

Chronic methamphetamine-induced neurodegeneration: Differential vulnerability of ventral tegmental area and substantia nigra pars compacta dopamine neurons

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ABSTRACT

Methamphetamine (meth) increases monoamine oxidase (MAO)-dependent mitochondrial stress in substantia nigra pars compacta (SNc) axons; chronic administration produces SNc degeneration that is prevented by MAO inhibition suggesting that MAO-dependent axonal mitochondrial stress is a causal factor. To test whether meth similarly increases mitochondrial stress in ventral tegmental area (VTA) axons, we used a genetically encoded redox biosensor to assess mitochondrial stress *ex vivo*. Meth increased MAO-dependent mitochondrial stress in both SNc and VTA axons. However, despite having the same meth-induced stress as SNc neurons, VTA neurons were resistant to chronic meth-induced degeneration indicating that meth-induced MAO-dependent mitochondrial stress in axons was necessary but not sufficient for degeneration. To determine whether L-type Ca^{2+} channel-dependent stress differentiates SNc and VTA axons, as reported in the soma, the L-type Ca^{2+} channel activator Bay K8644 was used. Opening L-type Ca^{2+} channels increased axonal mitochondrial stress in SNc but not VTA axons. To first determine whether mitochondrial stress was necessary for SNc degeneration, mice were treated with the mitochondrial antioxidant mitoTEMPO. Chronic meth-induced SNc degeneration was prevented by mitoTEMPO thereby confirming the necessity of mitochondrial stress. Similar to results with the antioxidant, both MAO inhibition and L-type Ca^{2+} channel inhibition also prevented SNc degeneration. Taken together the presented data demonstrate that both MAO- and L-type Ca^{2+} channel-dependent mitochondrial stress is necessary for chronic meth-induced degeneration.

1. Introduction

Methamphetamine (meth) is an addictive psychostimulant that also has the potential to cause significant damage to dopaminergic systems (Moratalla et al., 2017). Data from human meth abusers reveal decreased levels of striatal dopamine content, diminished expression of the dopamine transporter (DAT) and tyrosine hydroxylase (TH), and reduced DAT and vesicular monoamine transporter 2 (VMAT2) binding (Johanson et al., 2006; Kish et al., 2009; Kitamura et al., 2007; McCann et al., 1998, 2008; Moszczynska et al., 2004; Rumpf et al., 2017; Sekine et al., 2001; Volkow et al., 2001a, 2001b; Wilson et al., 1996). Morphological evidence indicating damage to the substantia nigra pars compacta (SNc) is also reported (Todd et al., 2013, 2016). The ability of meth to have deleterious effects to dopaminergic systems is particularly

concerning given that the number of people suffering from meth use disorders has been on the rise (Han et al., 2021; NSDUH, 2018; Shearer et al., 2020; Winkelman et al., 2018). In addition, meth abuse may increase the risk for developing Parkinson's disease (Callaghan et al., 2010, 2012; Curtin et al., 2015), the most common neurodegenerative movement disorder that is in large part characterized by progressive SNc degeneration.

Meth has two direct pharmacological sites of action in dopamine neurons: VMAT2 and DAT. Meth binds to and induces dysfunction of these two proteins resulting in the release of vesicularly packaged dopamine into the cytosol and efflux of cytosolic dopamine into the synapse (Freyberg et al., 2016; Sulzer et al., 2005). The ability of meth to increase synaptic concentrations of dopamine is largely responsible for the reinforcing and euphorogenic properties of meth, whereas the

Abbreviations: meth, methamphetamine; MAO, monoamine oxidase; SNc, substantia nigra pars compacta; VTA, ventral tegmental area; TH, tyrosine hydroxylase; DAT, dopamine transporter; VMAT2, vesicular monoamine transporter 2; aCSF, artificial cerebrospinal fluid.

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increased concentration of cytosolic dopamine may contribute to the deleterious effects of meth. The classical view of dopamine toxicity is that cytosolic dopamine can auto-oxidize, generating reactive quinones which would increase cytosolic stress (Sulzer and Zecca, 2000). Alternatively, cytosolic dopamine can also be metabolized by monoamine oxidase (MAO) enzymes which generate a potentially reactive aldehyde (Goldstein, 2020; Goldstein et al., 2013) and free electrons that were thought to enter the cytosol to generate hydrogen peroxide (Edmondson, 2014; Edmondson et al., 2009), both of which would also result in cytosolic stress. However, MAO enzymes are mitochondrially tethered and it was recently discovered that increasing cytosolic dopamine increases mitochondrial, not cytosolic oxidant stress (Graves et al., 2020). This MAO-dependent mitochondrial stress is a result of electrons generated through the deamination process being passed to the mitochondrial intermembrane space (Graves et al., 2020). Chronic (28 day) *in vivo* administration of meth results in SNc degeneration which is attenuated by MAO inhibition (Graves et al., 2021). These data suggest that meth-induced MAO-dependent mitochondrial stress is a key driver of degeneration. Neighboring ventral tegmental area (VTA) dopamine neurons also express MAO and would therefore be expected to undergo the same meth-induced MAO-dependent mitochondrial stress. If meth-induced MAO-dependent mitochondrial stress is a driver of degeneration then VTA dopamine neurons could also be vulnerable to chronic meth-induced degeneration.

The present study was designed to determine whether VTA dopamine neurons, like SNc neurons, are subject to meth-induced MAO-dependent mitochondrial stress and if so, whether VTA neurons are vulnerable or resistant to chronic meth-induced degeneration. To accomplish study goals, we used optical techniques to examine mitochondrial stress alongside immunohistochemical and stereological means to examine axonal and somatic loss. The present study demonstrates that both SNc and VTA neurons undergo similar MAO-dependent mitochondrial stress in axons when exposed to meth. However, unlike the SNc, the VTA was resistant to chronic meth-induced degeneration. This differential vulnerability can, at least in part, be attributed to the presence of L-type Ca^{2+} channel-dependent mitochondrial stress in SNc but not VTA neurons. We found the axonal compartment to be the site where there was both MAO- and L-type Ca^{2+} channel-dependent mitochondrial stress. Pharmacological strategies aimed at mitigating mitochondrial stress prevented meth-induced SNc degeneration. Presented data provide new insights into the differential vulnerability of SNc and VTA neurons with respect to chronic meth-induced degeneration and demonstrate the neuroprotective capacity of two clinically available medications.

2. Methods

2.1. Experimental subjects

Male mice were used throughout; investigations were restricted to male subjects because estrogen may afford neuroprotection to dopaminergic neurons. For example, female mice are less vulnerable to the damaging effects of an acute meth binge (Bourque et al., 2011a, 2011b; Liu and Bluzen, 2006) and are more resistant to dopaminergic degeneration in the MitoPark mouse model of Parkinson's disease (Chen et al., 2019). C57Bl/6 mice and mice expressing Cre recombinase under the tyrosine hydroxylase reporter (TH-Cre; hemizygous) on a C57Bl/6 background were bred in-house. All subjects were group housed, maintained on a 12-hr light/dark cycle, and provided free access to food and water throughout the study. Procedures were reviewed and approved by the University of Minnesota Animal Care and use Committee and in accordance with National Institutes of Health Guide for the Care and Use of Laboratory Animals. All experimental subjects were euthanized between 8 and 12 weeks of age.

2.2. *In vivo* drug treatments

Drugs administered *in vivo* include methamphetamine (5 mg/kg), the MAO inhibitor rasagiline (1 mg/kg), the L-type Ca^{2+} channel negative allosteric modulator isradipine (3 mg/kg/day), and the mitochondrial antioxidant mitoTEMPO (3 mg/kg). Mice administered saline injections were used as controls. Rasagiline is an irreversible MAO-B inhibitor; there are two MAO isoforms, MAO-A and MAO-B, both of which metabolize dopamine (Finberg and Rabey, 2016). It was previously thought that dopamine neurons only expressed MAO-A and MAO-B expression was reserved for astrocytes and serotonergic neurons (Levitt et al., 1982; Westlund et al., 1988), but more recent work provides evidence of MAO-A in dopamine neurons (Graves et al., 2020; Woodard et al., 2014). The MAO-B inhibitor rasagiline was used in the current report because 1) both rasagiline and the MAO-A inhibitor cloglyline attenuate meth-induced mitochondrial stress in SNc axons (Graves et al., 2020), 2) we recently demonstrated that MAO-B inhibition using rasagiline is neuroprotective (Graves et al., 2021) and 3) MAO-B inhibitors are clinically available and well tolerated with rasagiline being FDA approved for use in patients with Parkinson's disease. In contrast, selective MAO-A inhibitors are not well tolerated nor are they used clinically (Finberg and Rabey, 2016). Saline, meth, rasagiline, and mitoTEMPO were administered intraperitoneally at a volume of 10 ml/kg. Isradipine (3 mg/kg/day) was delivered via an osmotic minipump. Minipumps (Alzet model 2004) were filled with isradipine dissolved in vehicle (50% DMSO/50% PEG300) at a concentration to achieve 3 mg/kg/day dose and were implanted subcutaneously as previously described (Graves et al., 2021; Guzman et al., 2018; Ilijic et al., 2011); meth administration commenced two days post-op. Treatment groups included saline, meth, rasagiline + meth, mitoTEMPO + meth, and isradipine + meth. For rasagiline + meth and mitoTEMPO + meth treatment groups rasagiline or mitoTEMPO were administered as a pretreatment 30 min prior to the meth injection. *In vivo* drug administration utilized a 28 day paradigm with daily injections (Graves et al., 2021); subjects were euthanized within 12 h of the last injection.

2.3. Immunohistochemistry

Mice were anesthetized with an intraperitoneal injection of ketamine (50 mg/kg)/xylazine (4.5 mg/kg) and transcardially perfused with 4% paraformaldehyde in PBS. Brains were post-fixed overnight and cryoprotected with 30% sucrose in PBS. Thin sections (40 μm) were sliced using a microtome (Leica SM2010R). Every third section throughout the SNc and VTA was stained resulting in 11-12 slices for both the SNc and VTA. Every sixth section throughout the striatum and nucleus accumbens (sections between 1.2 and 0.4 μm anterior from bregma) was stained for a total of 4 striatal and 3 accumbal slices per subject. Sections were first treated with 20% formic acid for antigen retrieval, blocked with 5% normal donkey serum, and then stained for tyrosine hydroxylase (TH, primary antibody: rabbit anti-TH polyclonal AB152 Millipore 1:2000; secondary antibody: Alexa 555 donkey anti-rabbit A31572 Invitrogen 1:200). Sections spanning the SNc and VTA were counterstained with NeuN (primary antibody: mouse anti-NeuN monoclonal (1B7) ab104224 1:500; secondary antibody: Alexa 488 donkey anti-mouse A21202 Invitrogen, 1:200). Sections containing the striatum and nucleus accumbens were stained for TH as described above to visualize dopaminergic axons. Sections were mounted and coverslipped onto glass slides (Electron Microscopy Sciences) with ProLong Diamond Antifade Mountant (Invitrogen).

