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DOPAMINE: From Release and Modulation to Brain Diseases

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Inhibition of striatal dopamine release by the L-type calcium channel inhibitor isradipine co-varies with risk factors for Parkinson's

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Abstract

Ca^{2+} entry into nigrostriatal dopamine (DA) neurons and axons via L-type voltage-gated Ca^{2+} channels (LTCCs) contributes, respectively, to pacemaker activity and DA release and has long been thought to contribute to vulnerability to degeneration in Parkinson's disease. LTCC function is greater in DA axons and neurons from substantia nigra pars compacta than from ventral tegmental area, but this is not explained by channel expression level. We tested the hypothesis that LTCC control of DA release is governed rather by local mechanisms, focussing on candidate biological factors known to operate differently between types of DA neurons and/or be associated with their differing vulnerability to parkinsonism, including biological sex, α-synuclein, DA transporters (DATs) and calbindin-D28k (Calb1). We detected evoked DA release ex vivo in mouse striatal slices using fast-scan cyclic voltammetry and assessed LTCC support of DA release by detecting the inhibition of DA release by the LTCC inhibitors isradipine or CP8. Using genetic knockouts or pharmacological manipulations, we identified that striatal LTCC support of DA release depended on multiple intersecting factors, in a regionally and sexually divergent manner. LTCC function was promoted by factors associated with Parkinsonian risk, including male sex, α-synuclein, DAT and a dorsolateral co-ordinate, but limited by factors associated with protection, that is, female sex, glucocerebrosidase activity, Calb1 and ventromedial co-ordinate. Together, these data show that LTCC function in DA axons and isradipine effect are locally governed and suggest they vary in a manner that in turn might impact on, or reflect, the cellular stress that leads to parkinsonian degeneration.

KEYWORDS
Calb1, dopamine D2 receptor, dopamine release, dopamine transporter, L-type calcium channel, Parkinson's disease, sex differences, striatum, synuclein

1 | INTRODUCTION

Ca^{2+} entry via voltage-gated Ca^{2+} channels (VGCCs) is important for pacemaker activity in dopamine (DA) neurons within the substantia nigra pars compacta (SNc) and critical for release of DA in the striatum (Brimblecombe et al., 2015; Guzman et al., 2009). However, intracellular Ca^{2+} also generates metabolic stress that promotes vulnerability of SNc DA neurons to degeneration in Parkinson's disease (PD) (Surmeier et al., 2010). SNc DA neurons and their axons in dorsolateral striatum (DLS) are particularly vulnerable to parkinsonian degeneration, whereas DA neurons in ventral tegmental area (VTA) and their axons in ventral striatum (nucleus accumbens, NAc) are relatively spared. L-type VGCCs (LTCCs) support pacemaker activity in SNc predominantly via the Cav1.3 subtype (Guzman et al., 2009; Philippart et al., 2016) and by facilitating ATP production (Zampese et al., 2022) and also support DA release from axons in DLS (Brimblecombe et al., 2015). By contrast, in DA neurons in VTA and DA axons in NAc, LTCCs do not operate strongly (Brimblecombe et al., 2015; Guzman et al., 2009; Philippart et al., 2016; Zampese et al., 2022). However, this regional difference is not reflected by LTCC gene expression at the level of the soma at least: the dominant subtype of LTCCs in DA soma in SNc and VTA is thought to be the Cav1.3 rather than Cav1.2 subtype (Philippart et al., 2016), but Cav1.3 gene expression is more enriched in VTA than in SNc (Poulin et al., 2014). The identity of the subtype operating in axons is not yet known and cannot be determined pharmacologically. The potential discrepancies between expression and function suggests that regulatory mechanisms locally govern function. Here, we explored factors that locally regulate the LTCC support of DA release from striatal DA axons in DLS vs NAc core.

Several biological factors and mechanisms that have direct relevance to the biology of DA neurons and/or to PD are candidates for LTCC regulators. On a population level, after ageing, the biggest risk factor for PD is biological sex: men are twice as likely as women to develop PD (Wooten et al., 2004), but the biology underlying this difference is poorly understood. It has been established in cardiac myocytes that LTCC function is greater in males than females (Curl et al., 2008), but sex differences in LTCC function in central DA systems have not previously been explored. Here, we explored how LTCCs support DA release in both sexes of mice and define sex differences.

Several genetic mutations and polymorphisms confer an increased risk to PD (Bekris et al., 2010). These range from rare, high penetrance mutations, for example, in the genes for α-synuclein (*SNCA*) and PINK-1/Parkin, to more common, less penetrant single-nucleotide polymorphisms, discovered by GWAS including *GBA* (gene encoding glucocerebrosidase, GCase) as the most commonly associated genetic risk factor. Interestingly, variants in *SNCA* occur as both rare, high penetrance and common, low penetrance forms. Here, we tested whether α-synuclein or GCase levels could impact on LTCC support of DA release.

We also tested whether several known physiological regulators of DA output from SNc and VTA DA neurons might support a differential contribution of LTCCs to DA release. We focussed on calbindin-D28K (Calb1), the DAT and DA D_2-receptors (D_2Rs), each of which have been reported to interact with LTCCs in different systems. Calb1 is enriched in PD-resistant VTA neurons (Gerfen et al., 1987; Poulin et al., 2014), limits DA release in NAc (Brimblecombe et al., 2019) and, in cellular expression systems, can bind to and limit function of LTCCs (Lee et al., 2006). We report here that Calb1 limits LTCC support of DA release in a regionally and sexually divergent manner. DATs are enriched in SNc and VTA DA neurons, with highest expression levels reported in SNc, in both mouse and human brains (Ciliax et al., 1995; Poulin et al., 2014), and differential DAT expression has long been hypothesised to be a driving factor in sensitivity to PD on cellular and population levels (Lohr et al., 2017; Ritz et al., 2009). In cellular expression systems, DATs and other electrogenic monoamine transporters promote LTCC currents (Cameron et al., 2015). We report here that DATs play a role in facilitating how LTCCs support axonal DA release. Lastly, D2Rs are described as more enriched in SNc than VTA DA neurons (Greene et al., 2005; Poulin et al., 2014) and can inhibit LTCC function (via βγ-inhibition) in striatal GABAergic projection neurons (Hernández-López et al., 2000; Olson et al., 2005). Despite D2Rs not being associated with either risk or protection in PD (not least due to their broad expression pattern), they are well placed to modulate LTCC support of DA release. We report here that D2Rs operate a limiting factor on LTCC function in NAc. Overall, we reveal a network of factors that, by either inhibiting or facilitating LTCC function in

DA axons, contribute to a greater role for LTCCs in supporting DA release in DLS than NAc, and in males than females, and a correspondingly varying outcome of LTCC inhibitor isradipine on DA release.

2 | MATERIALS AND METHODS

2.1 | Animals

We used male and female adult mice aged 3–10 months. Wild-type mice were C57BL/6 J (Charles River, RRID:IMSR_JAX:000664). Calbindin-knockdown (CalbKD) animals were generated by crossing homozygous $DAT^{IREScre}$ (https://www.jax.org/strain/006660, RRID:IMSR_JAX:006660) mice with homozygous $Calb_{TM2}$ mice (loxP sites flanking Exon1) (Barski et al., 2002) donated by Prof. Meyer (University of Munich) (available from Jax https://www.jax.org/strain/031936 and EMMA: strain name B6.[R1]-Calb1tm2Mpin, RRID:IMSR_JAX:031936), generating double-heterozygous offspring with decreased calb1 expression in DAT-expressing cells as previously published (Brimblecombe et al., 2019). CalbWT control mice were age- and sex-matched heterozygous $DAT^{IREScre}$ mice. *SNCA*-OVX and *Snca*-null mice were generated in house as previously published (Janezic et al., 2013) (OVX https://www.jax.org/strain/023837, RRID:IMSR_JAX:023837; null https://www.jax.org/strain/003692, RRID:IMSR_JAX:003692). $Gba^{+/-}$ mice were purchased from Jax (https://www.jax.org/strain/002594, RRID:IMSR_JAX:002594).

2.2 | Slice preparation

Mice were killed by cervical dislocation, the brains removed and 300 μm coronal striatal slices prepared as described previously (Brimblecombe et al., 2015) in ice-cold HEPES-based buffer saturated with 95% O_2/5% CO_2, containing in mM: 120 NaCl, 20 $NaHCO_3$, 6.7 HEPES acid, 5 KCl, 3.3 HEPES salt, 2 $CaCl_2$, 2 $MgSO_4$, 1.2 KH_2PO_4, 10 glucose. Slices were incubated at room temperature for ≥1 h in HEPES-based buffer before experiments. All procedures were licenced to be carried out at the University of Oxford under the UK Animals (Scientific Procedures) Act 1986.

2.3 | Fast-scan cyclic voltammetry

Ex vivo DA release was monitored in acute coronal slices using fast-scan cyclic voltammetry (FCV) as previously described (Brimblecombe et al., 2015). Slices were superfused in a recording chamber with bicarbonate-buffered artificial cerebrospinal fluid (aCSF) saturated with 95% O_2/5%CO_2 at 31–33°C, containing in mM: 124 NaCl, 26 $NaHCO_3$, 3.8 KCl, .8–3.6 $CaCl_2$ (as stated), 1.3 $MgSO_4$, 1.3 KH_2PO_4, 10 glucose. Evoked extracellular DA concentration ($[DA]_o$) was monitored by FCV using a Millar voltammeter (Julian Millar, Barts and the London School of Medicine and Dentistry) and single-use carbon-fibre microelectrodes (7–10 μm diameter) fabricated in-house (tip length 50–100 μm). A triangular voltage waveform (range −700 to +1,300 mV vs. Ag/AgCl) was applied at 800 V/s at a scan frequency of 8 Hz. Electrodes were switched out of circuit between scans. Electrodes were calibrated using 2 μM DA, prepared immediately before calibration using stock solution (2.5 mM in .1 M $HClO_4$ stored at 4°C). Signals were attributed to DA due to the potentials of their characteristic oxidation (500–600 mV) and reduction (−200 mV) peaks. Step-by-step methods can be found at protocols.io (10.17504/protocols.io.4r3l27dxxg1y/v1).

2.4 | Electrical stimulation

DA recordings were obtained from dorsolateral quadrant of dorsal striatum, the dorsolateral striatum (DLS), or nucleus accumbens core (NAc). DA release was evoked by a local bipolar concentric Pt/Ir electrode (25 μm diameter; FHC inc. ME, USA) placed approximately 100 μm from the recording electrode. Stimulus pulses (200 μs duration) were given at .6 mA (perimaximal in drug-free control conditions). Stimulations were single pulses (1p) and were repeated at 2.5 min intervals. A stable pre-drug baseline of six stimulations before isradipine or CP8 was washed on for 12 stimulations. A stable baseline was determined during the experiment when the average peak oxidation for stimulus 5 and 6 was 95–105% of stimulus 1 and 2. All data were obtained in the presence of the nAChR antagonist, dihydro-β-erythroidine (DHβE, 1 μM) to exclude the strong effects of nAChRs on DA release (Cachope et al., 2012; Rice & Cragg, 2004; Threlfell et al., 2012; Zhou et al., 2001).

2.5 | Drugs and solutions

Reagents were purchased from Torcis or Sigma Aldrich (cocaine, water-soluble cholesterol). Stock solutions were made to 1,000–2,000× final concentrations in H_2O (DHβE, cocaine), DMSO (isradipine, L-741,626, CP8), .1 M HCl (nomifensine) or ethanol (lidocaine) and stored

at −20°C. Drugs were diluted to their required concentrations in aCSF immediately prior to use. Drug concentrations were chosen in accordance with previous studies (Acevedo-Rodriguez et al., 2014; Brimblecombe et al., 2015). Water soluble cholesterol or vehicle control Me-ß-cyclodextrin were dissolved in 10 ml aCSF, and slices were incubated and oxygenated for 30 min before transferring to recording chamber as previously described (Threlfell et al., 2021).

