Diagram (Figure 1) for abbreviations
Table 2: Brain tissue catechol ratio (pmol/mg) (means ± SEM) in rats treated with vehicle (VEH) or rotenone (ROT). P values are for independent-means t-tests comparing 2 treatments.

<table>
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<tr>
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<th>Process</th>
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<th>ROT</th>
<th>P</th>
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<tr>
<td>DA/DOPAL</td>
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<td>NE/DHPG</td>
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<td>13.93 ± 1.93</td>
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<td>DA/(DOPAL+ DOPAC+DHPG)</td>
<td>VMAT</td>
<td>1.67 ± 0.16</td>
<td>0.37 ± 0.19</td>
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<tr>
<td>DOPAC/DOPAL</td>
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<td>2.20 ± 0.87</td>
<td>0.036</td>
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<td>DA/DOPA</td>
<td>LAAAD</td>
<td>14.15 ± 1.47</td>
<td>1.13 ± 0.41</td>
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*See Concept Diagram (Figure 1) for abbreviations*