2.4. Stereological analyses

Sections were viewed on a Zeiss microscope with a motorized stage and a digital camera, controlled by StereoInvestigator software (version 2020.2.2, MicroBrightfield Inc). TH⁺ cells were stereologically counted throughout the SNc and VTA using the optical fractionator probe (Deniz

et al., 2018). SNc and VTA boundaries were traced under 2.5x/0.085 NA objective lens. Cell counting was performed on every 3rd section throughout SNc and VTA, a total of 11–12 slices under a 63x/1.4 NA lens using a 150 μm $\times$ 150 μm counting frame, a grid size of 250 μm $\times$ 275 μm , and 2 μm guard zones (top and bottom) for both the SNc and VTA. The above described parameters are summarized in Table 1 and resulted in a Gundersen coefficient of error ($m = 1$), a metric estimating counting precision (Gundersen et al., 1999), of 0.03–0.05. To control for the confound of phenotypic suppression, NeuN positive cells were also counted using aforementioned parameters.

SNc and VTA axons were similarly quantified using stereological methods but with the spaceballs probe (West, 2018). Regions of interest were traced in the nucleus accumbens or dorsal striatum using a 2.5x/0.085 NA objective lens with every 6th section used to result in 4 and 3 brain slices for the dorsal striatum and nucleus accumbens (sections between 1.2 and 0.4 μm anterior from bregma), respectively. Axon length was quantified for both the dorsal striatum and nucleus accumbens (NAc) under a 63x/1.4 NA lens. A hemispherical probe (radius of 7 μm for dorsal striatum and 6 μm for NAc) was used with a grid size of 275 μm $\times$ 275 μm , and 3 μm guard zones (top and bottom); parameters are summarized in Table 2 and resulted in a Gundersen coefficient of error ($m = 1$) of 0.04–0.06.

2.5. Stereotaxic surgeries and viral vectors

To examine mitochondrial stress, the redox sensitive probe roGFP, with a targeting sequence to achieve expression in the mitochondrial matrix (Sabharwal et al., 2013), was packaged in AAV construct with double inverted orientation (DIO) for use with TH-Cre mice. AAV9-DIO-mito-roGFP was generated by the University of Minnesota Viral Vector and Cloning Core. Isoflurane anesthesia was delivered using a low-flow precision vaporizer (Kent Scientific); subjects were first placed in an induction chamber with a flow rate of 5% to establish an anesthetic plane and subsequently placed in a stereotaxic frame (David Kopf Instruments) with a Cunningham adaptor (Harvard Apparatus). Once in the stereotaxic frame anesthesia was maintained at a flowrate of 1.5–2.5% and anesthetic depth was verified throughout surgeries. Subjects were administered 2 mg/kg meloxicam subcutaneously for prophylactic pain management after which an incision was made to expose the skull and a small hole drilled; 400 nl of AAV9-DIO-mito-roGFP virus was injected into the brain using a glass micropipette (Drummond Scientific Company) pulled on a P-1000 puller (Sutter Instrument). Injections were made based on stereotaxic coordinates; for SNc injections coordinates AP: $-3.5 \mu\text{m}$, ML: 1.1 μm , DV: 4.2 μm from bregma and for VTA: AP: $-3.3 \mu\text{m}$, ML: 0.9 μm , DV: 4.0 μm from bregma were targeted. Mice were administered meloxicam 2 mg/kg subcutaneously for 3 days post-op and subjects were euthanized >10 days post-op.

2.6. Ex vivo brain slice preparation

Mice were terminally anesthetized with an intraperitoneal injection of ketamine (50 mg/kg)/xylazine (4.5 mg/kg) and transcardially

Table 1
Optical fractionator stereological parameters.

	SNc	VTA
Sections		
Number of sections	11–12	11–12
Type	40 μm frozen	40 μm frozen
Actual thickness (mean $\pm$ SEM)	30.9 $\pm$ 0.5 μm	31.3 $\pm$ 1.5 μm
Sampling	every 3rd section	every 3rd section
Optical fractionator probe		
Counting frame	150 μm $\times$ 150 μm	150 μm $\times$ 150 μm
Grid size	250 μm $\times$ 275 μm	250 μm $\times$ 275 μm
Dissector height	13–24 μm	14–24 μm
Coefficient of error (Gundersen), $m = 1$	0.03–0.05	0.03–0.04

Table 2
Spaceballs stereological parameters.

	Dorsal Striatum	Nucleus Accumbens
Sections		
Number of sections	4	3
Type	40 μm frozen	40 μm frozen
Actual thickness (mean $\pm$ SEM)	30.3 $\pm$ 0.9 μm	30.9 $\pm$ 1.0 μm
Sampling	every 6th section	every 6th section
Spaceballs probe		
Grid size	275 μm $\times$ 275 μm	275 μm $\times$ 275 μm
Probe shape	hemisphere	hemisphere
Radius of spherical probe	7 μm	6 μm
Coefficient of error (Gundersen), $m = 1$	0.04–0.06	0.04–0.05

perfused using ice cold low Ca^{2+} /high sucrose artificial cerebrospinal fluid (aCSF; 49.0 mM NaCl, 2.5 mM KCl, 2.0 mM CaCl_2 , 10.0 mM MgCl_2 , 25.0 mM NaHCO_3 , 1.43 mM NaH_2PO_4 , 2.5 mM glucose, and 5.0 mM sucrose). Once perfused the brain was removed and coronal slices (220 μm thick) containing the striatum, nucleus accumbens, SNc, and/or VTA were collected using a vibratome (Leica VT1200S). Slices were placed in a holding chamber containing normal aCSF (124.0 mM NaCl, 3.0 mM KCl, 2.0 mM CaCl_2 , 1.0 mM MgCl_2 , 26.0 mM NaHCO_3 , 1.0 mM NaH_2PO_4 , and 16.66 mM glucose, pH 7.4; 310–320 mOsm) where they remained for a minimum of 30 min prior to imaging experiments; 95% O_2 /5% CO_2 was continuously bubbled throughout.

2.7. Two-photon laser scanning microscopy and mitochondrial stress

Mitochondrial stress was measured in *ex vivo* brain slices as previously described using the redox sensitive roGFP probe expressed in the mitochondrial matrix (Graves et al., 2020, 2021). Slices were transferred to a recording chamber with oxygenated aCSF continuously perfused and temperature maintained at 32–34 $^\circ\text{C}$. Fluorescence was measured using an Ultima Laser Scanning Microscope system (Bruker) with a Nikon FN-1 microscope, a 60X/1.00 NA lens, and PrairieView software. A two-photon laser (Chameleon Ultra II, Coherent Inc.) was used to excite roGFP with a 920 nm wavelength. A *t*-series consisting of 60 frames collected over ~ 20 s was acquired using a 10–12 μsec dwell time (0.195 μm $\times$ 0.195 μm pixels). After obtaining the test measurement the dynamic range of the probe was determined. This was accomplished by acquiring additional *t*-series after perfusion of 2 mM dithiothreitol, a reducing agent, and 200 μM aldrithiol, an oxidizing agent and relative oxidation calculated as previously described (Graves et al., 2020, 2021). Meth (10 μM) or Bay K8644 (10 μM) was bath perfused in aCSF. For experiments incorporating rasagiline slices were preincubated in the holding chamber with 1 μM rasagiline and bath perfused with meth (10 μM) + rasagiline (1 μM).

2.8. Statistical analyses

Prism (GraphPad Software) was used for all statistical analyses. Data are presented as box-and-whisker plots illustrating the median, quartiles, and range with individual dot plots overlaid. Mann-Whitney (two-tailed) and Kruskal-Wallis with Dunns *post-hoc* analyses were used to determine significance with $\alpha = 0.05$.

3. Results

3.1. Methamphetamine increased MAO-dependent mitochondrial stress in SNc and VTA axons

Dopamine is synthesized and packaged into vesicles by VMAT2; meth directly targets and induces dysfunction of VMAT2 thereby increasing cytosolic dopamine concentrations (Freyberg et al., 2016;

Sulzer et al., 2005). It was recently reported that increasing cytosolic dopamine in SNc axons using either meth or levodopa increases mitochondrial stress (Graves et al., 2020). The mechanism by which this occurs is cytosolic dopamine gets deaminated by mitochondrially tethered MAO enzymes which generates electrons; these electrons are then transferred to the mitochondrial intermembrane space resulting in oxidant stress (Graves et al., 2021). Consistent with this report, we show that meth increased mitochondrial stress in SNc axons and this meth-induced stress was attenuated by MAO inhibition (Fig. 1B). VTA neurons also synthesize and vesicularly package dopamine using VMAT2 and express MAO enzymes; it was therefore hypothesized that meth would similarly increase MAO-dependent mitochondrial stress in VTA axons. To test this, VTA axonal mitochondrial stress was measured in the nucleus accumbens. Consistent with SNc axons, meth also increased mitochondrial stress in VTA axons and this stress was attenuated by MAO inhibition (Fig. 1C). To determine whether meth-induced mitochondrial stress was specific to axons, as previously reported for SNc neurons (Graves et al., 2020), we examined somatic mitochondrial stress in both the SNc and VTA. Bath perfusion of meth had no effect on somatic mitochondrial stress in SNc and VTA dopamine neurons (Fig. 2), confirming the compartmentalization of meth-induced, MAO-dependent mitochondrial stress to axons in both neuronal populations.

3.2. VTA neurons were resistant to chronic methamphetamine-induced degeneration

Chronic 28-day meth (5 mg/kg) results in SNc degeneration and MAO inhibition is neuroprotective (Graves et al., 2021). Given that meth similarly increased axonal MAO-dependent mitochondrial stress in SNc and VTA axons (Fig. 1), we sought to determine whether chronic meth could also induce degeneration of VTA neurons. Mice were administered 5 mg/kg meth or saline daily for 28 consecutive days and euthanized within 12 h of the last injection. Chronic administration of meth had no discernible effect on the number of TH⁺ cells in the VTA (Fig. 3A). Given that meth increased axonal (Fig. 1) but not somatic mitochondrial stress (Fig. 2), we examined whether chronic meth administration resulted in VTA axonal loss. Axon length in the nucleus accumbens was stereologically quantified and showed no difference between mice treated with meth or saline (Fig. 3B). These data indicate that although meth increased MAO-dependent mitochondrial stress in VTA axons, this is not sufficient to cause axonal or somatic loss.

3.3. L-type Ca²⁺ channel activity increased mitochondrial stress in SNc but not VTA axons

Thus far experiments have demonstrated that despite having similar meth-induced MAO-dependent axonal mitochondrial stress as SNc neurons (Fig. 1), VTA neurons were resistant to chronic meth-induced degeneration (Fig. 3). At the somatic level, SNc neurons are reported to have mitochondrial stress resultant from Cav1.3 L-type Ca²⁺ channel-dependent Ca²⁺ oscillations whereas VTA neurons do not (Chan et al., 2007; Guzman et al., 2010; Surmeier et al., 2017). However, meth-induced mitochondrial stress is restricted to axons (Fig. 1) and does not occur in the soma (Fig. 2). We therefore sought to determine whether L-type Ca²⁺ channel activity also increases mitochondrial stress in axons. The redox biosensor roGFP was expressed in the mitochondrial matrix of SNc or VTA dopamine neurons and levels of axonal mitochondrial stress examined in the dorsal striatum, corresponding to SNc axons, or nucleus accumbens, corresponding to VTA axons. Levels of axonal mitochondrial stress were determined with aCSF perfusion and with Bay K8644, the L-type Ca²⁺ channel activator, added to the aCSF. SNc and VTA dopamine neurons undergo autonomous pacemaking activity which, in an intact brain, would propagate to axons and synaptic terminals; however, in the *ex vivo* brain slice preparation the axons are severed from the soma and therefore lack the normal pacemaking activity that would lead to opening of voltage-gated L-type Ca²⁺ channels. Bay K8644 was therefore implemented as a means to open L-type Ca²⁺ channels in axons. Consistent with what is seen in the soma (Guzman et al., 2010), L-type Ca²⁺ channel activity increased mitochondrial stress in SNc but not VTA axons (Fig. 4). This indicates that unlike MAO-dependent mitochondrial stress which is compartmentally restricted to axons, L-type Ca²⁺ channel-dependent mitochondrial stress occurs in both the soma (Guzman et al., 2010) and axons (Fig. 4A) of SNc neurons. Moreover, these data indicate that both MAO- and L-type Ca²⁺ channel-dependent mitochondrial stress are present in SNc but not VTA axons.