2.6 | CP8 synthesis

According to the method (Kang et al., 2013), 2-(3-chlorophenyl)ethylamine (357 µl, 2.58 mmol, 1.0 eq.) was added in one portion to a solution of cyclopentane isocyanate (290 mg, 2.58 mmol, 1.0 eq.) in CH_2Cl_2 (10 ml), and the reaction was stirred at room temperature for 5 h (completion was monitored by LRMS). Malonyl chloride (276 µl, 2.84 mmol, 1.1 eq.) was then added dropwise under vigorous stirring over 5 min. The resulting mixture was stirred for 1 h and concentrated in vacuo. The residue was purified by column chromatography on silica gel (EtOAc/pentane, 1:4) to afford the title product as a white solid (687 mg, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25–7.19 (m, 3H), 7.13 (dt, $J = 6.7$, 2.1 Hz, 1H), 5.20–5.07 (m, 1H), 4.13–4.03 (m, 2H), 3.62 (s, 2H), 2.92–2.84 (m, 2H), 1.94 (tqd, $J = 8.0$, 4.9, 2.0 Hz, 4H), 1.89–1.79 (m, 2H), 1.61–1.57 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.9, 164.6, 151.1, 140.0, 134.5, 123.0, 129.2, 127.3, 127.1, 54.6, 42.8, 40.3, 33.8, 28.8, 25.7; LRMS m/z (ESI) 333.1 [M-H]. The data were in accordance with those reported in the literature (Kang et al., 2013).

2.7 | Data and statistical analysis

FCV data were acquired and analysed using Axoscope 10.5 (Molecular devices) and Excel macros written locally. Data are expressed as mean ± standard error of the mean (SEM), and N = number of slices (typically 1 slice used per animal for any given experiment) from a minimum of 3 animals. Data from each slice were obtained by averaging four recordings prior to drug wash-on (control) versus average of four recordings after 20 min. Typically, data are shown normalised to control conditions to standardise for variation in initial evoked extracellular DA concentrations ($[DA]_o$). Population means were compared using one- or two-way ANOVA with Dunnett's post-test and unpaired t-test where appropriate using GraphPad Prism (9.3.1). Curve fits were performed in GraphPad Prism. Data for all figures are available at Zenodo (10.5281/zenodo.7801867).

2.8 | Classification tree

A classification tree was performed in R(4.2.1) (10.5281/zenodo.7801867). The normalised effect of isradipine for each individual experiment was annotated with the experimental condition: sex and genotype of mouse and presence of drug prior to washing on isradipine (fig. 5 data tab in xlsx file 10.5281/zenodo.7801867). These data were sorted using a classification tree using Gini impurity algorithm with a cut off of .91 normalised effect of isradipine, which corresponds to 2 s.d. above the mean effect of isradipine in DLS of male WT mouse (i.e., the original recording conditions where we identify a significant effect of isradipine on decreasing DA; Brimblecombe et al., 2015). It should be noted that .91 is also 2 s.d. below mean normalised effect of isradipine in male WT NAc (2.4 mM $[Ca^{2+}]_o$), which was our original recording conditions when we did not identify an effect of isradipine. Due to the structure of the classification tree whereby, factors were effectively sorted with no outliers (i.e., the leaves [final nodes] were mostly pure with probabilities of 0 or 1), we determined it was unsuitable to run a logistic regression analysis on these data.

2.9 | Western blots

Mice were culled by cervical dislocation, brains extracted and dissected on ice. The striatum and midbrain were extracted from both hemispheres and snap frozen. Samples were prepared for western blot as previously described (Connor-Robson et al., 2019). Briefly, tissue was homogenised on ice in RIPA buffer containing protease inhibitors (Roche) and protein concentration determined by BCA assay. Following dilution, Laemmli buffer was added, and samples were boiled for 5 min at 95°C. Samples were loaded and run on 4–15% criterion-TGX gels and transferred to PDVF membranes (BioRad). Membranes were blocked for 1 h at room temperature and then probed with primary antibody (calb1: 1:1,000 Cell Signalling #13176) overnight at 4°C. After incubation with HRP conjugated secondary antibodies, the blot was developed using chemiluminescent HRP substrate.

3 | RESULTS

3.1 | Sex difference in the LTCC support of DA release

We and others have to date principally explored LTCC function in males rather than females (Brimblecombe

et al., 2015; Guzman et al., 2009, 2018; Ilijic et al., 2011). There is an ~2:1 prevalence of PD in males and females, and it is therefore important to understand the biology of LTCCs in both sexes, in order to gain a fuller understanding of disease aetiology but also drug action. Here, we tested whether LTCCs support DA release in DLS of female mice, which they do in male mice (Brimblecombe et al., 2015). We first assessed DA release and uptake parameters. We found no difference in peak evoked extracellular concentrations of DA ($[DA]_o$) evoked by single electrical stimulus pulses (1p) (Figure 1a,b, t-test $t_{14} = .14$ $P = .89$) but did find faster DA clearance rates in females (Figure 1a,c, T-test $t_{10} = 4.15$ $P = .002$), consistent with greater DAT levels in the striatum of female mice (Costa et al., 2021). We then tested the effect of LTCC inhibitor isradipine (5 μM) on 1p-evoked $[DA]_o$ in DLS and confirmed a decrease in evoked $[DA]_o$ in male mice (Figure 1d, comparison of normalised peak $[DA]_o$, one-sample t-test $t_2 = 5.6$, $P = .03$, N = 3) as published previously (Brimblecombe et al., 2015) but, by contrast, not in female mice (Figure 1d, comparison of normalised peak $[DA]_o$, one-sample t-test $t_4 = .87$, $P = .44$, N = 5), revealing a statistically significant difference between the two sexes (Figure 1e, two-way ANOVA: sex X drug interaction, $F_{16,67} = 3.0$, $P < .001$; main effect of sex, $F_{1,67} = 44.9$, $P < .001$).

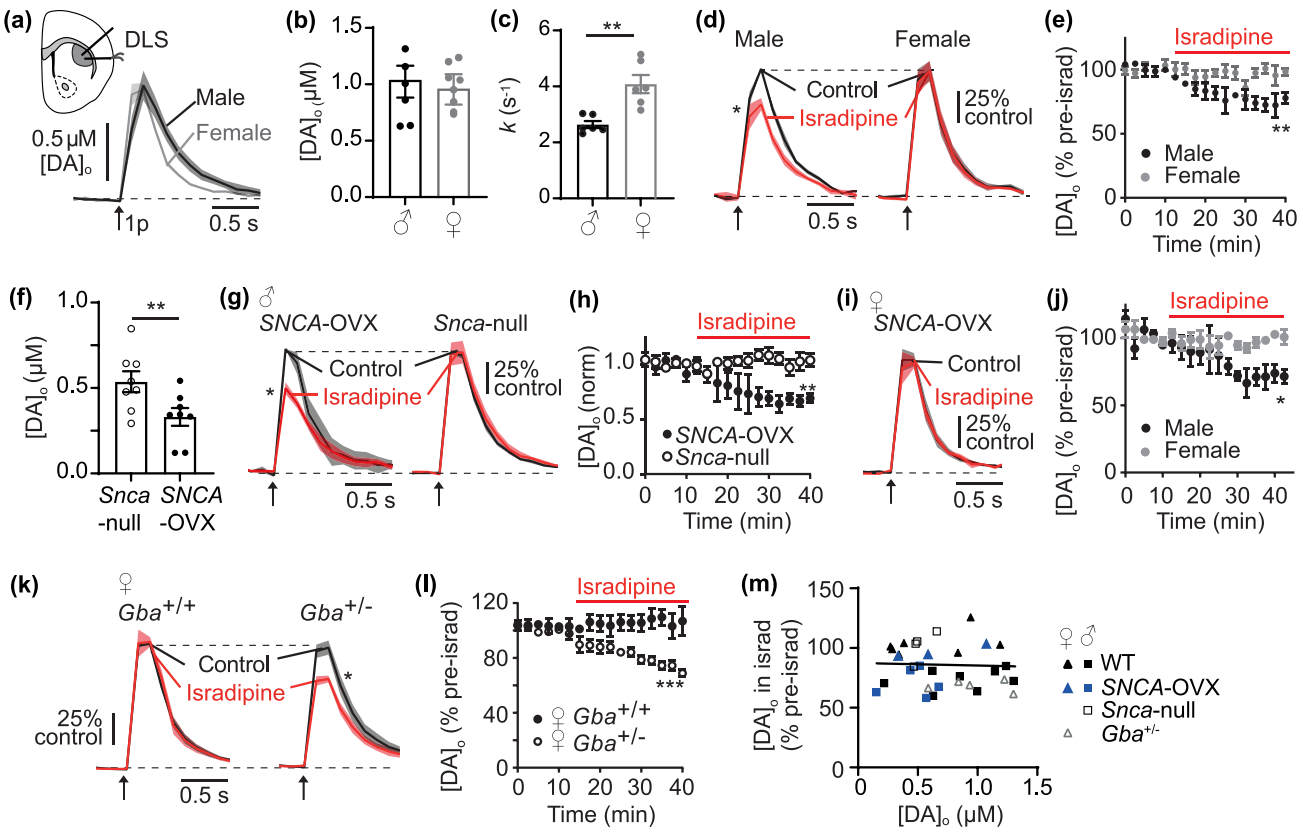


FIGURE 1 Male sex, presence of α-synuclein and knockdown of *Gba* promote LTCC support of DA release in DLS. (a) Mean $[DA]_o$ ± SEM versus time evoked by a single pulse (1p, *arrow*) in DLS of male (*black*) versus female (*grey*) wild-type mice. (b) Summary of mean peak evoked $[DA]_o$ ± SEM, N = 6 mice per sex. (c) Decay constants of falling phases of mean 1p-evoked DA transients in DLS. N = 6 mice per sex. (d) Mean transients for $[DA]_o$ ± SEM versus time evoked by 1p (*arrow*) in control conditions (*black*) versus isradipine (5 μM, *red*) in male (*left*) and female (*right*) normalised to own control. (e) Peak $[DA]_o$ ± SEM as % of pre-isradipine control levels versus time. N = 3 male, 5 female. (f) Summary of peak $[DA]_o$ evoked by 1p in DLS from male *SNCA*-OVX vs *Snca*-null mice. N = 8 mice. (g) Mean $[DA]_o$ ± SEM versus time evoked by 1p (*arrow*) in control versus isradipine in *SNCA*-OVX (*left*) and *Snca*-null (*right*), normalised to control. (h) Peak $[DA]_o$ ± SEM as % of pre-isradipine control levels versus time. N = 4 mice. (i) Mean $[DA]_o$ ± SEM versus time evoked by 1p (*arrow*) in control and isradipine in female SNCA-OVX mice, normalised to control. N = 3 mice. (j) Peak $[DA]_o$ ± SEM as % of pre-isradipine control levels versus time of female vs male *SNCA*-OVX mice. (k) Mean $[DA]_o$ ± SEM versus time evoked by 1p (*arrow*) in DLS in control and isradipine in female wild-type ($Gba^{+/+}$) versus *Gba*-het ($Gba^{+/-}$) mice, normalised to control. (l) Peak $[DA]_o$ ± SEM as % of pre-isradipine control levels versus time for female $Gba^{+/+}$ (N = 3) versus $Gba^{+/-}$ mice (N = 5). (m) Peak $[DA]_o$ in isradipine (as % of pre-isradipine control levels) versus peak $[DA]_o$ (μM) in pre-isradipine control conditions plotted for all data shown in conditions in A-L. Male = squares; female = triangles; genotypes indicated by colour. *<.05, **<.01, ***<.001.