3.4. Mitochondrial stress was necessary for chronic methamphetamine-induced SNc degeneration and both MAO and L-type Ca²⁺ channel inhibition were neuroprotective

Both SNc and VTA axons are subject to meth-induced MAO-dependent mitochondrial stress (Fig. 1) yet VTA neurons remained resistant to

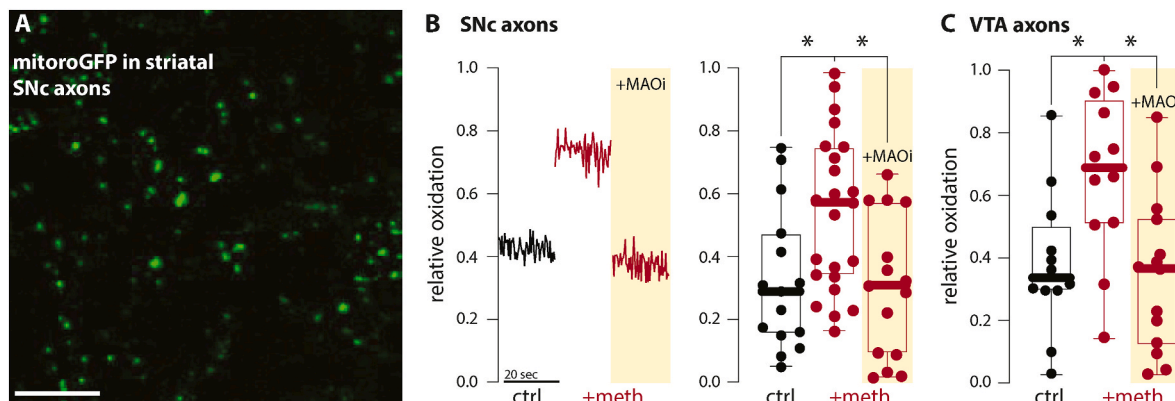


Fig. 1. Meth increased mitochondrial stress in SNc and VTA axons. **(A)** Sample image in the dorsal striatum showing the redox biosensor roGFP (targeted to the mitochondrial matrix) expressed in SNc axons; scale bar denotes 10 μm. **(B)** Sample fluorescent traces (left) depicting differences in axonal mitochondrial stress between aCSF (ctrl), meth (+meth, 10 μM), and +meth with the MAO inhibitor rasagiline (+MAOi, 1 μM) in *ex vivo* brain slices. Quantified data (right) indicate that compared to ctrl, +meth increased SNc axonal mitochondrial stress which was attenuated by +MAOi; ctrl n = 15 brain slices/5 mice, +meth n = 23 brain slices/6 mice, +MAOi n = 15 brain slices/3 mice. Data analyzed using Kruskal-Wallis ($H(2) = 9.796$, $p = 0.0075$) with Dunn's *post-hoc* (ctrl vs. +meth, $p = 0.0383$, ctrl vs. +MAOi, $p > 0.9999$, +meth vs. +MAOi, $p = 0.0193$). **(C)** +meth similarly increased mitochondrial stress in VTA axons in the nucleus accumbens and +MAOi attenuated the increased mitochondrial stress; ctrl n = 12 brain slices/4 mice, +meth n = 12 brain slices/4 mice, +MAOi n = 14 brain slices/3 mice. Data analyzed using Kruskal-Wallis ($H(2) = 9.308$, $p = 0.0095$) with Dunn's *post-hoc* (control vs. +meth, $p = 0.0356$, control vs. +MAOi, $p > 0.9999$, +meth vs. +MAOi, $p = 0.0163$). * $p < 0.05$.

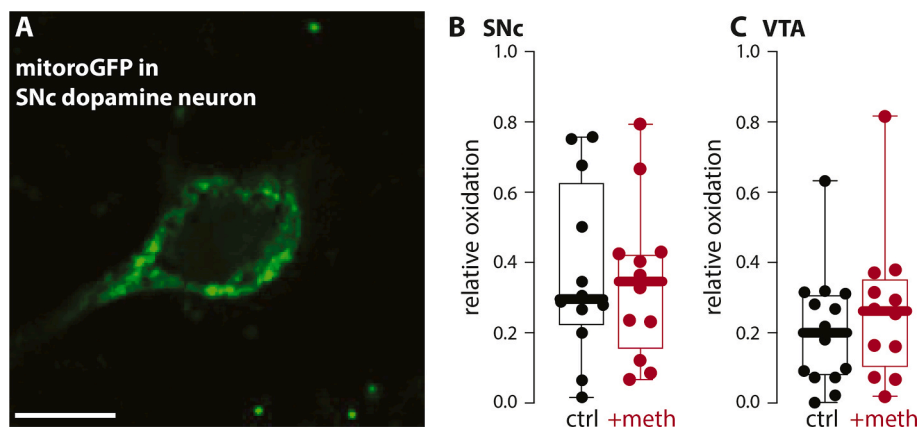


Fig. 2. Meth did not increase somatic mitochondrial stress in SNc or VTA dopamine neurons. **(A)** Sample image showing roGFP (targeted to the mitochondrial matrix) expressed in a SNc dopamine neuron; scale bar denotes 10 μ m. **(B)** Meth (+meth; 10 μ M) had no effect on somatic mitochondrial stress in SNc neurons; ctrl n = 12 neurons/4 mice, +meth n = 12 neurons/4 mice. Data analyzed using Mann-Whitney test ($U = 72$, $p > 0.9999$, two-tailed). **(C)** +meth also had no effect on somatic mitochondrial stress in VTA dopamine neurons ctrl n = 14 neurons/5 mice, +meth n = 12 neurons/4 mice. Data analyzed using Mann-Whitney test ($U = 72$, $p = 0.5604$, two-tailed).

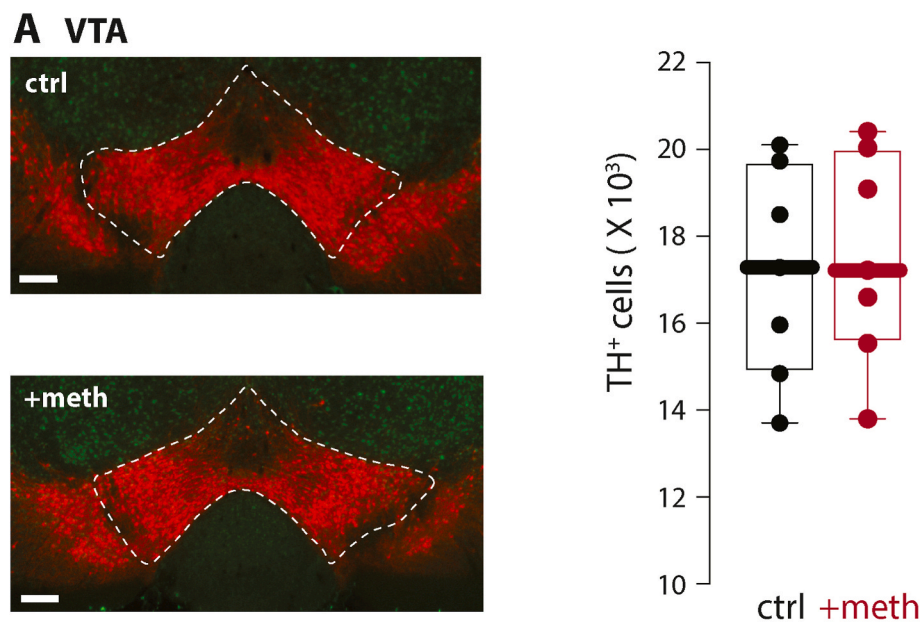


Fig. 3. VTA neurons were resistant to chronic meth-induced degeneration. **(A)** Sample images (left) depicting tyrosine hydroxylase expressing (TH⁺) neurons in VTA of saline (ctrl; 10 ml/kg)- and meth (+meth; 5 mg/kg)-treated mice with VTA boundary indicated by dashed line; scale bars denote 200 μ m; TH stained in red with NeuN counterstained in green. Quantified data (right) indicate that chronic meth had no effect on number of TH⁺ cells in the VTA; ctrl n = 7 and +meth n = 7 mice. Data analyzed using Mann-Whitney test ($U = 22$, $p = 0.8048$, two-tailed). **(B)** Sample image (left) showing boundary of nucleus accumbens; scale bar denotes 500 μ m. Sample images (middle) depicting TH⁺ axons in the NAc of ctrl and +meth mice; scale bars denote 20 μ m. Quantified data (right) show no difference in axon length between ctrl and +meth mice; ctrl n = 5 and +meth n = 5 mice. Data analyzed using Mann-Whitney test ($U = 12$, $p > 0.9999$, two-tailed).

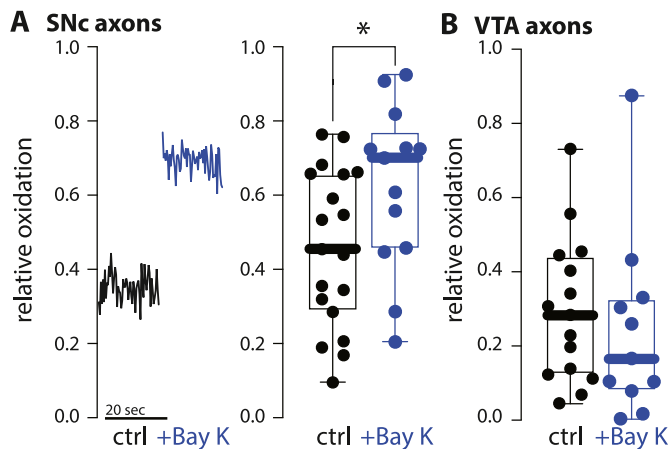


Fig. 4. L-type Ca^{2+} channel-dependent mitochondrial stress was present in SNc but not VTA axons. (A) Sample fluorescent traces (left) depicting differences in axonal mitochondrial stress between aCSF (ctrl) and Bay K8644 (+Bay K, 10 μM , bath perfusion) in *ex vivo* brain slices. Quantified data (right) indicate that compared to ctrl, +Bay K increased mitochondrial stress in SNc axons; ctrl $n = 19$ brain slices/6 mice, +Bay K $n = 13$ brain slices/3 mice. Data analyzed using Mann-Whitney test ($U = 70$, $p = 0.0407$, two-tailed). (B) +Bay K did not increase VTA axonal mitochondrial stress; ctrl $n = 15$ brain slices/4 mice, +Bay K $n = 11$ brain slices/3 mice. Data analyzed by Mann-Whitney test ($U = 61$, $p = 0.2812$, two-tailed). * $p < 0.05$.

chronic meth-induced degeneration (Fig. 3). While the presence of additional L-type Ca^{2+} channel-dependent mitochondrial stress in SNc but not VTA axons (Fig. 4) supports the hypothesis that mitochondrial stress plays a causal role in chronic meth-induced SNc degeneration, evidence for this remains indirect. To directly test whether mitochondrial stress is necessary for chronic meth-induced SNc degeneration, mice were pretreated with the mitochondrial antioxidant mitoTEMPO prior to each meth injection. First, 28 days of 5 mg/kg meth resulted in SNc degeneration as evidenced by decreased number of TH^+ cells (Fig. 5A and B); to confirm that this reflected overt degeneration and not mere phenotypic suppression, sections were counterstained for NeuN, a non-specific neuronal marker. Like the number of TH^+ cells, the number of NeuN stained cells were similarly decreased in the SNc (Fig. 5C). Pretreatment with mitoTEMPO (3 mg/kg) was neuroprotective, confirming that mitochondrial oxidant stress was necessary for chronic meth-induced degeneration (Fig. 5A and B). A recent report demonstrates that pretreatment with the MAO inhibitor rasagiline (1 mg/kg) is also neuroprotective (Graves et al., 2021); this finding was reproduced here (Fig. 5A and B). Lastly, L-type Ca^{2+} channel activity increases mitochondrial stress in both the somatic (Guzman et al., 2010) and axonal (Fig. 4A) compartments of SNc neurons; treatment with the L-type Ca^{2+} channel negative allosteric modulator isradipine, which decreases somatic mitochondrial stress in SNc neurons (Guzman et al., 2010), was also neuroprotective when administered throughout the 28-day treatment paradigm (Fig. 5A and B). Together these data demonstrate that mitochondrial stress was a driver of degeneration and the presence of both MAO- and L-type Ca^{2+} channel-dependent stress were necessary for chronic meth-induced SNc degeneration.

Although MAO and L-type Ca^{2+} channel inhibition prevented

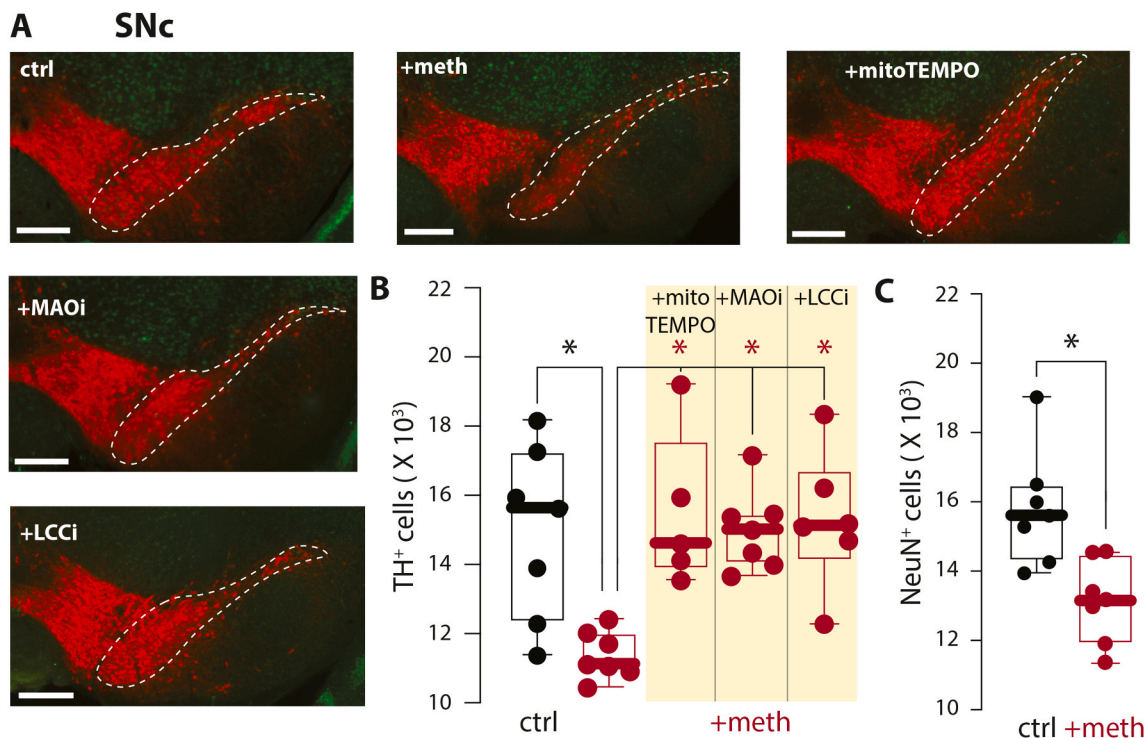


Fig. 5. The mitochondrial antioxidant mitoTEMPO, MAO and L-type Ca^{2+} channel inhibition prevented chronic meth-induced degeneration of SNc neurons. (A) Sample images depicting chronic meth (+meth; 5 mg/kg)-induced loss of TH^+ cells in the SNc and neuroprotection by pretreatment of mitochondrial antioxidant mitoTEMPO (+mitoTEMPO; 3 mg/kg), the MAO inhibitor rasagiline (+MAOi; 1 mg/kg) and the L-type Ca^{2+} channel inhibitor isradipine (+LCCi; 3 mg/kg/day); scale bars denote 200 μm with SNc boundary drawn with dashed line; TH stained in red with NeuN counterstained in green. (B) Stereological quantification of TH^+ cells indicated a loss of TH^+ cells in SNc of chronic meth-treated mice and this loss was prevented by + mitoTEMPO, +MAOi, and +LCCi; ctrl $n = 7$, +meth $n = 7$, +mitoTEMPO $n = 5$, +MAOi $n = 7$, and +LCCi $n = 6$ mice. Data analyzed using Kruskal-Wallis ($H(4) = 14.28$, $p = 0.0065$) with Dunn's *post-hoc* (ctrl vs + meth, $p = 0.0366$, +meth vs. +MAOi, $p = 0.0401$, +meth vs. +LCCi, $p = 0.0201$, +meth vs + mitoTEMPO, $p = 0.0481$, all other comparisons $p > 0.9999$). (C) Stereological quantification of NeuN stained cells confirmed SNc cell loss; ctrl $n = 7$ and +meth $n = 7$ mice. Data analyzed using Mann-Whitney test ($U = 4$, $p = 0.0070$, two-tailed). * $p < 0.05$.

somatic loss (Fig. 5), the cellular compartment where these two sources of stress can converge is in axons. To ascertain the damaging effects of chronic meth on axons and the neuroprotective capacity of MAO and L-type Ca^{2+} channel inhibition, sections were collected spanning the dorsal striatum, stained for TH, and axon length stereologically quantified. Chronic meth treatment resulted in a significant loss of SNc axons and MAO and L-type Ca^{2+} channel inhibition attenuated this loss (Fig. 6).

4. Discussion

The current report highlights the differential vulnerability of SNc and VTA dopamine neurons. VTA dopamine neurons were resistant to chronic meth-induced degeneration despite having the same meth-induced MAO-dependent axonal mitochondrial stress as SNc neurons. When paired with data demonstrating that the mitochondrial antioxidant mitoTEMPO and the MAO inhibitor rasagiline prevented SNc degeneration, it can be concluded that meth-induced MAO-dependent mitochondrial stress in axons is necessary but not sufficient for degeneration. An important feature that differentiates SNc and VTA neurons is the L-type Ca^{2+} channel-dependent mitochondrial stress. In SNc neurons Ca^{2+} oscillations driven by L-type Ca^{2+} channel activity contribute to mitochondrial stress in the somatic compartment of SNc but not VTA dopamine neurons (Chan et al., 2007; Guzman et al., 2010, 2018). However, meth-induced MAO-dependent stress was restricted to axons and did not occur in the soma. We now show that L-type Ca^{2+} channel activity also contributed to mitochondrial stress in SNc axons, making the axonal compartment a site of convergence for meth-induced MAO- and L-type Ca^{2+} channel-dependent stress. Mitigating either source of mitochondrial stress pharmacologically was neuroprotective. Altogether the presented data argue that chronic meth-induced degeneration is driven by mitochondrial stress, at least two sources of mitochondrial stress are necessary for degeneration, and the cellular compartment where at least two sources of mitochondrial stress can be found is in axons.

4.1. Meth increased MAO-dependent mitochondrial stress in both SNc and VTA axons but VTA neurons were resistant to degeneration

It was recently shown that chronic 28-day meth treatment (5 mg/kg) produces SNc degeneration and that MAO inhibition is neuroprotective (Graves et al., 2021); these findings were reproduced in the current report. Meth disrupts VMAT2 function causing increased cytosolic dopamine concentrations (Freyberg et al., 2016; Sulzer et al., 2005). Classically it has been thought that cytosolic dopamine could be harmful in three ways: cytosolic dopamine could 1) auto-oxidize forming reactive quinones (Sulzer and Zecca, 2000), 2) be metabolized by MAO which increases cytosolic hydrogen peroxide via release of free electrons generated by the deamination process (Edmondson, 2014; Edmondson et al., 2009) and/or 3) release a second potentially reactive byproduct from MAO metabolism, 3,4-dihydroxyphenylacetaldehyde (DOPAL) (Goldstein, 2020). All three of these paths would yield cytosolic stress. However, in both *ex vivo* brain slices and dopamine differentiated human stem cells, increasing cytosolic dopamine increases mitochondrial, not cytosolic oxidant stress (Graves et al., 2020). This mitochondrial stress is a result of MAO metabolism of dopamine, but the electrons generated are transferred to the mitochondrial intermembrane space, not the cytosol, thereby providing bioenergetic support but also increasing mitochondrial oxidant stress. SNc and VTA dopamine neurons synthesize and package dopamine into vesicles using VMAT2 and through interactions with VMAT2 meth increases cytosolic dopamine concentrations in both neuronal populations. MAO expression also does not differentiate the two dopaminergic neuronal populations as both express and utilize MAO enzymes for dopamine metabolism. Consistent with this, meth increased MAO-dependent mitochondrial stress in both SNc and VTA axons. However, somatic mitochondrial stress in either SNc or VTA neurons was not significantly altered by meth. The reason for this compartmentalization is thought to be because axons and axon terminals are primary sites of neurotransmitter synthesis, vesicular packaging, and storage in neurons; consistent with this, VMAT2, tyrosine hydroxylase, and tissue content of dopamine are all higher in axonal than somatic regions (Nirenberg et al., 1995; Pickel et al., 1996; Salvatore and Pruetz, 2012; Salvatore et al., 2009). Therefore, axons and axon terminals would be primary sites for meth-induced VMAT2 dysfunction leading to elevated cytosolic dopamine concentrations,