3.2 | Genetic contributors to PD promote LTCC support of DA release

Mutations in the *SNCA* gene including common, lower penetrance SNPs identified by GWAS (Alegre-Abarrategui et al., 2019) are associated with rare monogenic forms of PD, while mutations in *GBA* are the most common genetic factor in PD. We tested whether α-synuclein facilitates LTCC support of DA release, using a BAC transgenic mouse line that overexpresses human wild-type α-synuclein (*SNCA*-OVX) (Janezic et al., 2013), versus the background control line that are null for mouse α-synuclein (*Snca*-null). Mean peak $[DA]_o$ evoked by 1p in DLS of *SNCA*-OVX mice was ~30% less than *Snca*-null mice (Figure 1f, unpaired t-test $t_{14} = 2.55$ $P = .023$) as seen previously (Janezic et al., 2013; Roberts et al., 2020; Threlfell et al., 2021). In DLS of male *SNCA*-OVX mice, isradipine decreased 1p-evoked $[DA]_o$ by ~30%, (Figure 1g, comparison of peak $[DA]_o$ $t_3 = 6.6$ $P = .007$ N = 4) as seen in WT (Figure 1d) (Brimblecombe et al., 2015). However, in male *Snca*-null mice, isradipine had no effect on 1p-evoked $[DA]_o$ (Figure 1g, comparison of normalised peak $[DA]_o$ $t_3 = .25$ $P = .82$ N = 4), showing a statistically significant difference between the two genotypes (Figure 1h, two-way ANVOA: genotype X drug interaction, $F_{17,102} = 3.0$, $P = .0003$; main effect of genotype, $F_{1,6} = 4.89$ $P = .002$) and suggesting a dependence of LTCC function on α-synuclein.

We did not see an LTCC role in DA release in females in WT mice (see Figure 1c) but tested whether LTCC support of DA release in females could be established in either of two different PD-related mouse lines. First, we explored whether overexpression of α-synuclein could result in LTCC support of DA release. However, in female *SNCA*-OVX mice, isradipine did not decrease $[DA]_o$ in DLS (Figure 1i, comparison of normalised peak $[DA]_o$ $t_2 = 2.2$ $P = .16$), indicating a significant difference between male and female *SNCA*-OVX mice (Figure 1j, two-way ANVOA: genotype X drug interaction, $F_{17,102} = 1.89$, $P = .027$; main effect of genotype, $F_{1,6} = 7.85$ $P = .03$). Second, since mutations in the GCase *GBA* gene are associated with PD and seen more commonly in women with PD than men (Li et al., 2021), we explored whether LTCC support of DA release varied with GCase levels in females, by comparing mice that were heterozygous for the mouse *Gba* gene ($Gba^{+/-}$) with wild-type littermate homozygous controls ($Gba^{+/+}$). Isradipine did not modify 1p-evoked $[DA]_o$ in DLS $Gba^{+/+}$ female controls, as seen for other wild-type females, but strikingly, isradipine did inhibit 1p-evoked $[DA]_o$ in female $Gba^{+/-}$ mice (Figure 2k, comparison of normalised peak $[DA]_o$, $Gba^{+/+}$(WT), $t_2 = .33$ $P = .77$; $Gba^{+/-}$, $t_4 = 12.03$ $P = .0003$), showing a significant difference in drug effect between these two genotypes (Figure 1l: two-way ANVOA: genotype X drug interaction $F_{16,96} = 6.4$ $P < .0001$; main effect of genotype, $F_{1,6} = 16.2$ $P = .007$; absolute levels of evoked $[DA]_o$ for these recording sites were similar between genotypes pre-isradipine, at 1.09 ± .09 for $Gba^{+/+}$ and .98 ± .15 μM for $Gba^{+/-}$). These observations indicate that a role for LTCCs in supporting DA release is exposed when GCase function is compromised.

We note that across all experiments and variables, we found no correlation between 1p-evoked $[DA]_o$ levels and the subsequent effect of isradipine (Figure 1m, simple linear regression, slope does not significantly differ from zero $F_{1,28} = .049$ $P = .83$), confirming that effect size was not a function of different initial release probability in different conditions.

3.3 | Sex differences and calb1 interact to govern LTCC function

VTA and SNc DA neurons differ in a number of key aspects that might contribute to their differing sensitivities to parkinsonian degeneration. One notable difference is the greater expression of calbindin-D28K (Calb1) in VTA neurons, which has been suspected to be protective against PD, although evidence is mixed (Airaksinen et al., 1997; Yuan et al., 2013). We used cre-lox P recombination to selectively knockdown Calb1 in DA neurons in a cross of DAT-Cre mice with floxed Calb1 mice as previously (Brimblecombe et al., 2019), which does not affect evoked $[DA]_o$ or DA uptake in DLS, but it has profound impact in NAc, consistent with the regional endogenous expression profile of Calb1. We found here that in control mice (DAT-Cre hets, referred to as CalbWT), isradipine decreased 1p-evoked $[DA]_o$ in males in DLS but not NAc and did not modify evoked $[DA]_o$ in females in DLS (Figure 2a) as per wild-type mice (see Figure 1). By contrast, in CalbKD mice, isradipine decreased 1p-evoked $[DA]_o$ in male DLS and NAc, and in female DLS (Figure 2b). Overall, Calb1 knockdown significantly increased the effect of isradipine on DA release (Figure 2c, two-way ANOVA effect of genotype $F_{1,14} = 31.17$ $P < .001$) and enabled LTCCs to support DA release in both male NAc and female DLS, even at lower $[Ca^{2+}]_o$ in NAc (Figure 2c). We tested whether there was a gross upregulation of Calb1 levels in the striatum of wild-type female mice relative to males, leading to the occlusion of LTCC function seen in WT female mice. However, western blot analysis of protein levels in whole striatal tissue did not detect a difference in Calb1

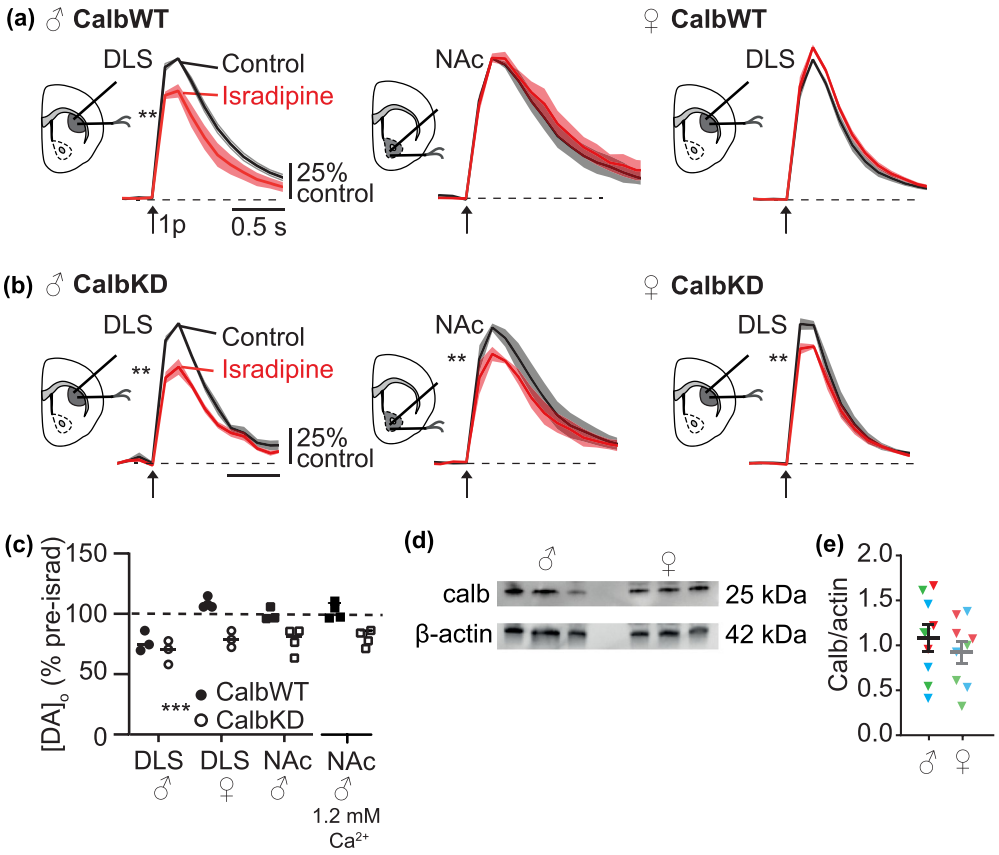


FIGURE 2 Calb1 limits LTCC function in NAc in male mice, and in DLS in female mice. (a, b) Mean $[DA]_o \pm$ SEM versus time evoked by single pulses (1p, *arrow*) in control and isradipine (5 μM, *red*) in (a) CalbWT mice or (b) CalbKD mice, in male DLS (*left*), male NAc (*centre*), and female DLS (*right*) normalised to their own control conditions. $^{**}P < .01$ comparison of peak $[DA]_o$. (c) Summary of peak $[DA]_o$ remaining after isradipine (% of pre-isradipine control) in CalbWT and CalbKD striatum. $^{**}P < .01$ effect of genotype 2-way ANOVA. N = 3 pairs DLS male and female N = 4 NAc. (d) Representative examples of Western blots for three males and three females showing actin (42 kDa) and Calb1 (25 kDa). (e) Calbindin-D28K/β-actin protein ratios in male and female striatum. Mean ± SEM; colours group technical replicates (i.e., blue points correspond to blot shown in c and are samples from which stats correspond to, N = 3 brains, repeated for three blots, illustrated by red, green and blue).

expression in striatum of females versus males (Figure 2d,e, unpaired t-test $T_4 = .18$ $P = .86$).

3.4 | DAT function promotes LTCC support of DA release

Translocation of DA across the membrane by DAT involves the co-transport of 2 Na^+ and 1 Cl^-, an electrogenic process that can depolarise DA neuron membranes, independently of DA translocation (Ingram et al., 2002) and in cellular expression systems can activate LTCCs (Cameron et al., 2015). We tested whether DATs operate in DA axons to support LTCC function. We inhibited DATs using cocaine (5 μM) or nomifensine (10 μM), monoamine uptake inhibitors, which are also known to prevent the electrogenic effects of DAT (Sonders et al., 1997), and tested the impact of isradipine on 1p-evoked $[DA]_o$ in DLS in male wild-type mice. We found that in the presence of cocaine or nomifensine, the effect of LTCC inhibitor isradipine in the DLS was no longer apparent (Figure 3a–c, two-way ANOVA: isradipine X pre-condition interaction $F_{32,192} = 3.57$, $P < .001$; comparison of peaks one-sample t-test: control $t_3 = 6.48$, $P = .0074$, cocaine $t_4 = .37$ $P = .73$, nomifensine $t_4 = 1.39$ $P = .23$). The loss of isradipine effect in the presence of cocaine was not due to any potential effects of cocaine as an inhibitor of voltage-gated Na^+-channels (Na_v) because application of the Na_v blocker and analogue lidocaine did not similarly prevent the effect of isradipine. Rather, in the presence of lidocaine, isradipine decreased 1p-evoked $[DA]_o$ as seen in standard conditions (Figure 3c, one-way ANOVA $F_{2,9} = 11.5$ $P = .03$, Dunnett's post-test lidocaine vs. control $P = .83$, cocaine vs. control $P = .003$, nomifensine vs. control $P = .014$). These data show that inhibition of DATs occludes LTCCs

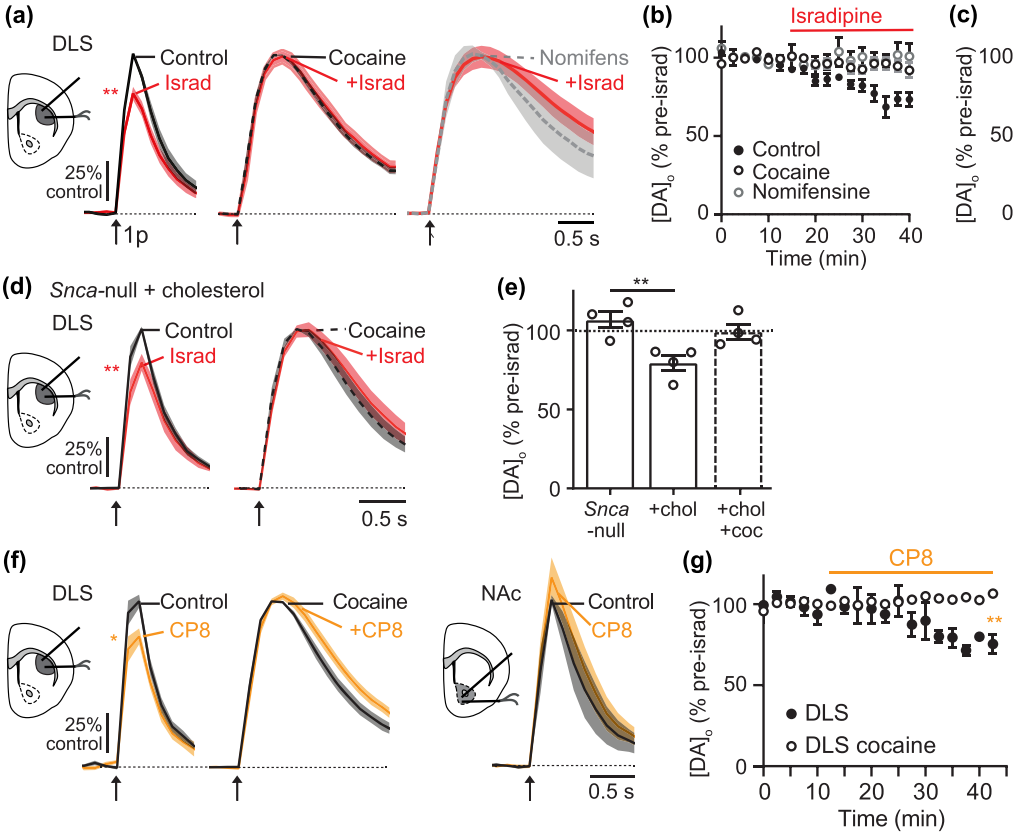


FIGURE 3 LTCC support of DA release is facilitated by DATs. (a) Mean $[DA]_o \pm$ SEM versus time evoked by single pulses (1p, *arrow*) in male mice in DLS in control versus isradipine (5 μM, *red*) alone (*left*), or in the presence of cocaine (*centre*), or nomifensine (*right*). (b) Peak $[DA]_o \pm$ SEM (normalised to pre-isradipine level) versus time in control (*black*), cocaine (unfilled) or nomifensine (grey unfilled). (c) Summary of peak $[DA]_o$ remaining after isradipine (% of pre-isradipine control) in DLS of male mice in control (Ctrl) N = 3, lidocaine (*Lido*) N = 3, cocaine (*Coc*) N = 5 or nomifensine (*Nom*) N = 5. (d) Mean $[DA]_o \pm$ SEM versus time evoked by 1p (*arrow*) in DLS in control versus isradipine in *SNCA*-null tissue incubated in cholesterol alone (*left*) versus with cocaine (*right*). (e) Summary of peak $[DA]_o$ remaining after isradipine (% of pre-isradipine control) in DLS of male *Snca*-null mice in control N = 4, cholesterol incubation N = 4 or cholesterol incubation + cocaine. N = 4. (f) Mean $[DA]_o \pm$ SEM versus time evoked by 1p (*arrow*) in control and CP8 (10 μM, *yellow*) in DLS in control conditions (*left*), in DLS in the prior presence of cocaine (*centre*) or in NAc in control conditions (*right*). (g) Peak $[DA]_o \pm$ SEM (normalised to pre-CP8 level) versus time in DLS in control (*black*) versus cocaine (unfilled), N = 4. $^*P < .05$, $^{**}P < .01$.

from contributing to DA release and suggest that DATs support LTCC function in DLS.

We tested whether a potentiation of DAT function could facilitate LTCC support of DA release where it was not previously observable. We have previously shown that DAT function in *Snca*-null mice can be augmented by cholesterol (Threlfell et al., 2021) and exploited this action to see whether in *Snca*-null male mice an effect of isradipine could be brought about after cholesterol incubation and its allied increase in DAT function. Indeed, after incubation in cholesterol, isradipine significantly decreased 1p-evoked $[DA]_o$ (Figure 3d, control: $t_3 = 4.2$ $P = .025$). To confirm that this outcome depended on DAT function, we repeated in the presence of cocaine to inhibit DAT. In the presence of cocaine, cholesterol incubation no longer supported an effect of isradipine on DA release (Figure 3d, cocaine: $t_3 = .17$ $P = .87$, Figure 3e, one-way ANOVA $F_{3,12} = 6.29$ $P = .0083$, Dunnett's post-test *Snca*-null control vs. cholesterol $P = .0034$, vs. chol +cocaine $P = .54$), suggesting that a DAT-dependent increase in LTCC function underlay this impact of cholesterol.

We excluded an alternative hypothesis that DAT function promotes the effect of isradipine not by increasing LTCC function but by changing the ability of isradipine to bind to LTCCs. It is known that at hyperpolarised potentials, isradipine does not effectively bind to LTCC (Helton et al., 2005; Rey et al., 2020). Since inhibitors of DATs will reduce DAT-associated depolarising electrogenic currents and maintain DA axons in a more hyperpolarised state, they might prevent isradipine from binding to LTCC. We therefore tested whether the actions of an alternative LTCC antagonist to isradipine, CP8, which binds LTCCs in a voltage-independent

manner (Kang et al., 2013; Rey et al., 2020), were also prevented in the presence of cocaine. We found first that CP8 decreased 1p-evoked $[DA]_o$ in DLS of male mice, but not in male NAc (Figure 3f,g, t-test comparison on peak DLS: $t_2 = 5.19$ $P = .035$, DLS + cocaine $t_3 = 1.39$ $P = .26$, NAc: $t_2 = 1.54$ $P = .26$; two-way ANOVA post-test time point 10 vs. 40 min DLS: $P = .0052$. NAc [not shown]: $P = .78$, DLS + cocaine $P = .99$), paralleling the effects of isradipine. Further in parallel with the effects of isradipine, CP8 did not reduce 1p-evoked $[DA]_o$ in DLS in the presence of cocaine (Figure 3g, two-way ANOVA post-test time point 10 vs. 40 min DLS: $P = .0052$. NAc: $P = .78$, DLS + cocaine $P = .99$). These data indicate that DATs indeed support LTCC function in contributing to DA release and do not simply support the voltage-sensitive binding and action of isradipine.

3.5 | D2Rs limit LTCC function in NAc

We tested whether D2R activity modifies how LTCCs support DA release by assessing the impact of isradipine on 1p-evoked $[DA]_o$ in the presence of D2R inhibition. In DLS, isradipine decreased DA release similarly by ~25% in the presence and absence of D2R inhibitor L-741,626 (Figure 4a,b, comparison of peaks one sample t-test control $t_3 = 6.48$ $P = .0074$; L-741 $t_4 = 4.87$ $P = .0082$). However in NAc, D2R inhibition unsilenced a role for LTCCs in supporting DA release: it enabled an inhibition of $[DA]_o$ by subsequent application of isradipine (Figure 4c,d, comparison of peaks one sample t-test control: $t_3 = 1.78$, $P = .19$; L-741: $t_3 = 27.56$ $P = .0001$), indicating that D2R activity is limiting LTCC function in NAc but not DLS (Figure 4e, two-way ANOVA region X L-741: $F_{1,14} = 8.13$, $P = .013$, main effect of region: $F_{1,14} = 16.8$, $P = .001$, main effect of L-741 $F_{1,14} = 14.5$ $P = .002$).

Given this apparent role for D2Rs in limiting LTCC support of DA release in NAc, we tested a hypothesis that the mechanism through which cocaine limits LTCC function in DLS might be elevation of DA levels leading to an enhancement of D2R-activation. We inhibited D2Rs with L-741,626, to see if this allowed isradipine to decrease evoked $[DA]_o$ in the presence of cocaine. However, isradipine did not decrease DA release in DLS in the presence of cocaine and L-741,626, indicating that the control of LTCC support of DA release by DATs in DLS is independent of D2R regulation (Figure 4f, t-test $t_7 = 1.65$ $P = .14$).

We tested finally whether D2Rs or DATs interact with the effects of Calb1 in NAc on LTCC support of DA release. In NAc of CalbKD mice where LTCCs support DA release (see Figure 2), D2R-inhibition did not augment the impact of isradipine on DA release compared to without D2R inhibition (Figure 4g–i, Dunnets post-test $t_9 = .3$ $P = .94$) indicating either that the impact of CalbKD and D2R inhibition of LTCC role has reached a ceiling or that the mechanism through which D2Rs are governing LTCC impact involves Calb1. The inhibition of DATs in NAc of CalbKD mice with cocaine occluded the effect of isradipine on DA release (Figure 4g–i, $t_2 = .73$ $P = .53$). These data indicate that LTCC support of DA release in NAc in CalbKD mice can be facilitated by DATs (Figure 4i, one-way ANOVA Sidak post-test CalbKD vs cocaine $t_9 = 3.9$ $P = .007$ CalbKD vs. L471 $t_9 = .3$ $p = .94$). These features of DAT support but no D2R control of LTCC function in NAc in CalbKD mice mirror the regulation of LTCC function in DLS of wild-type mice. Together, they also reveal that while Calb1 and D2Rs each limit LTCC function in wild-type NAc they do not do so in DLS (in the presence of normal DAT function).

Here, we have shown that the apparent function of LTCCs in supporting DA release varies with a large range of biological conditions, some of which show apparent co-dependencies. To better understand how these factors, of genotype, sex, region and molecular regulators interact to govern whether LTCCs support DA release, and by extension set the conditions when isradipine will decrease DA release, we employed a classification tree algorithm with Gini impurity, for each experimental replicate, to understand the relative influence of each factor (Figure 5a). The classification tree illustrated that DAT activity (*cocaine data*) is a key means of supporting L-function; the presence of cocaine is a robust limiter of isradipine's effect. Conversely, *Calb1* and *Gba* strongly limit LTCC function; knockdown of either promoted isradipine action. In contrast, in wild-type, *SNCA*-OVX and *Snca*-null mice, LTCC function co-varied with sex, striatal region, D2-receptor state or cholesterol level (Figure 5a). The branching of the tree into multiple pure leaves (each end node had probability of 0 or 1, no outliers) facilitated a straightforward classification of factors into either facilitators or inhibitors of LTCC function (Figure 5b).