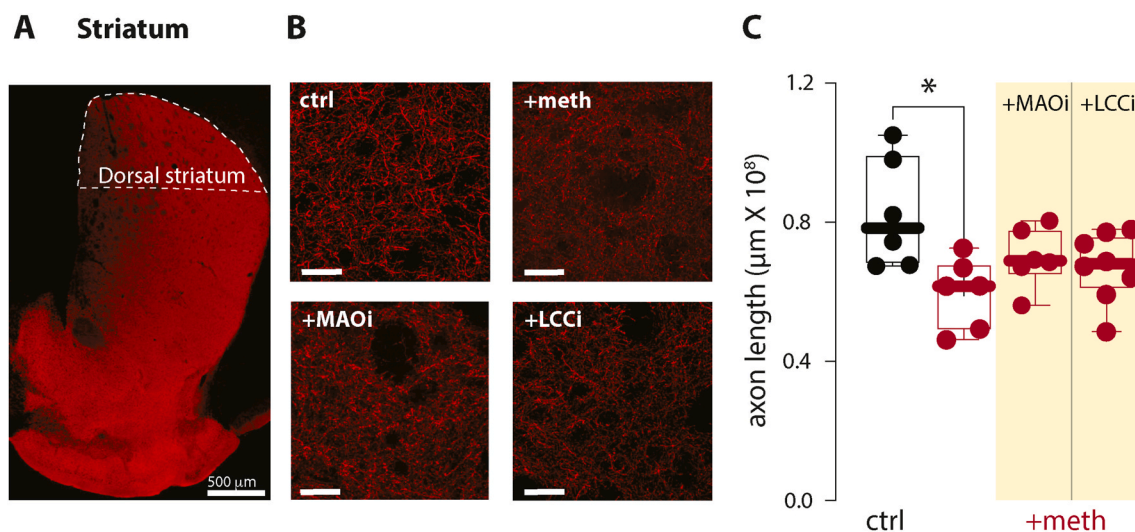


Fig. 6. MAO and L-type Ca^{2+} channel inhibition attenuated chronic meth-induced loss of SNc axons. (A) Sample image showing boundary of dorsal striatum; scale bar denotes 500 μm. (B) Sample images depicting chronic meth (+meth; 5 mg/kg)-induced SNc axon loss; axons in the dorsal striatum and neuroprotection by the MAO inhibitor rasagiline (+MAOi; 1 mg/kg) and the L-type Ca^{2+} channel inhibitor isradipine (+LCCi; 3 mg/kg/day); axons in the dorsal striatum were stained for TH; scale bars 20 μm. (C) Stereological quantification of TH⁺ axon length indicated a loss of TH⁺ axons in the dorsal striatum of chronic meth treated mice and this loss was prevented by +MAOi and +LCCi; ctrl n = 6, +meth n = 6, +MAOi n = 6, +LCCi n = 8 mice. Data analyzed using Kruskal-Wallis ($H(3) = 8.472$, $p = 0.0372$) with Dunn's *post-hoc* (control vs +meth, $p = 0.0248$, +meth vs. +MAOi, $p = 0.5366$, ctrl vs. +LCCi, $p = 0.4858$, all other comparisons $p > 0.9999$). * $p < 0.05$.

which in turn increases mitochondrial oxidant stress.

Chronic 28-day administration of meth resulted in degeneration of SNc neurons and MAO inhibition was neuroprotective, consistent with a prior report (Graves et al., 2021). However, despite having the same meth-induced MAO-dependent mitochondrial stress as SNc neurons, VTA neurons were resistant to chronic meth-induced degeneration. This raised the question as to whether mitochondrial stress was necessary for degeneration or if the stress was secondary to an alternative causal factor. MitoTEMPO is an antioxidant that accumulates in mitochondria; the neuroprotection afforded by mitoTEMPO pretreatment confirms that mitochondrial stress is necessary and when paired with the finding that MAO inhibition is neuroprotective indicates that meth-induced MAO-dependent mitochondrial stress, while necessary, is not sufficient to produce degeneration.

Two MAO isoforms exist (MAO-A and MAO-B) and are expressed in the brain. Classically it has been thought that MAO-A expression was reserved for dopaminergic and noradrenergic neurons with MAO-B expressed in astrocytes and serotonergic neurons (Finberg and Rabey, 2016; Youdim et al., 2006); however, there is evidence for both MAO-A and MAO-B in dopaminergic neurons (Graves et al., 2020; Woodard et al., 2014). Rasagiline, the MAO inhibitor used in the current and previous (Graves et al., 2021) report is an irreversible MAO-B inhibitor. Whether there is differential isoform contribution to chronic meth-induced degeneration is unclear. *In vivo* the rasagiline dose used in the current study (1 mg/kg) inhibits 97% of MAO-B and 18% of MAO-A activity (Huang et al., 1999); a contribution of MAO-A inhibition to the neuroprotective effect of rasagiline can therefore not be excluded. MAO-A inhibition also attenuates meth-induced mitochondrial stress in SNc axons (Graves et al., 2020). From this it would be hypothesized that *in vivo* administration of a MAO-A inhibitor would, like the MAO-B inhibitor rasagiline, attenuate chronic meth-induced degeneration. In contrast to this prediction, MAO-A inhibition exacerbates meth toxicity in acute binge paradigms with enhanced decreases in tissue content of dopamine and TH expression but not DAT expression (Kuhn et al., 2008; Thomas et al., 2008, 2009; Wagner and Walsh, 1991).

Our results also contrast with acute binge models in that chronic administration resulted in SNc cell loss whereas acute binge models predominantly produce axonal deficits without somatic loss (Blaker et al., 2019; Lohr et al., 2015; Ricaurte et al., 1982) with few exceptions (Ares-Santos et al., 2014; Sonsalla et al., 1996). However, the chronic model used in the current report is in accordance with acute binge models in that both the current study and binge studies find VTA neurons to be largely resistant to the deleterious effects of meth (Harvey et al., 2000; Kousik et al., 2014; Zhang and Angulo, 1996). A similar pattern of resistance and vulnerability is seen in Parkinson's disease (Gibb and Lees, 1991; Hirsch et al., 1988). Parkinson's disease is the most common neurodegenerative movement disorder with hallmark motor symptoms arising due to the progressive degeneration of SNc neurons (Surmeier et al., 2017). In contrast, VTA neurons remain resistant to degeneration with loss occurring at late stages of disease (Gibb and Lees, 1991; Hirsch et al., 1988). The MitoPark mouse model of Parkinson's disease also reproduces this differential vulnerability with VTA neurons being more resistant to degeneration than SNc neurons (Ekstrand et al., 2007; Ricke et al., 2020). Lastly, in both the acute binge and the chronic model used in the current report, the first step towards toxicity is increased cytosolic dopamine resulting from VMAT2 dysfunction. Decreasing VMAT2 expression exacerbates and increasing VMAT2 expression attenuates meth toxicity from an acute binge (Fumagalli et al., 1999; Guillot et al., 2008a, 2008b; Lohr et al., 2015). Further supporting a role of cytosolic dopamine being a driver of toxicity, inhibiting dopamine synthesis also attenuates meth-induced axonal loss in binge models (Albers and Sonsalla, 1995; Axt et al., 1990; Schmidt et al., 1985; Wagner et al., 1983).

However, the presence of cytosolic dopamine in and of itself does not necessarily result in SNc degeneration. Rasagiline attenuates meth-induced mitochondrial stress in axons but as a result cytosolic stress is

increased (Graves et al., 2020); occlusion of MAO activity presumably results in auto-oxidation becoming the dominant metabolic outcome. From this it can be inferred that during the 28-day administration paradigm, the meth-treated group is subjected to chronic mitochondrial stress whereas the treatment group receiving the MAO inhibitor as a pretreatment prior to meth is subjected to chronic cytosolic stress. Given that MAO inhibition was neuroprotective, attenuating both somatic and axonal loss, this suggests that even vulnerable SNc dopamine neurons are able to tolerate, to at least some extent, increases in cytosolic stress caused by MAO inhibition. Furthermore, our results suggest that increasing cytosolic dopamine, whether by meth administration or by inhibiting metabolism with a MAO inhibitor, is not inherently degenerative. In order for degeneration to occur multiple sources of mitochondrial stress, for example MAO- and L-type Ca^{2+} channel-dependent mitochondrial stress, are required.

4.2. L-type Ca^{2+} channel activity increased mitochondrial stress in SNc but not VTA axons

One key difference between SNc and VTA dopamine neurons thought to contribute to the differential vulnerability in Parkinson's disease is Ca^{2+} driven differences in somatic mitochondrial stress (Zampese and Surmeier, 2020). In SNc neurons pacemaking activity is associated with Ca^{2+} oscillations, driven primarily by $\text{Ca}_v1.3$ L-type Ca^{2+} channels, that contribute to somatic mitochondrial stress and although VTA neurons have similar pacemaking properties, there is less cytosolic Ca^{2+} and somatic mitochondrial stress (Guzman et al., 2009, 2010, 2018). Whether these differences in intracellular Ca^{2+} reflect differences in levels of $\text{Ca}_v1.3$ L-type Ca^{2+} channel protein expression is unclear; however, somatic L-type Ca^{2+} currents are larger in SNc than VTA dopamine neurons (Philippart et al., 2016). Additionally, compared to the VTA, SNc dopamine neurons have limited Ca^{2+} buffering capacity with low expression of buffering proteins such as calbindin (Alfa-hel-Kakunda and Silverman, 1997; Damier et al., 1999; Fu et al., 2012; Liang et al., 1996; Neuhoff et al., 2002; Poulin et al., 2014; Sequier et al., 1990). Using pharmacological strategies, it has been shown that L-type Ca^{2+} channels modulate dopamine release in both the striatum and nucleus accumbens (Brimblecombe et al., 2015; Koizumi et al., 1995; Okita et al., 2000; Rocchitta et al., 2005), suggesting that SNc and VTA axons express L-type Ca^{2+} channels. However, direct evidence of axonal L-type Ca^{2+} channel expression in SNc and VTA axons is, to the best of our knowledge, lacking. Whether differences in L-type Ca^{2+} currents, expression of L-type Ca^{2+} channels (particularly $\text{Ca}_v1.3$) and/or expression of Ca^{2+} buffering proteins differentiate SNc and VTA axons is unknown. In the present study we show that L-type Ca^{2+} channel activation in SNc axons, but not VTA axons increased mitochondrial stress. SNc axons therefore mirror the Ca^{2+} -induced mitochondrial stress observed in the somatic compartment which is distinct from the MAO-dependent mitochondrial stress present in axons but not in the soma. This indicates that the presence or absence of mitochondrial stress induced by L-type Ca^{2+} channels determines whether neurons are vulnerable or resistant to chronic meth-induced degeneration. Consistent with this, the L-type Ca^{2+} channel negative allosteric modulator isradipine was neuroprotective. Given that meth-induced mitochondrial stress was specific to axons and did not occur in the soma, it suggests that the deleterious effects of meth occur at sites where multiple sources of mitochondrial stress are present, i.e. axons. SNc neurons which have both MAO- and L-type Ca^{2+} channel-dependent axonal mitochondrial stress were vulnerable to chronic meth-induced degeneration whereas VTA neurons, which have MAO- but not L-type Ca^{2+} channel-dependent mitochondrial stress in axons (Fig. 7), were resistant. This suggests that the degenerative process is likely to begin in axonal compartments and progresses to somatic loss in a dying-back pattern of degeneration.