4 | DISCUSSION

We reveal here that LTCC support of DA release from DA axons is regulated by multiple, endogenous factors in a regionally and sexually divergent manner. In DLS, LTCCs operate more evidently in males, and their support of DA release is facilitated by α-synuclein and DATs and permitted by the absence of Calb1. In NAc, LTCC

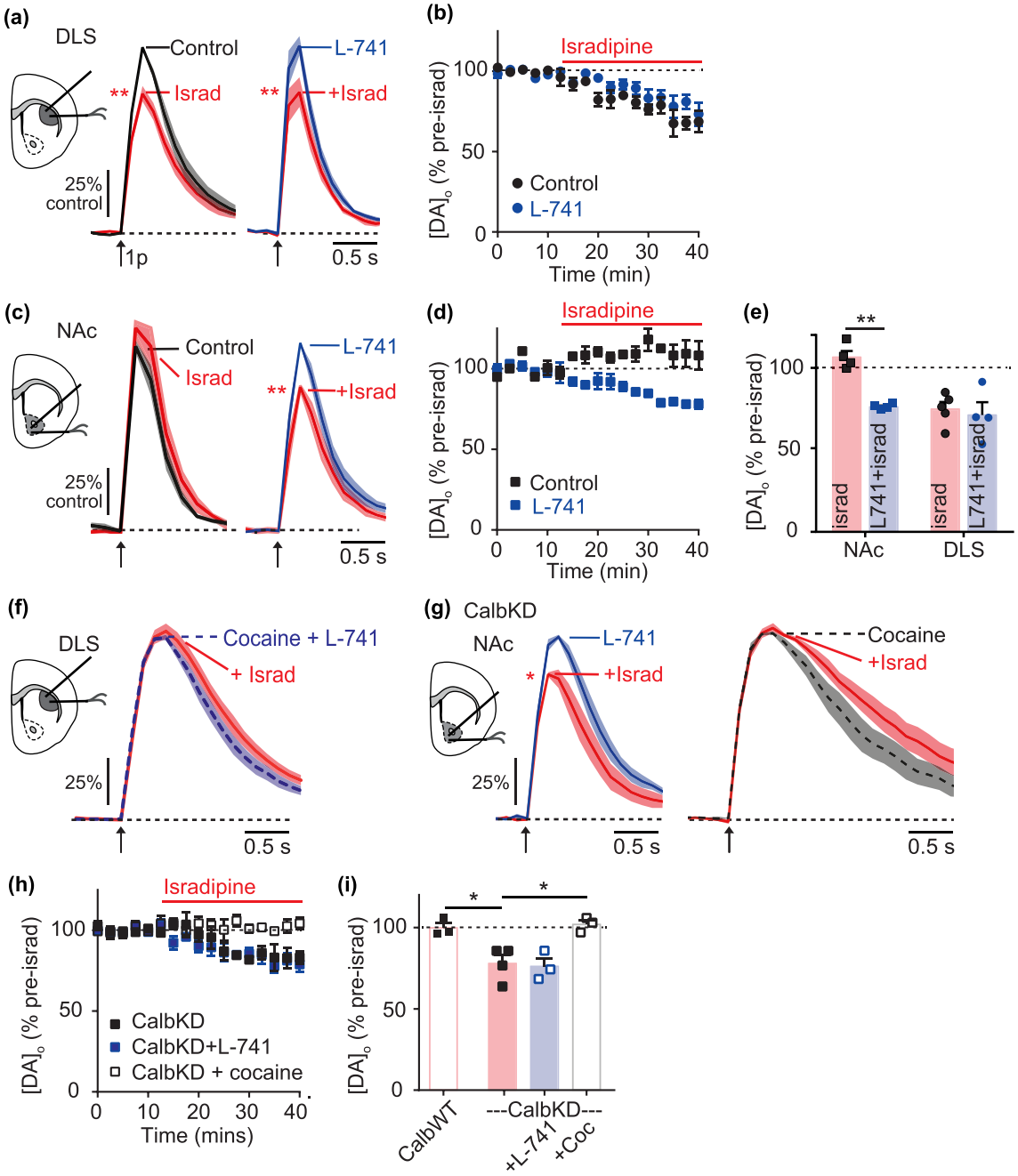
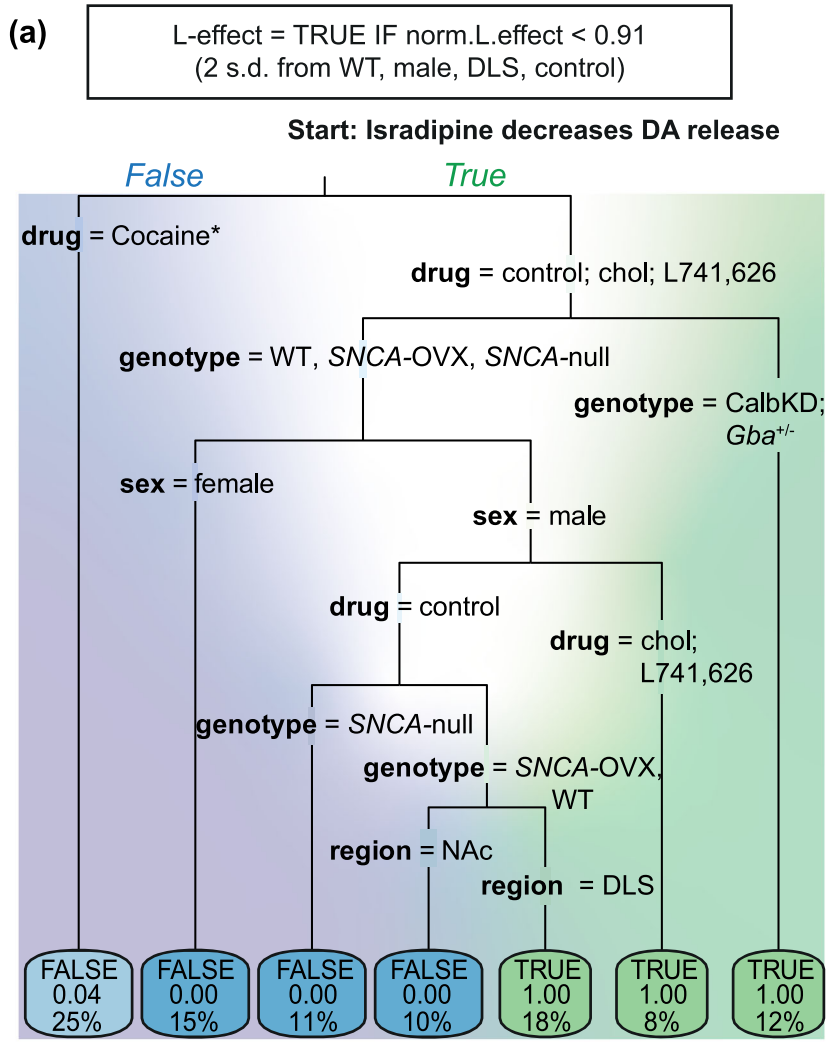


FIGURE 4 LTCC support of DA release is silenced by D2R. (a, c) Mean $[DA]_o \pm$ SEM versus time evoked by single pulses (1p, *arrow*) in wild-type male mice in DLS (a) or NAc (c) in control versus isradipine (5 μM, *red*) alone (*left*) or in the presence of D2R-antagonist L-741,626 (2 μM, *right*). (b, d) Peak $[DA]_o \pm$ SEM (normalised to pre-isradipine level) versus time in control (*black*) or L-741,626 (*blue*) in DLS (B) or NAc (D). (e) Summary of peak $[DA]_o$ remaining after isradipine (% of pre-isradipine control), in NAc and CPu without (red) or with L-741 (blue) (N = 4). (F) Mean $[DA]_o \pm$ SEM versus time evoked by 1p (*arrow*) in DLS in the presence of cocaine + L-741,626 (*blue dashed*) alone, or with isradipine (*red*). (g) Mean $[DA]_o \pm$ SEM versus time evoked by 1p (*arrow*) in NAc from CalbKD mice with and without isradipine in the prior presence of either L-741,626 (*left*) or cocaine (*right*). (h) Peak $[DA]_o \pm$ SEM (% of pre-isradipine level) versus time during isradipine application in NAc from CalbKD mice in control conditions (*black*), or the presence of L-741,626 (*blue*), or cocaine (*unfilled*). (i) Summary of peak $[DA]_o$ remaining after isradipine (% of pre-isradipine control) in NAc in CalbWT (N = 3) or CalbKD mice, N = 4 with L-741 (N = 3) or cocaine (N = 3). $^*P < .05$, $^{**}P < .01$.

support of DA release is prevented by Calb1 and D2Rs. Intriguingly, regulators that promote LTCC function are associated with increased risk of degeneration in PD, while some of those that limit function are associated with reduced neuronal vulnerability, suggesting that intersecting mechanisms regulating DA handing and



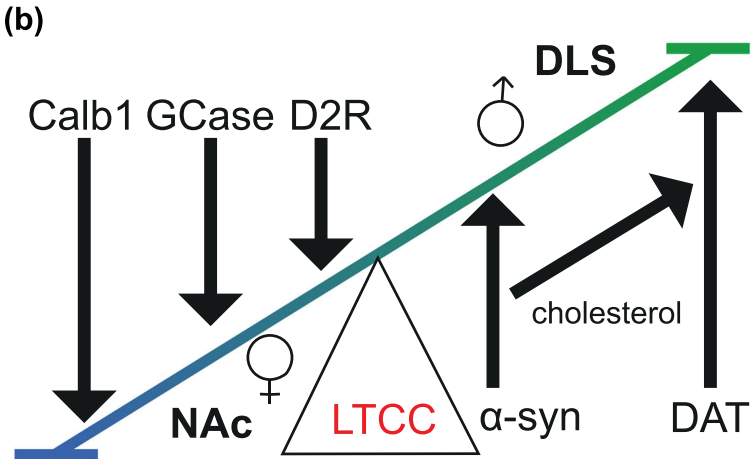


FIGURE 5 Segregation of factors to facilitators or attenuators of LTCC support of DA release. (a) Decision tree segregating experimental conditions of sex, genotype and drug condition using the Gini index. Blue to green background shading shows predictive power of a FALSE (blue) or TRUE (green) outcome, respectively. White area shows high uncertainty. Labels on branches indicate recording condition. Left branches for 'isradipine decreases DA release' are FALSE. Labels on final boxes (leaves) indicate outcome: TRUE/FALSE (*top*), probability of outcome being correct, that is, 0 or 1, indicate all data from that branch has been correctly sorted (*middle*) and % of datapoints contained within each leaf (*bottom*). *Cocaine is the presence of cholesterol or L-741,626. (b) Cartoon illustrating which endogenous factors limit (downward arrows) or promote (upward arrows) LTCC support of DA release.

LTCC function might together contribute to the metabolic load of vulnerable neurons. We believe that the pattern of isradipine-sensitive DA release reflects a vulnerable state of the axons, and therefore, the sensitivity of DA release to isradipine is a phenotype of vulnerable DA axons.

4.1 | Sex differences interact with multiple factors to govern LTCC function

LTCCs have been suggested to confer vulnerability to degeneration of SNc DA neurons in PD (Surmeier, 2018), a disease with a higher incidence in men than women

(Wooten et al., 2004). Yet the relative roles of LTCCs in DA neurons in males versus females has been unknown. We reveal there is a greater role for LTCCs in supporting DA release in male DLS than in female, and this raises the speculation that differential LTCC function might contribute to the greater incidence of PD in men than women. Future studies should assess function in both sexes if we are to better understand how to exploit Ca^{2+} biology for a neuroprotective strategy. We believe that including both sexes in pre-clinical research could provide powerful insights into the aetiology of Parkinson's.

Alpha-synuclein is well known to be a contributing factor in PD; however, its precise physiological and pathological functions remain incompletely characterised (Alegre-Abarrategui et al., 2019). Here, we identify in male mice that axonal LTCC support of DA release is dependent on α-synuclein. It should be noted that some commercially available wild-type mouse strains including the Harlan C57Bl6/6S (also listed as C57BL/6JOlaHsd) are null for α-synuclein due to a spontaneous deletion (Specht & Schoepfer, 2001), which likely limits LTCC function in that strain also. The mechanism is yet to be determined, but we note a cascade of potentially interacting factors: We previously found that α-synuclein impacts on brain cholesterols and other lipids that shape DAT activity (Threlfell et al., 2021) and found here that cholesterol reinstates an LTCC function lost after α-synuclein deletion in a DAT-dependent manner.

Mutations in the *GBA* gene are the most common genetic factors in PD (Smith & Schapira, 2022) and appear especially important in female patients with PD (Li et al., 2021). Here, we show that *GBA* knockdown led to isradipine decreasing DA release in the DLS of $Gba^{+/-}$ female mice, indicating that intact GCase activity normally limits how LTCCs support DA release in female mice. Although we have not identified the cellular pathway responsible for GCase limiting LTCC control of DA, there are a number of possibilities (Do et al., 2019). A downregulation of GCase (via $Gba^{+/-}$) might alter the lipid composition of the membrane, allowing LTCC to contribute to release, in a mechanism complementary to that of α-synuclein regulation of LTCC described above; alternatively following a decrease in GCase levels, Ca^{2+} might become rate-limiting in the fusion of lysosome to autophagosomes, leading to a compromise in axonal function and DA release following LTCC-inhibition (Tian et al., 2015); potentially, dysregulation of lysosomal function might promote ER stress and mitochondrial stress, which may lead to an increase in LTCC signalling to increase ATP production (Zampese et al., 2022). These candidate mechanisms could be tested in future research.

The fast Ca^{2+} buffer calbindin-D28k (Calb1) has been considered previously as potentially neuroprotective, but evidence for a neuroprotective role has been mixed. It remains possible that the toxin models typically used are too aggressive to reveal neuroprotection (Airaksinen et al., 1997; Yuan et al., 2013). In any event, we found that Calb1 was important for both regional and sex differences in LTCC regulation of DA release. Under our standard experimental conditions, LTCCs do not contribute to DA release in male NAc (Brimblecombe et al., 2015), and we show here that Calb1 plays a key limiting role. We found also that Calb1 prevents LTCCs from supporting DA release in DLS of females, despite finding previously that Calb1 does not limit the levels of DA released (Brimblecombe et al., 2019). The mechanisms by which Calb1 limits how LTCCs support DA release have not been fully elucidated and are likely to depend on a number of factors, due to the global signalling nature of Ca^{2+} and the ability of Calb1 to act as a sink and a source for Ca^{2+} (Schwaller, 2020; Schwaller et al., 2002). Potential mechanisms include Calb1 buffering Ca^{2+} after entry via LTCCs; Calb1 acting as a Ca^{2+} shuttle to facilitate Ca^{2+}-dependent inactivation (CDI) of LTCC (Lee et al., 2006); or Calb1 reducing activation of LTCC via inhibition of DAT function (see below). The mechanisms through which Calb1 and LTCC interact in a sex-specific manner are also likely to be complex. Sexual dimorphism in LTCC function has been documented in cardiac tissue (Curl et al., 2008; Machuki et al., 2019; Prabhavathi et al., 2014), while in the brain, Calb1 function can be regulated by sex-linked genes and hormones at transcriptional and protein level (Abel & Rissman, 2012). The mechanisms operating in DA neurons are not yet known but could have important clinical implications for sex-specific incidence of disease and responsiveness to medication.

4.2 | DAT promotes and D2R limits LTCC support of DA release

We found that LTCC function is supported by DATs, in males in DLS in wild-types and in NAc after calb1 knockdown. Correspondingly, electrogenic monoamine transporters including the DAT are documented to promote Ca^{2+} entry via LTCCs in expression systems (Cameron et al., 2015). We speculate that a similar electrogenic mechanism is responsible in DA axons. It is intriguing to note that in NAc, DA uptake rates are greater after calb1 knockdown than in controls (Brimblecombe et al., 2019), supporting a hypothesis that LTCC support of DA release in NAc after Calb1 knockdown might result from the associated impact of elevated DAT on depolarisation, in

addition to a more simple explanation that there is reduced Ca^{2+} buffering. The greater LTCC function in DLS than NAc (Brimblecombe et al., 2015) parallels the higher DAT levels in source DA neurons in SNc than VTA (Poulin et al., 2014). However, DAT function alone is not the sole predictor of LTCC function, as LTCC support of DA release in DLS was not different between wild-type and DAT-$Cre^{+/-}$ mice (CalbWT), despite DAT-$Cre^{+/-}$ mice having lower DAT levels and function (Bäckman et al., 2006; Brimblecombe et al., 2019). Alternative explanations for DAT regulation of LTCC function could yet include that LTCCs might regulate DA release in a DAT-dependent manner, so when DAT is inhibited, LTCC dependence is occluded. Our findings together with other literature add to the growing body of evidence that DATs serve more functions than simply clearing extracellular DA, as they participate in the underlying control of DA release (Brimblecombe et al., 2019; Condon et al., 2019; Kile et al., 2010; Siciliano et al., 2018; Venton et al., 2006). It remains to be understood why females appear to have enhanced DAT function yet limited LTCC control of DA release. Understanding this discrepancy in the future could offer important mechanistic insights.

We also found that LTCC support of DA release is inhibited by D2Rs, in male NAc. LTCCs have vice versa been shown to regulate D2Rs (Dragicevic et al., 2014; Duda et al., 2016; Zhang & Sulzer, 2012), and therefore, together these data show that there is reciprocal regulation between LTCCs and D2Rs. D2Rs have been shown previously to inhibit LTCC function in striatal GABAergic projection neurons and cultured midbrain neurons (Hernández-López et al., 2000; Olson et al., 2005; Yasumoto et al., 2004), but this effect is somewhat surprising for DA release because D2Rs do not appear to be activated sufficiently in this preparation to be limiting DA release for these stimuli (Condon et al., 2019; Cragg, 2003; Kennedy et al., 1992), suggesting that there is limited tonic activity at D2Rs. An explanation for this disparity could be that for 1p-evoked DA release, there is no net effect of inhibiting D2Rs on $[DA]_o$ due to the wide range of ion channels and intracellular cascades affected by D2Rs, including voltage-gated K^+ channel-dependent mechanisms (Fulton et al., 2011), N- and PQ-type Ca^{2+} channels (Cardozo & Bean, 1995) and LTCC-dependent mechanisms (Hernández-López et al., 2000) (reviewed in Ford, 2014). These multiple outcomes of D2R activity might also provide an explanation for why D2Rs were not detected to limit LTCC function in DLS. From these collected observations, it is evident that roles of LTCCs in DA release, and the effects of isradipine, are plastic; they are variable between DA neuron types; they vary with the activity of a range of biological regulatory mechanisms, and experimental conditions, and whether the subjects are males or females.

The mechanisms by which LTCCs are supporting DA release, and differently with diverse factors, are not yet resolved. Incoming Ca^{2+} via LTCCs could directly contribute to the Ca^{2+} required for exocytosis or could contribute to other processes that modify axonal physiology and DA release probability. LTCCs lack the synprint site possessed by other types of VGCCs, which tethers VGCCs to the exocytotic machinery (Mochida et al., 2003), suggesting LTCCs might not be located in sufficient proximity to catalyse exocytosis directly. Besides catalysing vesicular exocytosis, there are other potential mechanisms through which LTCCs might support DA release including (but not limited to) supporting AP propagation, intracellular signalling cascades or metabolic support of DA axons.

How APs propagate throughout the vast, branched, unmyelinated and varicose DA axonal arbour (Matsuda et al., 2009) is not well understood but is likely to be highly energy consuming (Bolam & Pissadaki, 2012) and subject to branch point failure. LTCCs have been shown able to modulate AP conductance in optic nerves prior to myelination (Alix et al., 2008) and might modulate AP shape, axonal excitability and AP propagation, and consequently, recruitment of DA release sites in DA axons. A change in AP propagation might also couple to Ca^{2+}-dependent signalling cascades including those regulated by GPCRs that regulate DA release (Fulton et al., 2011). In cardiac myocytes, LTCC function is integrated with physiological need by adrenergic regulation: upon ß-adrenergic stimulation, activation of PKA leads to phosphorylation of Cav1.2 and enhanced calcium-dependent inactivation (CDI) of Ca^{2+} channels, limiting influx of Ca^{2+} during APs (Morales et al., 2019). LTCCs might similarly undergo CDI in DA axons, with resulting loss of effect of isradipine on DA release.

There is the possibility that Ca^{2+} entry via LTCCs and downstream activation of RyRs drives mitochondrial generation of ATP (Zampese et al., 2022) that in turn might support axon function and DA release. ATP production, downstream of Ca^{2+} entry via LTCCs, is an attractive potential explanation for the detrimental effects of isradipine on DA release, particularly given that we hypothesise that the situations where isradipine decreases DA release reflects a physiologically vulnerable state of the axon. The different factors we report that modulate LTCC support of DA release might change axonal demand for ATP, thus driving differing dependencies on Ca^{2+} entry via LTCCs to signal to mitochondria to support the required production of ATP. LTCC function might be a reporter for axons with higher metabolic demand or, since this process contributes a metabolic stress (Pacelli et al., 2015), might drive axonal metabolic stress. Female rodents have been found to have decreased ageing-related ROS production and better mitochondrial function in the brain, compared to males

(Gaignard et al., 2015; Guevara et al., 2011), which may be linked to the difference in LTCC function we report here. These hypotheses are not mutually exclusive but are yet to be tested for DA axons. In turn, if a putative LTCC-driven ATP generation is causing a state of vulnerability in PD, for example, via generation of oxidative stress, then the inhibition of LTCCs might be neuroprotective. However, if LTCC function is a readout of axonal vulnerability, by reporting high demand for energy supply, then the inhibition of LTCCs might add a further strain to an already exhausted axonal population. Understanding the energetic demands of dopaminergic axons is non-trivial, but interventions to decrease their energetic demand rather than limit their energetic supply seems more likely to be neuroprotective.

In summary, we show that the factors that govern the variable role of axonal LTCCs in supporting DA release in DLS versus NAc include α-synuclein, DATs, calb1 and D2Rs. These factors can vary between striatal regions and support LTCC function in DLS while preventing LTCC function in NAc, thereby accounting for the disparity between LTCC expression and function in different DA neurons. It is further noteworthy that factors that promote LTCC support of DA release such as male sex, α-synuclein, DAT and the absence of Calb1 correspond to risk factors for parkinsonian degeneration, understanding how these factors interact may allow the stratification of patient cohorts to identify those who would most benefit from targeting LTCC as a neuroprotective strategy with isradipine (Parkinson Study Group STEADY-PD III Investigators, 2020). By facilitating LTCC function these factors might drive metabolic damage to vulnerable neurons, corroborating the hypothesis that LTCCs might then offer a target for a neuroprotective therapy. Alternatively, LTCC support of DA release might be only a readout of vulnerability, a 'canary in the coalmine', when in turn manipulation of LTCC function would not protect vulnerable neurons. The utility of inhibiting LTCCs as a neuroprotective strategy against Parkinson's requires there to be a net benefit from a number of competing mechanisms. The reduction in a Ca^{2+}-associated oxidative stress in somatodendritic or axonal compartments might be beneficial, but only if this outweighs the detrimental effects of loss of DA function associated with a decrease in evoked striatal DA release and potential loss of ATP generation. A fuller understanding of the interconnected underlying biology of these Ca^{2+}-dependent mechanisms in DA axons will better inform neuroprotective strategies for PD targeting LTCCs and beyond.

AUTHOR CONTRIBUTIONS

Katherine R. Brimblecombe: Conceptualization; data curation; formal analysis; investigation; visualization; writing—original draft; writing—review and editing. **Natalie Connor-Robson:** Data curation; formal analysis; visualization. **Carole J. R. Bataille:** Data curation; methodology; resources; validation. **Bradley M Roberts:** Data curation; formal analysis. **Caitlin Gracie:** Data curation; formal analysis; investigation. **Bethan O'Connor:** Data curation; formal analysis. **Gayathri Karthik:** Data curation; formal analysis. **Angela J. Russell:** Resources; supervision; validation. **Richard Wade-Martins:** Resources. **Stephanie J. Cragg:** Conceptualization; funding acquisition; project administration; resources; supervision; writing—original draft; writing–review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to disclose.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/ejn.16180.

DATA AVAILABILITY STATEMENT

Data for all figures and code are available at Zenodo (10.5281/zenodo.7801867). Step-by-step experimental protocols are available online (at 10.17504/protocols.io.4r3l27dxxg1y/v1).