In acute binge paradigms axons are particularly vulnerable (Blaker et al., 2019; Lohr et al., 2015; Ricaurte et al., 1982). Dopamine is vesicularly packaged and released from axon terminals making it logical

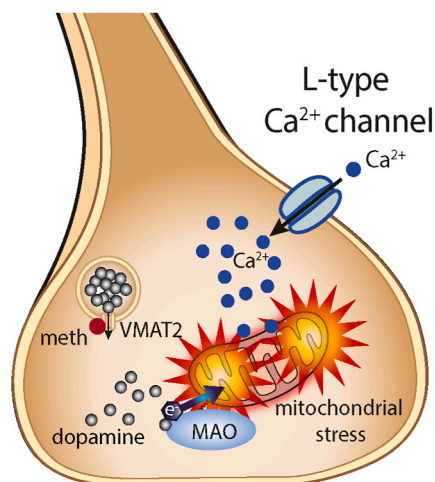
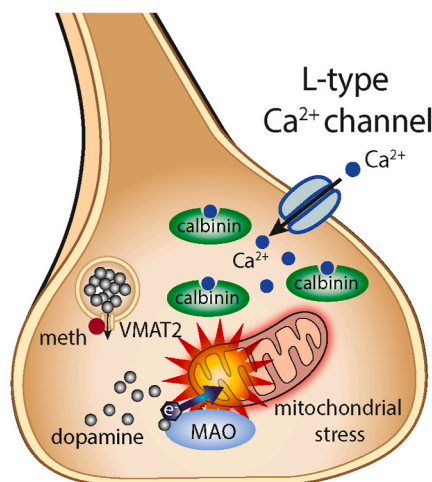
A SNc axons**A** VTA axons

Fig. 7. Differential sources of mitochondrial stress in substantia nigra pars compacta and ventral tegmental area axons. **(A)** Schematic depiction illustrating observed sources of mitochondrial stress in substantia nigra pars compacta (SNc) axons. Methamphetamine (meth) increases cytosolic dopamine via interactions with VMAT2; cytosolic dopamine is then metabolized by mitochondrially tethered monoamine oxidase (MAO) enzymes and electrons generated by MAO deamination of dopamine are transferred to the mitochondrial intermembrane space resulting in mitochondrial stress. Additionally, Ca^{2+} influx through L-type Ca^{2+} channels increase mitochondrial stress in SNc axons. **(B)** Schematic depiction illustrating observed sources of mitochondrial stress in ventral tegmental area (VTA) axons. Like in SNc axons, meth increases cytosolic dopamine resulting in MAO-dependent mitochondrial stress. However, bath application of the L-type Ca^{2+} channel activator Bay K8644 had no effect on mitochondrial stress suggesting that Ca^{2+} influx through L-type Ca^{2+} channels may be insufficient to impact mitochondria or is being buffered by Ca^{2+} binding proteins such as calbindin. The cellular compartment where L-type Ca^{2+} channel- and meth-induced MAO-dependent mito-

chondrial stress occur is in axons (meth did not increase somatic mitochondrial stress). SNc dopamine neurons were vulnerable to chronic meth-induced degeneration indicating that at least two sources of mitochondrial stress (i.e. MAO- and L-type Ca^{2+} channel-dependent) are required for degeneration as blocking either MAO or L-type Ca^{2+} channels was neuroprotective.

that axons and synaptic terminals would be hotspots for meth-induced MAO-dependent mitochondrial stress. However, somatodendritic dopamine release is also an important component of dopamine neuron function (Rice and Patel, 2015) and *in vitro* neurites are particularly vulnerable to the deleterious effects of meth (Cubells et al., 1994; Larsen et al., 2002). Further study is necessary to determine whether meth similarly increases MAO-dependent dendritic mitochondrial stress in dopaminergic neurons; if so, dendrites could be a second anatomical site where MAO- and L-type Ca^{2+} channel-dependent mitochondrial stress converge. If this is the case, a dying-back pattern beginning at both axons and dendrites would be hypothesized resulting in a loss of distal processes (i.e. dendrites and axons) that, with repeated administration, would progress to overt somatic loss as seen with the current 28 day treatment paradigm.

4.3. Isradipine for neuroprotection and addiction pharmacotherapy

Substance use disorders are chronic diseases with high rates of relapse and limited treatment options. Meth is a particularly concerning drug of abuse given that it is not only addictive but also neurotoxic, so much so that meth abuse is associated with an increased risk for developing Parkinson's disease (Callaghan et al., 2010, 2012; Curtin et al., 2015). Additionally, meth abuse results in cognitive deficits that are correlated with decreased DAT binding suggesting that meth-induced cognitive dysfunction may be a consequence of degeneration (McCann et al., 2008). The current report demonstrates the neurodegenerative effect of chronic meth administration, but degeneration also continues despite abstinence (Graves et al., 2021; Kousik et al., 2014). It is unclear whether the damage from meth continues to progress indefinitely if left untreated although in human subjects protracted abstinence may afford some degree of recovery (Volkow et al., 2001a). An ideal treatment strategy for patients with meth use disorders would be one that provides neuroprotection and combats relapse. Isradipine prevented degeneration during chronic meth administration and is also protective when administered during abstinence (Graves et al., 2021). This clinically available medication also blocks acquisition and facilitates extinction training in a conditioned place preference paradigm and attenuates cue-induced reinstatement of cocaine-seeking in rodent models (Addy et al., 2018; Degoulet et al., 2016). In human

subjects isradipine administration decreases some meth-induced positive subjective and reinforcing effects associated with its abuse liability in dependent individuals (Johnson et al., 1999, 2005). L-type Ca^{2+} channel inhibition, using the clinically available isradipine, may therefore serve as a dual-purpose pharmacotherapy that protects against both neurodegeneration and relapse in abstinent individuals.

5. Conclusions

Mitochondrial stress is thought to be a causal factor of many neurodegenerative diseases and underlie the differential vulnerability of neuronal populations (Goldberg et al., 2012; Guzman et al., 2010; Lin and Beal, 2006; Sanchez-Padilla et al., 2014); here we see that mitochondrial stress is necessary for chronic meth-induced SNc degeneration and differentiates the vulnerability of SNc and VTA neurons. Presented data also demonstrate that meth-induced MAO- and L-type Ca^{2+} channel-dependent mitochondrial stress both occur in SNc axons and are each necessary for chronic meth-induced degeneration. Lastly, the clinically available MAO inhibitor rasagiline and L-type Ca^{2+} channel negative allosteric modulator isradipine were neuroprotective with the latter potentially able to also serve as addiction pharmacotherapy.

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Author contributions

SMG and YD designed experiments and drafted the manuscript. SMG, YD, and YBL performed experiments and analyzed data. All authors reviewed and edited the manuscript.

Declaration of competing interest

The authors declare no conflict of interest.