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REFERENCES

Abel, J. L., & Rissman, E. F. (2012). Location, location, location: Genetic regulation of neural sex differences. *Reviews in Endocrine & Metabolic Disorders*, *13*, 151–161. https://doi.org/10.1007/s11154-011-9186-0

Acevedo-Rodriguez, A., Zhang, L., Zhou, F., Gong, S., Gu, H., de Biasi, M., Zhou, F.-M., & Dani, J. A. (2014). Cocaine inhibition of nicotinic acetylcholine receptors influences dopamine release. *Front Synaptic Neurosci*, *6*, 19. https://doi.org/10.3389/fnsyn.2014.00019

Airaksinen, M. S., Thoenen, H., & Meyer, M. (1997). Vulnerability of midbrain dopaminergic neurons in Calbindin-D 28k -deficient mice: Lack of evidence for a neuroprotective role of endogenous Calbindin in MPTPtreated and weaver mice. *The European Journal of Neuroscience*, *9*, 120–127. https://doi.org/10.1111/j.1460-9568.1997.tb01360.x

Alegre-Abarrategui, J., Brimblecombe, K. R., Roberts, R. F., Velentza-Almpani, E., Tilley, B. S., Bengoa-Vergniory, N., & Proukakis, C. (2019). Selective vulnerability in α-synucleinopathies. *Acta Neuropathologica*, *138*, 681–704. https://doi.org/10.1007/s00401-019-02010-2

Alix, J. J. P., Dolphin, A. C., & Fern, R. (2008). Vesicular apparatus, including functional calcium channels, are present in developing rodent optic nerve axons and are required for normal node of Ranvier formation. *The Journal of Physiology*, *586*, 4069–4089. https://doi.org/10.1113/jphysiol.2008.155077

Bäckman, C. M., Malik, N., Zhang, Y., Shan, L., Grinberg, A., Hoffer, B. J., Westphal, H., & Tomac, A. C. (2006). Characterization of a mouse strain expressing Cre recombinase from the 3′ untranslated region of the dopamine transporter locus. *Genesis*, *44*, 383–390. https://doi.org/10.1002/dvg.20228

Barski, J. J., Mörl, K., & Meyer, M. (2002). Conditional inactivation of the calbindin D-28k (Calb1) gene by Cre/loxP-mediated recombination. *Genesis*, *32*, 165–168. https://doi.org/10.1002/gene.10045

Bekris, L. M., Mata, I. F., & Zabetian, C. P. (2010). The genetics of Parkinson disease. *Journal of Geriatric Psychiatry and Neurology*, *23*, 228–242. https://doi.org/10.1177/0891988710383572

Bolam, J. P., & Pissadaki, E. K. (2012). Living on the edge with too many mouths to feed: Why dopamine neurons die. *Movement Disorders*, *27*, 1478–1483. https://doi.org/10.1002/mds.25135

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. *The Journal of Physiology*, *593*, 929–946. https://doi.org/10.1113/jphysiol.2014.285890

Brimblecombe, K. R., Vietti-Michelina, S., Platt, N. J., Kastli, R., Hnieno, A., Gracie, C. J., & Cragg, S. J. (2019). Calbindin-D28K limits dopamine release in ventral but not dorsal striatum by regulating Ca 2+ availability and dopamine transporter function. *ACS Chemical Neuroscience*, *10*, 3419–3426. https://doi.org/10.1021/acschemneuro.9b00325

Cachope, R., Mateo, Y., Mathur, B. N., Irving, J., Wang, H.-L., Morales, M., Lovinger, D. M., & Cheer, J. F. (2012). Selective activation of cholinergic interneurons enhances accumbal phasic dopamine release: Setting the tone for reward processing. *Cell Reports*, *2*, 33–41. https://doi.org/10.1016/j.celrep.2012.05.011

Cameron, K. N., Solis, E., Ruchala, I., de Felice, L. J., & Eltit, J. M. (2015). Amphetamine activates calcium channels through dopamine transporter-mediated depolarization. *Cell Calcium*, *58*, 457–466. https://doi.org/10.1016/j.ceca.2015.06.013

Cardozo, D. L., & Bean, B. P. (1995). Voltage-dependent calcium channels in rat midbrain dopamine neurons: Modulation by dopamine and GABAB receptors. *Journal of Neurophysiology*, *74*, 1137–1148. https://doi.org/10.1152/jn.1995.74.3.1137

Ciliax, B. J., Heilman, C., Demchyshyn, L. L., Pristupa, Z. B., Ince, E., Hersch, S. M., Niznik, H. B., & Levey, A. I. (1995). The dopamine transporter: Immunochemical characterization and localization in brain. *The Journal of Neuroscience*, *75*, 1714–1723. https://doi.org/10.1523/JNEUROSCI.15-03-01714.1995

Condon, M. D., Platt, N. J., Zhang, Y. F., Roberts, B. M., Clements, M. A., Vietti-Michelina, S., Tseu, M. Y., Brimblecombe, K. R., Threlfell, S., Mann, E. O., & Cragg, S. J. (2019). Plasticity in striatal dopamine release is governed by release-independent depression and the dopamine transporter. *Nature Communications*, *10*, 1, 4263–15. https://doi.org/10.1038/s41467-019-12264-9

Connor-Robson, N., Booth, H., Martin, J. G., Gao, B., Li, K., Doig, N., Vowles, J., Browne, C., Klinger, L., Juhasz, P., Klein, C., Cowley, S. A., Bolam, P., Hirst, W., & Wade-Martins, R. (2019). An integrated transcriptomics and proteomics analysis reveals functional endocytic dysregulation caused by mutations in LRRK2. *Neurobiology of Disease*, *127*, 512–526. https://doi.org/10.1016/j.nbd.2019.04.005

Costa, K. M., Schenkel, D., & Roeper, J. (2021). Sex-dependent alterations in behavior, drug responses and dopamine transporter expression in heterozygous DAT-Cre mice. *Scientific Reports*, *11*, 3334. https://doi.org/10.1038/s41598-021-82600-x

Cragg, S. J. (2003). Variable dopamine release probability and short-term plasticity between functional domains of the primate striatum. *The Journal of Neuroscience*, *23*, 4378–4385. https://doi.org/10.1523/JNEUROSCI.23-10-04378.2003

Curl, C. L., Delbridge, L. M. D., & Wendt, I. R. (2008). Sex differences in cardiac muscle responsiveness to Ca2+ and L-type Ca2+ channel modulation. *European Journal of Pharmacology*, *586*, 288–292. https://doi.org/10.1016/j.ejphar.2008.02.053

Do, J., McKinney, C., Sharma, P., & Sidransky, E. (2019). Glucocerebrosidase and its relevance to Parkinson disease. *Molecular Neurodegeneration*, *141*(14), 1, 36–16. https://doi.org/10.1186/s13024-019-0336-2

Dragicevic, E., Poetschke, C., Duda, J., Schlaudraff, F., Lammel, S., Schiemann, J., Fauler, M., Hetzel, A., Watanabe, M., Lujan, R., Malenka, R. C., Striessnig, J., & Liss, B. (2014). Cav1.3 channels control D2-autoreceptor responses via NCS-1 in substantia nigra dopamine neurons. *Brain*, *137*, 2287–2302. https://doi.org/10.1093/brain/awu131

Duda, J., Pötschke, C., & Liss, B. (2016). Converging roles of ion channels, calcium, metabolic stress, and activity pattern of *Substantia nigra* dopaminergic neurons in health and Parkinson's disease. *Journal of Neurochemistry*, *139*, 156–178. https://doi.org/10.1111/jnc.13572

Ford, C. P. (2014). The role of D2-autoreceptors in regulating dopamine neuron activity and transmission. *Neuroscience*, *282*, 13–22. https://doi.org/10.1016/j.neuroscience.2014.01.025

Fulton, S., Thibault, D., Mendez, J. A., Lahaie, N., Tirotta, E., Borrelli, E., Bouvier, M., Tempel, B. L., & Trudeau, L. E. (2011). Contribution of Kv1.2 voltage-gated potassium channel to D2 autoreceptor regulation of axonal dopamine overflow. *The Journal of Biological Chemistry*, *286*, 9360–9372. https://doi.org/10.1074/jbc.M110.153262

Gaignard, P., Savouroux, S., Liere, P., Pianos, A., Thérond, P., Schumacher, M., Slama, A., & Guennoun, R. (2015). Effect of sex differences on brain mitochondrial function and its suppression by ovariectomy and in aged mice. *Endocrinology*, *156*, 2893–2904. https://doi.org/10.1210/en.2014-1913

Gerfen, C. R., Baimbridge, K. G., & Thibault, J. (1987). The neostriatal mosaic: III. Biochemical and developmental dissociation of patch-matrix mesostriatal systems. *The Journal of Neuroscience*, *7*, 3935–3944. https://doi.org/10.1523/JNEUROSCI.07-12-03935.1987

Greene, J. G. J. G., Dingledine, R., Grenamyre, J. T., & Greenamyre, J. T. (2005). Gene expression profiling of rat midbrain dopamine neurons: Implications for selective vulnerability in parkinsonism. *Neurobiology of Disease*, *18*, 19–31. https://doi.org/10.1016/j.nbd.2004.10.003

Guevara, R., Gianotti, M., Oliver, J., & Roca, P. (2011). Age and sex-related changes in rat brain mitochondrial oxidative status. *Experimental Gerontology*, *46*, 923–928. https://doi.org/10.1016/j.exger.2011.08.003

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. *The Journal of Clinical Investigation*, *128*, 2266–2280. https://doi.org/10.1172/JCI95898

Guzman, J. N., Sánchez-Padilla, J., Chan, C. S., & Surmeier, D. J. (2009). Robust pacemaking in substantia nigra dopaminergic neurons. *The Journal of Neuroscience*, *29*, 11011–11019. https://doi.org/10.1523/JNEUROSCI.2519-09.2009

Helton, T. D., Xu, W., & Lipscombe, D. (2005). Neuronal L-type calcium channels open quickly and are inhibited slowly. *The Journal of Neuroscience*, *25*, 10247–10251. https://doi.org/10.1523/JNEUROSCI.1089-05.2005

Hernández-López, S., Tkatch, T., Perez-Garci, E., Galarraga, E., Bargas, J., Hamm, H., & Surmeier, D. J. (2000). D2 dopamine receptors in striatal medium spiny neurons reduce L-type Ca2+ currents and excitability via a novel PLCβ1–IP3–Calcineurin-signaling cascade. *The Journal of Neuroscience*, *20*, 8987–8995. https://doi.org/10.1523/JNEUROSCI.20-24-08987.2000

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. *Neurobiology of Disease*, *43*, 364–371. https://doi.org/10.1016/j.nbd.2011.04.007

Ingram, S. L., Prasad, B. M., & Amara, S. G. (2002). Dopamine transporter-mediated conductances increase excitability of midbrain dopamine neurons. *Nature Neuroscience*, *5*, 971–978. https://doi.org/10.1038/nn920

Janezic, S., Threlfell, S., Dodson, P. D., Dowie, M. J., Taylor, T. N., Potgieter, D., Parkkinen, L., Senior, S. L., Anwar, S., Ryan, B., Deltheil, T., Kosillo, P., Cioroch, M., Wagner, K., Ansorge, O., Bannerman, D. M., Bolam, J. P., Magill, P. J., Cragg, S. J., & Wade-Martins, R. (2013). Deficits in dopaminergic transmission precede neuron loss and dysfunction in a new Parkinson model. *Proceedings of the National Academy of Sciences of the United States of America*, *110*, E4016–E4025. https://doi.org/10.1073/pnas.1309143110

Kang, S., Cooper, G., Dunne, S. F., Luan, C.-H., Surmeier, D. J., & Silverman, R. B. (2013). Structure-activity relationship of N,N'-disubstituted pyrimidinetriones as Ca(V)1.3 calcium channel-selective antagonists for Parkinson's disease. *Journal of Medicinal Chemistry*, *56*, 4786–4797. https://doi.org/10.1021/jm4005048

Kennedy, R. T., Jones, S. R., & Wightman, R. M. (1992). Dynamic observation of dopamine autoreceptor effects in rat striatal slices. *Journal of Neurochemistry*, *59*, 449–455. https://doi.org/10.1111/j.1471-4159.1992.tb09391.x

Kile, B. M., Guillot, T. S., Venton, B. J., Wetsel, W. C., Augustine, G. J., & Wightman, R. M. (2010). Synapsins differentially control dopamine and serotonin release. *The Journal of Neuroscience*, *30*, 9762–9770. https://doi.org/10.1523/JNEUROSCI.2071-09.2010

Lee, D., Obukhov, A. G., Shen, Q., Liu, Y., Dhawan, P., Nowycky, M. C., & Christakos, S. (2006). Calbindin-D28k decreases L-type calcium channel activity and modulates intracellular calcium homeostasis in response to K+ depolarization in a rat beta cell line RINr1046-38. *Cell Calcium*, *39*, 475–485. https://doi.org/10.1016/j.ceca.2006.01.010

Li, Q., Jing, Y., Lun, P., Liu, X., & Sun, P. (2021). Association of gender and age at onset with glucocerebrosidase associated Parkinson's disease: A systematic review and meta-analysis. *Neurological Sciences*, *42*, 2261–2271. https://doi.org/10.1007/s10072-021-05230-1

Lohr, K. M., Masoud, S. T., Salahpour, A., & Miller, G. W. (2017). Membrane transporters as mediators of synaptic dopamine dynamics: Implications for disease. *The European Journal of Neuroscience*, *45*, 20–33. https://doi.org/10.1111/ejn.13357

Machuki, J. O., Zhang, H.-Y., Geng, J., Fu, L., Adzika, G. K., Wu, L., Shang, W., Wu, J., Kexue, L., Zhao, Z., & Sun, H. (2019). Estrogen regulation of cardiac cAMP-L-type Ca2+ channel pathway modulates sex differences in basal contraction and responses to β2AR-mediated stress in left ventricular apical myocytes. *Cell Communication and Signaling: CCS*, *17*, 34. https://doi.org/10.1186/s12964-019-0346-2

Matsuda, W., Furuta, T., Nakamura, K. C., Hioki, H., Fujiyama, F., Arai, R., & Kaneko, T. (2009). Single nigrostriatal dopaminergic neurons form widely spread and highly dense axonal arborizations in the neostriatum. *The Journal of Neuroscience*, *29*, 444–453. https://doi.org/10.1523/JNEUROSCI.4029-08.2009

Mochida, S., Westenbroek, R. E., Yokoyama, C. T., Zhong, H., Myers, S. J., Scheuer, T., Itoh, K., & Catterall, W. A. (2003). Requirement for the synaptic protein interaction site for reconstitution of synaptic transmission by P/Q-type calcium channels. *Proceedings of the National Academy of Sciences of the United States of America*, *100*, 2819–2824. https://doi.org/10.1073/pnas.262787699

Morales, D., Hermosilla, T., & Varela, D. (2019). Calcium-dependent inactivation controls cardiac L-type Ca2+ currents under β-adrenergic stimulation. *The Journal of General Physiology*, *151*, 786–797. https://doi.org/10.1085/jgp.201812236

Olson, P. A., Tkatch, T., Hernandez-Lopez, S., Ulrich, S., Ilijic, E., Mugnaini, E., Zhang, H., Bezprozvanny, I., & Surmeier, D. J.

(2005). G-protein-coupled receptor modulation of striatal CaV1.3 L-type Ca2+ channels is dependent on a Shank-binding domain. *The Journal of Neuroscience*, *25*, 1050–1062. https://doi.org/10.1523/JNEUROSCI.3327-04.2005

Pacelli, C., Giguère, N., Bourque, M.-J., Lévesque, M., Slack, R. S., & Trudeau, L.-É. (2015). Elevated mitochondrial bioenergetics and axonal arborization size are key contributors to the vulnerability of dopamine neurons. *Current Biology*, *25*, 2349–2360. https://doi.org/10.1016/j.cub.2015.07.050

Parkinson Study Group STEADY-PD III Investigators. (2020). Isradipine versus placebo in early Parkinson disease. *Annals of Internal Medicine*, *172*, 591–598. https://doi.org/10.7326/M19-2534

Philippart, F., Destreel, G., Merino-Sepúlveda, P., Henny, P., Engel, D., & Seutin, V. (2016). Differential somatic Ca2+ channel profile in midbrain dopaminergic neurons. *The Journal of Neuroscience*, *36*, 7234–7245. https://doi.org/10.1523/JNEUROSCI.0459-16.2016

Poulin, J.-F., Zou, J., Drouin-Ouellet, J., Kim, K.-Y. A., Cicchetti, F., & Awatramani, R. B. (2014). Defining midbrain dopaminergic neuron diversity by single-cell gene expression profiling. *Cell Reports*, *9*, 930–943. https://doi.org/10.1016/j.celrep.2014.10.008

Prabhavathi, K., Selvi, K. T., Poornima, K. N., & Sarvanan, A. (2014). Role of biological sex in normal cardiac function and in its disease outcome—A review. *Journal of Clinical and Diagnostic Research*, *8*, BE01–BE04. https://doi.org/10.7860/JCDR/2014/9635.4771

Rey, S., Maton, G., Satake, S., Llano, I., Kang, S., Surmeier, D. J., Silverman, R. B., & Collin, T. (2020). Physiological involvement of presynaptic L-type voltage-dependent calcium channels in GABA release of cerebellar molecular layer interneurons. *Journal of Neurochemistry*, *155*, 390–402. https://doi.org/10.1111/jnc.15100

Rice, M. E., & Cragg, S. J. (2004). Nicotine amplifies reward-related dopamine signals in striatum. *Nature Neuroscience*, *7*, 583–584. https://doi.org/10.1038/nn1244

Ritz, B. R., Manthripragada, A. D., Costello, S., Lincoln, S. J., Farrer, M. J., Cockburn, M., & Bronstein, J. (2009). Dopamine transporter genetic variants and pesticides in Parkinson's disease. *Environmental Health Perspectives*, *117*, 964–969. https://doi.org/10.1289/ehp.0800277

Roberts, B. M., Doig, N. M., Brimblecombe, K. R., Lopes, E. F., Siddorn, R. E., Threlfell, S., Connor-Robson, N., Bengoa-Vergniory, N., Pasternack, N., Wade-Martins, R., Magill, P. J., & Cragg, S. J. (2020). GABA uptake transporters support dopamine release in dorsal striatum with maladaptive downregulation in a parkinsonism model. *Nature Communications*, *11*, 4958. https://doi.org/10.1038/s41467-020-18247-5

Schwaller, B. (2020). Cytosolic Ca2+ buffers are inherently Ca2+ signal modulators. *Cold Spring Harbor Perspectives in Biology*, *12*, a035543. https://doi.org/10.1101/cshperspect.a035543

Schwaller, B., Meyer, M., & Schiffmann, S. (2002). "New" functions for "old" proteins: The role of the calcium-binding proteins calbindin D-28k, calretinin and parvalbumin, in cerebellar physiology. Studies with knockout mice. *Cerebellum*, *1*, 241–258.

Siciliano, C. A., Saha, K., Calipari, E. S., Fordahl, S. C., Chen, R., Khoshbouei, H., & Jones, S. R. (2018). Amphetamine reverses escalated cocaine intake via restoration of dopamine transporter conformation. *The Journal of Neuroscience*, *38*, 484–497. https://doi.org/10.1523/JNEUROSCI.2604-17.2017

Smith, L., & Schapira, A. H. V. (2022). GBA variants and Parkinson disease: Mechanisms and treatments. *Cells*, *11*(8):1261. https://doi.org/10.3390/cells11081261

Sonders, M. S., Zhu, S. J., Zahniser, N. R., Kavanaugh, M. P., & Amara, S. G. (1997). Multiple ionic conductances of the human dopamine transporter: The actions of dopamine and psychostimulants. *The Journal of Neuroscience*, *17*, 960–974. https://doi.org/10.1523/JNEUROSCI.17-03-00960.1997

Specht, C. G., & Schoepfer, R. (2001). Deletion of the alpha-synuclein locus in a subpopulation of C57BL/6J inbred mice. *BMC Neuroscience*, *2*, 11. https://doi.org/10.1186/1471-2202-2-11

Surmeier, D. J. (2018). Determinants of dopaminergic neuron loss in Parkinson's disease. *The FEBS Journal*, *285*, 3657–3668. https://doi.org/10.1111/febs.14607

Surmeier, D. J., Guzman, J. N., & Sanchez-Padilla, J. (2010). Calcium, cellular aging, and selective neuronal vulnerability in Parkinson's disease. *Cell Calcium*, *47*, 175–182. https://doi.org/10.1016/j.ceca.2009.12.003

Threlfell, S., Lalic, T., Platt, N. J., Jennings, K. A., Deisseroth, K., & Cragg, S. J. (2012). Striatal dopamine release is triggered by synchronized activity in cholinergic interneurons. *Neuron*, *75*, 58–64. https://doi.org/10.1016/j.neuron.2012.04.038

Threlfell, S., Mohammadi, A. S., Ryan, B. J., Connor-Robson, N., Platt, N. J., Anand, R., Serres, F., Sharp, T., Bengoa-Vergniory, N., Wade-Martins, R., Ewing, A., Cragg, S. J., & Brimblecombe, K. R. (2021). Striatal dopamine transporter function is facilitated by converging biology of α-synuclein and cholesterol. *Frontiers in Cellular Neuroscience*, *15*, 658244. https://doi.org/10.3389/fncel.2021.658244

Tian, X., Gala, U., Zhang, Y., Shang, W., Nagarkar Jaiswal, S., di Ronza, A., Jaiswal, M., Yamamoto, S., Sandoval, H., Duraine, L., Sardiello, M., Sillitoe, R. V., Venkatachalam, K., Fan, H., Bellen, H. J., & Tong, C. (2015). A voltage-gated calcium channel regulates lysosomal fusion with endosomes and autophagosomes and is required for neuronal homeostasis. *PLoS Biology*, *13*, e1002103. https://doi.org/10.1371/journal.pbio.1002103

Venton, B. J., Seipel, A. T., Phillips, P. E. M., Wetsel, W. C., Gitler, D., Greengard, P., Augustine, G. J., & Wightman, R. M. (2006). Cocaine increases dopamine release by mobilization of a synapsin-dependent reserve pool. *The Journal of Neuroscience*, *26*, 3206–3209. https://doi.org/10.1523/JNEUROSCI.4901-04.2006

Wooten, G. F., Currie, L. J., Bovbjerg, V. E., Lee, J. K., & Patrie, J. (2004). Are men at greater risk for Parkinson's disease than women? *Journal of Neurology, Neurosurgery, and Psychiatry*, *75*, 637–639. https://doi.org/10.1136/jnnp.2003.020982

Yasumoto, F., Negishi, T., Ishii, Y., Kyuwa, S., Kuroda, Y., & Yoshikawa, Y. (2004). Dopamine receptor 2 regulates L-type voltage-gated calcium channel in primary cultured mouse midbrain neural network. *Cellular and Molecular Neurobiology*, *246*(24), 877–882. https://doi.org/10.1007/s10571-004-6927-6

Yuan, H.-H., Chen, R.-J., Zhu, Y.-H., Peng, C.-L., & Zhu, X.-R. (2013). The neuroprotective effect of overexpression of

calbindin-D(28k) in an animal model of Parkinson's disease. *Molecular Neurobiology*, *47*, 117–122. https://doi.org/10.1007/s12035-012-8332-3

Zampese, E., Wokosin, D. L., Gonzalez-Rodriguez, P., Guzman, J. N., Tkatch, T., Kondapalli, J., Surmeier, W. C., D'Alessandro, K. B., de Stefani, D., Rizzuto, R., Iino, M., Molkentin, J. D., Chandel, N. S., Schumacker, P. T., & Surmeier, D. J. (2022). Ca2+ channels couple spiking to mitochondrial metabolism in substantia nigra dopaminergic neurons. *Science Advances*, *8*, eabp8701. https://doi.org/10.1126/sciadv.abp8701

Zhang, H., & Sulzer, D. (2012). Regulation of striatal dopamine release by presynaptic auto- and heteroreceptors. *Basal Ganglia*, *2*, 5–13. https://doi.org/10.1016/j.baga.2011.11.004

Zhou, F. M., Liang, Y., & Dani, J. A. (2001). Endogenous nicotinic cholinergic activity regulates dopamine release in the striatum. *Nature Neuroscience*, *4*, 1224–1229. https://doi.org/10.1038/nn769

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