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References

- Addy, N.A., Nunes, E.J., Hughley, S.M., Small, K.M., Baracz, S.J., Haight, J.L., Rajadhyaksha, A.M., 2018. The L-type calcium channel blocker, isradipine, attenuates cue-induced cocaine-seeking by enhancing dopaminergic activity in the ventral tegmental area to nucleus accumbens pathway. *Neuropsychopharmacology* 43, 2361–2372.
- Albers, D.S., Sonsalla, P.K., 1995. Methamphetamine-induced hyperthermia and dopaminergic neurotoxicity in mice: pharmacological profile of protective and nonprotective agents. *J. Pharmacol. Exp. Therapeut.* 275, 1104–1114.
- Alfahel-Kakunda, A., Silverman, W.F., 1997. Calcium-binding proteins in the substantia nigra and ventral tegmental area during development: correlation with dopaminergic compartmentalization. *Brain Res. Dev. Brain Res.* 103, 9–20.
- Ares-Santos, S., Granado, N., Espadas, I., Martinez-Murillo, R., Moratalla, R., 2014. Methamphetamine causes degeneration of dopamine cell bodies and terminals of the nigrostriatal pathway evidenced by silver staining. *Neuropsychopharmacology* 39, 1066–1080.
- Axt, K.J., Commins, D.L., Vosmer, G., Seiden, L.S., 1990. alpha-Methyl-p-tyrosine pretreatment partially prevents methamphetamine-induced endogenous neurotoxin formation. *Brain Res.* 515, 269–276.
- Blaker, A.L., Rodriguez, E.A., Yamamoto, B.K., 2019. Neurotoxicity to dopamine neurons after the serial exposure to alcohol and methamphetamine: protection by COX-2 antagonism. *Brain Behav. Immun.*
- Bourque, M., Dluzen, D.E., Di Paolo, T., 2011a. Male/Female differences in neuroprotection and neuromodulation of brain dopamine. *Front. Endocrinol.* 2, 35.
- Bourque, M., Liu, B., Dluzen, D.E., Di Paolo, T., 2011b. Sex differences in methamphetamine toxicity in mice: effect on brain dopamine signaling pathways. *Psychoneuroendocrinology* 36, 955–969.
- Brimblecombe, K.R., Gracie, C.J., Platt, N.J., Cragg, S.J., 2015. Gating of dopamine transmission by calcium and axonal N-, Q-, T- and L-type voltage-gated calcium channels differs between striatal domains. *J. Physiol.* 593, 929–946.
- Callaghan, R.C., Cunningham, J.K., Sajeev, G., Kish, S.J., 2010. Incidence of Parkinson's disease among hospital patients with methamphetamine-use disorders. *Mov. Disord.* 25, 2333–2339.
- Callaghan, R.C., Cunningham, J.K., Sykes, J., Kish, S.J., 2012. Increased risk of Parkinson's disease in individuals hospitalized with conditions related to the use of methamphetamine or other amphetamine-type drugs. *Drug Alcohol Depend.* 120, 35–40.
- Chan, C.S., Guzman, J.N., Ilijic, E., Mercer, J.N., Rick, C., Tkatch, T., Meredith, G.E., Surmeier, D.J., 2007. 'Rejuvenation' protects neurons in mouse models of Parkinson's disease. *Nature* 447, 1081–1086.
- Chen, Y.H., Wang, V., Huang, E.Y., Chou, Y.C., Kuo, T.T., Olson, L., Hoffer, B.J., 2019. Delayed dopamine dysfunction and motor deficits in female Parkinson model mice. *Int. J. Mol. Sci.* 20.
- Cubells, J.F., Rayport, S., Rajendran, G., Sulzer, D., 1994. Methamphetamine neurotoxicity involves vacuolation of endocytic organelles and dopamine-dependent intracellular oxidative stress. *J. Neurosci.* 14, 2260–2271.
- Curtin, K., Fleckenstein, A.E., Robison, R.J., Crookston, M.J., Smith, K.R., Hanson, G.R., 2015. Methamphetamine/amphetamine abuse and risk of Parkinson's disease in Utah: a population-based assessment. *Drug Alcohol Depend.* 146, 30–38.
- Damier, P., Hirsch, E.C., Agid, Y., Graybiel, A.M., 1999. The substantia nigra of the human brain. I. Nigrosomes and the nigral matrix, a compartmental organization based on calbindin D(28K) immunohistochemistry. *Brain* 122 (Pt 8), 1421–1436.
- Degoulet, M., Stelly, C.E., Ahn, K.C., Morikawa, H., 2016. L-type Ca(2+)-channel blockade with antihypertensive medication disrupts VTA synaptic plasticity and drug-associated contextual memory. *Mol. Psychiatry* 21, 394–402.
- Deniz, O.G., Altun, G., Kaplan, A.A., Yurt, K.K., von Bartheld, C.S., Kaplan, S., 2018. A concise review of optical, physical and isotropic fractionator techniques in neuroscience studies, including recent developments. *J. Neurosci. Methods* 310, 45–53.
- Edmondson, D.E., 2014. Hydrogen peroxide produced by mitochondrial monoamine oxidase catalysis: biological implications. *Curr. Pharmacol. Des.* 20, 155–160.
- Edmondson, D.E., Binda, C., Wang, J., Upadhyay, A.K., Mattevi, A., 2009. Molecular and mechanistic properties of the membrane-bound mitochondrial monoamine oxidases. *Biochemistry* 48, 4220–4230.
- Ekstrand, M.I., Terzioglu, M., Galter, D., Zhu, S., Hofstetter, C., Lindqvist, E., Thams, S., Bergstrand, A., Hansson, F.S., Trifunovic, A., Hoffer, B., Cullheim, S., Mohammed, A. H., Olson, L., Larsson, N.G., 2007. Progressive parkinsonism in mice with respiratory-chain-deficient dopamine neurons. *Proc. Natl. Acad. Sci. U. S. A.* 104, 1325–1330.
- Finberg, J.P., Rabey, J.M., 2016. Inhibitors of MAO-A and MAO-B in psychiatry and neurology. *Front. Pharmacol.* 7, 340.
- Freyberg, Z., Sonders, M.S., Aguilar, J.I., Hiranita, T., Karam, C.S., Flores, J., Pizzo, A.B., Zhang, Y., Farino, Z.J., Chen, A., Martin, C.A., Kopajtic, T.A., Fei, H., Hu, G., Lin, Y. Y., Mosharov, E.V., McCabe, B.D., Freyberg, R., Wimalasena, K., Hsin, L.W., Sames, D., Krantz, D.E., Katz, J.L., Sulzer, D., Javitch, J.A., 2016. Mechanisms of amphetamine action illuminated through optical monitoring of dopamine synaptic vesicles in *Drosophila* brain. *Nat. Commun.* 7, 10652.
- Fu, Y., Yuan, Y., Halliday, G., Rusznak, Z., Watson, C., Paxinos, G., 2012. A cytoarchitectonic and chemoarchitectonic analysis of the dopamine cell groups in the substantia nigra, ventral tegmental area, and retrorubral field in the mouse. *Brain Struct. Funct.* 217, 591–612.
- Fumagalli, F., Gainetdinov, R.R., Wang, Y.M., Valenzano, K.J., Miller, G.W., Caron, M.G., 1999. Increased methamphetamine neurotoxicity in heterozygous vesicular monoamine transporter 2 knock-out mice. *J. Neurosci.* 19, 2424–2431.
- Gibb, W.R., Lees, A.J., 1991. Anatomy, pigmentation, ventral and dorsal subpopulations of the substantia nigra, and differential cell death in Parkinson's disease. *J. Neurol. Neurosurg. Psychiatry* 54, 388–396.
- Goldberg, J.A., Guzman, J.N., Estep, C.M., Ilijic, E., Kondapalli, J., Sanchez-Padilla, J., Surmeier, D.J., 2012. Calcium entry induces mitochondrial oxidant stress in vagal neurons at risk in Parkinson's disease. *Nat. Neurosci.* 15, 1414–1421.
- Goldstein, D.S., 2020. The catecholaldehyde hypothesis: where MAO fits in. *J. Neural. Transm.* 127, 169–177.
- Goldstein, D.S., Sullivan, P., Holmes, C., Miller, G.W., Alter, S., Strong, R., Mash, D.C., Kopin, I.J., Sharabi, Y., 2013. Determinants of buildup of the toxic dopamine metabolite DOPAL in Parkinson's disease. *J. Neurochem.* 126, 591–603.
- Graves, S.M., Schwarzschild, S.E., Tai, R.A., Chen, Y., Surmeier, D.J., 2021. Mitochondrial oxidant stress mediates methamphetamine neurotoxicity in substantia nigra dopaminergic neurons. *Neurobiol. Dis.* 156, 105409.
- Graves, S.M., Xie, Z., Stout, K.A., Zampese, E., Burbulla, L.F., Shih, J.C., Kondapalli, J., Patriarchi, T., Tian, L., Brichta, L., Greengard, P., Krainc, D., Schumacker, P.T., Surmeier, D.J., 2020. Dopamine metabolism by a monoamine oxidase mitochondrial shuttle activates the electron transport chain. *Nat. Neurosci.* 23, 15–20.
- Guillot, T.S., Richardson, J.R., Wang, M.Z., Li, Y.J., Taylor, T.N., Ciliax, B.J., Zachrisson, O., Mercer, A., Miller, G.W., 2008a. PACAP38 increases vesicular monoamine transporter 2 (VMA2) expression and attenuates methamphetamine toxicity. *Neuropeptides* 42, 423–434.
- Guillot, T.S., Shepherd, K.R., Richardson, J.R., Wang, M.Z., Li, Y., Emson, P.C., Miller, G. W., 2008b. Reduced vesicular storage of dopamine exacerbates methamphetamine-induced neurodegeneration and astrogliosis. *J. Neurochem.* 106, 2205–2217.
- Gundersen, H.J., Jensen, E.B., Kieu, K., Nielsen, J., 1999. The efficiency of systematic sampling in stereology—reconsidered. *J. Microsc.* 193, 199–211.
- Guzman, J.N., Ilijic, E., Yang, B., Sanchez-Padilla, J., Wokosin, D., Galtieri, D., Kondapalli, J., Schumacker, P.T., Surmeier, D.J., 2018. Systemic isradipine treatment diminishes calcium-dependent mitochondrial oxidant stress. *J. Clin. Invest.* 128, 2266–2280.
- Guzman, J.N., Sanchez-Padilla, J., Chan, C.S., Surmeier, D.J., 2009. Robust pacemaking in substantia nigra dopaminergic neurons. *J. Neurosci.* 29, 11011–11019.
- Guzman, J.N., Sanchez-Padilla, J., Wokosin, D., Kondapalli, J., Ilijic, E., Schumacker, P. T., Surmeier, D.J., 2010. Oxidant stress evoked by pacemaking in dopaminergic neurons is attenuated by DJ-1. *Nature* 468, 696–700.
- Han, B., Cotto, J., Etz, K., Einstein, E.B., Compton, W.M., Volkow, N.D., 2021. Methamphetamine overdose deaths in the US by sex and race and ethnicity. *JAMA Psychiatry* 78, 564–567.
- Harvey, D.C., Lacan, G., Melegan, W.P., 2000. Regional heterogeneity of dopaminergic deficits in vervet monkey striatum and substantia nigra after methamphetamine exposure. *Exp. Brain Res.* 133, 349–358.
- Hirsch, E., Graybiel, A.M., Agid, Y.A., 1988. Melanized dopaminergic neurons are differentially susceptible to degeneration in Parkinson's disease. *Nature* 334, 345–348.
- Huang, W., Chen, Y., Shohami, E., Weinstock, M., 1999. Neuroprotective effect of rasagiline, a selective monoamine oxidase-B inhibitor, against closed head injury in the mouse. *Eur. J. Pharmacol.* 366, 127–135.
- Ilijic, E., Guzman, J.N., Surmeier, D.J., 2011. The L-type channel antagonist isradipine is neuroprotective in a mouse model of Parkinson's disease. *Neurobiol. Dis.* 43, 364–371.
- Johanson, C.E., Frey, K.A., Lundahl, L.H., Keenan, P., Lockhart, N., Roll, J., Galloway, G. P., Koeppe, R.A., Kilbourn, M.R., Robbins, T., Schuster, C.R., 2006. Cognitive function and nigrostriatal markers in abstinent methamphetamine abusers. *Psychopharmacology (Berlin)* 185, 327–338.
- Johnson, B.A., Roache, J.D., Ait-Daoud, N., Wallace, C., Wells, L., Dawes, M., Wang, Y., 2005. Effects of isradipine, a dihydropyridine-class calcium-channel antagonist, on d-methamphetamine's subjective and reinforcing effects. *Int. J. Neuropsychopharmacol.* 8, 203–213.
- Johnson, B.A., Roache, J.D., Bordnick, P.S., Ait-Daoud, N., 1999. Isradipine, a dihydropyridine-class calcium channel antagonist, attenuates some of d-methamphetamine's positive subjective effects: a preliminary study. *Psychopharmacology (Berlin)* 144, 295–300.
- Kish, S.J., Fitzmaurice, P.S., Boileau, I., Schmunk, G.A., Ang, L.C., Furukawa, Y., Chang, L.J., Wickham, D.J., Sherwin, A., Tong, J., 2009. Brain serotonin transporter in human methamphetamine users. *Psychopharmacology (Berlin)* 202, 649–661.
- Kitamura, O., Tokunaga, I., Gotohda, T., Kubo, S., 2007. Immunohistochemical investigation of dopaminergic terminal markers and caspase-3 activation in the striatum of human methamphetamine users. *Int. J. Leg. Med.* 121, 163–168.
- Koizumi, S., Kataoka, Y., Inoue, K., Kohzuma, M., Niwa, M., Taniyama, K., 1995. Contribution of L-type Ca2+ channels to long-term enhancement of high K(+)-evoked release of dopamine from rat striatal slices. *Neurosci. Lett.* 187, 123–126.
- Kousik, S.M., Carvey, P.M., Napier, T.C., 2014. Methamphetamine self-administration results in persistent dopaminergic pathology: implications for Parkinson's disease risk and reward-seeking. *Eur. J. Neurosci.* 40, 2707–2714.
- Kuhn, D.M., Francescutti-Verbeem, D.M., Thomas, D.M., 2008. Dopamine disposition in the presynaptic process regulates the severity of methamphetamine-induced neurotoxicity. *Ann. N. Y. Acad. Sci.* 1139, 118–126.

- Larsen, K.E., Fon, E.A., Hastings, T.G., Edwards, R.H., Sulzer, D., 2002. Methamphetamine-induced degeneration of dopaminergic neurons involves autophagy and upregulation of dopamine synthesis. *J. Neurosci.* 22, 8951–8960.
- Levitt, P., Pintar, J.E., Breakefield, X.O., 1982. Immunocytochemical demonstration of monoamine oxidase B in brain astrocytes and serotonergic neurons. *Proc. Natl. Acad. Sci. U. S. A.* 79, 6385–6389.
- Liang, C.L., Sinton, C.M., German, D.C., 1996. Midbrain dopaminergic neurons in the mouse: co-localization with Calbindin-D28K and calretinin. *Neuroscience* 75, 523–533.
- Lin, M.T., Beal, M.F., 2006. Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. *Nature* 443, 787–795.
- Liu, B., Dlužen, D.E., 2006. Effect of estrogen upon methamphetamine-induced neurotoxicity within the impaired nigrostriatal dopaminergic system. *Synapse* 60, 354–361.
- Lohr, K.M., Stout, K.A., Dunn, A.R., Wang, M., Salahpour, A., Guillot, T.S., Miller, G.W., 2015. Increased vesicular monoamine transporter 2 (VMAT2; Slc18a2) protects against methamphetamine toxicity. *ACS Chem. Neurosci.* 6, 790–799.
- McCann, U.D., Kuwabara, H., Kumar, A., Palermo, M., Abbey, R., Brasic, J., Ye, W., Alexander, M., Dannals, R.F., Wong, D.F., Ricaurte, G.A., 2008. Persistent cognitive and dopamine transporter deficits in abstinent methamphetamine users. *Synapse* 62, 91–100.
- McCann, U.D., Wong, D.F., Yokoi, F., Villemagne, V., Dannals, R.F., Ricaurte, G.A., 1998. Reduced striatal dopamine transporter density in abstinent methamphetamine and methcathinone users: evidence from positron emission tomography studies with [¹¹C]WIN-35,428. *J. Neurosci.* 18, 8417–8422.
- Moratala, R., Khairnar, A., Simola, N., Granado, N., Garcia-Montes, J.R., Porceddu, P.F., Tizabi, Y., Costa, G., Morelli, M., 2017. Amphetamine-related drugs neurotoxicity in humans and in experimental animals: main mechanisms. *Prog. Neurobiol.* 155, 149–170.
- Moszczynska, A., Fitzmaurice, P., Ang, L., Kalasinsky, K.S., Schmunk, G.A., Peretti, F.J., Aiken, S.S., Wickham, D.J., Kish, S.J., 2004. Why is parkinsonism not a feature of human methamphetamine users? *Brain* 127, 363–370.
- Neuhoff, H., Neu, A., Liss, B., Roeper, J., 2002. I(h) channels contribute to the different functional properties of identified dopaminergic subpopulations in the midbrain. *J. Neurosci.* 22, 1290–1302.
- Nirenberg, M.J., Liu, Y., Peter, D., Edwards, R.H., Pickel, V.M., 1995. The vesicular monoamine transporter 2 is present in small synaptic vesicles and preferentially localizes to large dense core vesicles in rat solitary tract nuclei. *Proc. Natl. Acad. Sci. U. S. A.* 92, 8773–8777.
- NSDUH, Center for Behavioral Health Statistics and Quality, 2018. 2017 National Survey on Drug Use and Health: Detailed Tables. Substance Abuse and Mental Health Services Administration, Rockville, MD.
- Okita, M., Watanabe, Y., Taya, K., Utsumi, H., Hayashi, T., 2000. Presynaptic L-type Ca²⁺ channels on excessive dopamine release from rat caudate putamen. *Physiol. Behav.* 68, 641–649.
- Philippart, F., Destreel, G., Merino-Sepulveda, P., Henny, P., Engel, D., Seutin, V., 2016. Differential somatic Ca²⁺ channel profile in midbrain dopaminergic neurons. *J. Neurosci.* 36, 7234–7245.
- Pickel, V.M., Nirenberg, M.J., Milner, T.A., 1996. Ultrastructural view of central catecholaminergic transmission: immunocytochemical localization of synthesizing enzymes, transporters and receptors. *J. Neurocytol.* 25, 843–856.
- Poulin, J.F., Zou, J., Drouin-Ouellet, J., Kim, K.Y., Cicchetti, F., Awatramani, R.B., 2014. Defining midbrain dopaminergic neuron diversity by single-cell gene expression profiling. *Cell Rep.* 9, 930–943.
- Ricaurte, G.A., Guillery, R.W., Seiden, L.S., Schuster, C.R., Moore, R.Y., 1982. Dopamine nerve terminal degeneration produced by high doses of methylamphetamine in the rat brain. *Brain Res.* 235, 93–103.
- Rice, M.E., Patel, J.C., 2015. Somatodendritic dopamine release: recent mechanistic insights. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 370.
- Ricke, K.M., Pass, T., Kimoloi, S., Fahrman, K., Jungst, C., Schauss, A., Baris, O.R., Aradjanski, M., Trifunovic, A., Eriksson Faelker, T.M., Bergami, M., Wiesner, R.J., 2020. Mitochondrial dysfunction combined with high calcium load leads to impaired antioxidant defense underlying the selective loss of nigral dopaminergic neurons. *J. Neurosci.* 40, 1975–1986.
- Rocchitta, G., Migheli, R., Mura, M.P., Grella, G., Esposito, G., Marchetti, B., Miele, E., Desole, M.S., Miele, M., Serra, P.A., 2005. Signaling pathways in the nitric oxide and iron-induced dopamine release in the striatum of freely moving rats: role of extracellular Ca²⁺ and L-type Ca²⁺ channels. *Brain Res.* 1047, 18–29.
- Rumpf, J.J., Albers, J., Fricke, C., Mueller, W., Classen, J., 2017. Structural abnormality of substantia nigra induced by methamphetamine abuse. *Mov. Disord.* 32, 1784–1788.
- Sabharwal, S.S., Waypa, G.B., Marks, J.D., Schumacker, P.T., 2013. Peroxiredoxin-5 targeted to the mitochondrial intermembrane space attenuates hypoxia-induced reactive oxygen species signalling. *Biochem. J.* 456, 337–346.
- Salvatore, M.F., Pruet, B.S., 2012. Dichotomy of tyrosine hydroxylase and dopamine regulation between somatodendritic and terminal field areas of nigrostriatal and mesoaccumbens pathways. *PLoS One* 7, e29867.
- Salvatore, M.F., Pruet, B.S., Spann, S.L., Dempsey, C., 2009. Aging reveals a role for nigral tyrosine hydroxylase ser31 phosphorylation in locomotor activity generation. *PLoS One* 4, e8466.
- Sanchez-Padilla, J., Guzman, J.N., Ilijic, E., Kondapalli, J., Galtieri, D.J., Yang, B., Schieber, S., Oertel, W., Wokosin, D., Schumacker, P.T., Surmeier, D.J., 2014. Mitochondrial oxidant stress in locus coeruleus is regulated by activity and nitric oxide synthase. *Nat. Neurosci.* 17, 832–840.
- Schmidt, C.J., Ritter, J.K., Sonsalla, P.K., Hanson, G.R., Gibb, J.W., 1985. Role of dopamine in the neurotoxic effects of methamphetamine. *J. Pharmacol. Exp. Therapeut.* 233, 539–544.
- Sekine, Y., Iyo, M., Ouchi, Y., Matsunaga, T., Tsukada, H., Okada, H., Yoshikawa, E., Futatsubashi, M., Takei, N., Mori, N., 2001. Methamphetamine-related psychiatric symptoms and reduced brain dopamine transporters studied with PET. *Am. J. Psychiatr.* 158, 1206–1214.
- Sequier, J.M., Hunziker, W., Andressen, C., Celio, M.R., 1990. Calbindin D-28k protein and mRNA localization in the rat brain. *Eur. J. Neurosci.* 2, 1118–1126.
- Shearer, R.D., Howell, B.A., Bart, G., Winkelman, T.N.A., 2020. Substance use patterns and health profiles among US adults who use opioids, methamphetamine, or both, 2015–2018. *Drug Alcohol Depend.* 214, 108162.
- Sonsalla, P.K., Jochowitz, N.D., Zeevalk, G.D., Oostveen, J.A., Hall, E.D., 1996. Treatment of mice with methamphetamine produces cell loss in the substantia nigra. *Brain Res.* 738, 172–175.
- Sulzer, D., Sonders, M.S., Poulsen, N.W., Galli, A., 2005. Mechanisms of neurotransmitter release by amphetamines: a review. *Prog. Neurobiol.* 75, 406–433.
- Sulzer, D., Zecca, L., 2000. Intraneuronal dopamine-quinone synthesis: a review. *Neurotox. Res.* 1, 181–195.
- Surmeier, D.J., Obeso, J.A., Halliday, G.M., 2017. Selective neuronal vulnerability in Parkinson disease. *Nat. Rev. Neurosci.* 18, 101–113.
- Thomas, D.M., Francescutti-Verbeem, D.M., Kuhn, D.M., 2008. The newly synthesized pool of dopamine determines the severity of methamphetamine-induced neurotoxicity. *J. Neurochem.* 105, 605–616.
- Thomas, D.M., Francescutti-Verbeem, D.M., Kuhn, D.M., 2009. Increases in cytoplasmic dopamine compromise the normal resistance of the nucleus accumbens to methamphetamine neurotoxicity. *J. Neurochem.* 109, 1745–1755.
- Todd, G., Noyes, C., Flavel, S.C., Della Vedova, C.B., Spyropoulos, P., Chatterton, B., Berg, D., White, J.M., 2013. Illicit stimulant use is associated with abnormal substantia nigra morphology in humans. *PLoS One* 8, e56438.
- Todd, G., Pearson-Dennett, V., Wilcox, R.A., Chau, M.T., Thoirs, K., Thewlis, D., Vogel, A.P., White, J.M., 2016. Adults with a history of illicit amphetamine use exhibit abnormal substantia nigra morphology and parkinsonism. *Park. Relat. Disord.* 25, 27–32.
- Volkow, N.D., Chang, L., Wang, G.J., Fowler, J.S., Franceschi, D., Sedler, M., Gatley, S.J., Miller, E., Hitzemann, R., Ding, Y.S., Logan, J., 2001a. Loss of dopamine transporters in methamphetamine abusers recovers with protracted abstinence. *J. Neurosci.* 21, 9414–9418.
- Volkow, N.D., Chang, L., Wang, G.J., Fowler, J.S., Leonido-Yee, M., Franceschi, D., Sedler, M.J., Gatley, S.J., Hitzemann, R., Ding, Y.S., Logan, J., Wong, C., Miller, E.N., 2001b. Association of dopamine transporter reduction with psychomotor impairment in methamphetamine abusers. *Am. J. Psychiatr.* 158, 377–382.
- Wagner, G.C., Lucot, J.B., Schuster, C.R., Seiden, L.S., 1983. Alpha-methyltyrosine attenuates and reserpine increases methamphetamine-induced neuronal changes. *Brain Res.* 270, 285–288.
- Wagner, G.C., Walsh, S.L., 1991. Evaluation of the effects of inhibition of monoamine oxidase and senescence on methamphetamine-induced neuronal damage. *Int. J. Dev. Neurosci.* 9, 171–174.
- West, M.J., 2018. Space balls revisited: stereological estimates of length with virtual isotropic surface probes. *Front. Neuroanat.* 12, 49.
- Westlund, K.N., Denney, R.M., Rose, R.M., Abell, C.W., 1988. Localization of distinct monoamine oxidase A and monoamine oxidase B cell populations in human brainstem. *Neuroscience* 25, 439–456.
- Wilson, J.M., Kalasinsky, K.S., Levey, A.I., Bergeron, C., Reiber, G., Anthony, R.M., Schmunk, G.A., Shannak, K., Haycock, J.W., Kish, S.J., 1996. Striatal dopamine nerve terminal markers in human, chronic methamphetamine users. *Nat. Med.* 2, 699–703.
- Winkelman, T.N.A., Admon, L.K., Jennings, L., Shippee, N.D., Richardson, C.R., Bart, G., 2018. Evaluation of amphetamine-related hospitalizations and associated clinical outcomes and costs in the United States. *JAMA Netw. Open* 1, e183758.
- Woodard, C.M., Campos, B.A., Kuo, S.H., Nirenberg, M.J., Nestor, M.W., Zimmer, M., Mosharov, E.V., Sulzer, D., Zhou, H., Paull, D., Clark, L., Schadt, E.E., Sardi, S.P., Rubin, L., Eggan, K., Brock, M., Lipnick, S., Rao, M., Chang, S., Li, A., Noggle, S.A., 2014. iPSC-derived dopamine neurons reveal differences between monozygotic twins discordant for Parkinson's disease. *Cell Rep.* 9, 1173–1182.
- Youdim, M.B., Edmondson, D., Tipton, K.F., 2006. The therapeutic potential of monoamine oxidase inhibitors. *Nat. Rev. Neurosci.* 7, 295–309.
- Zampese, E., Surmeier, D.J., 2020. Calcium, bioenergetics, and Parkinson's disease. *Cells* 9.
- Zhang, Y., Angulo, J.A., 1996. Contrasting effects of repeated treatment vs. withdrawal of methamphetamine on tyrosine hydroxylase messenger RNA levels in the ventral tegmental area and substantia nigra zona compacta of the rat brain. *Synapse* 24, 218–223.