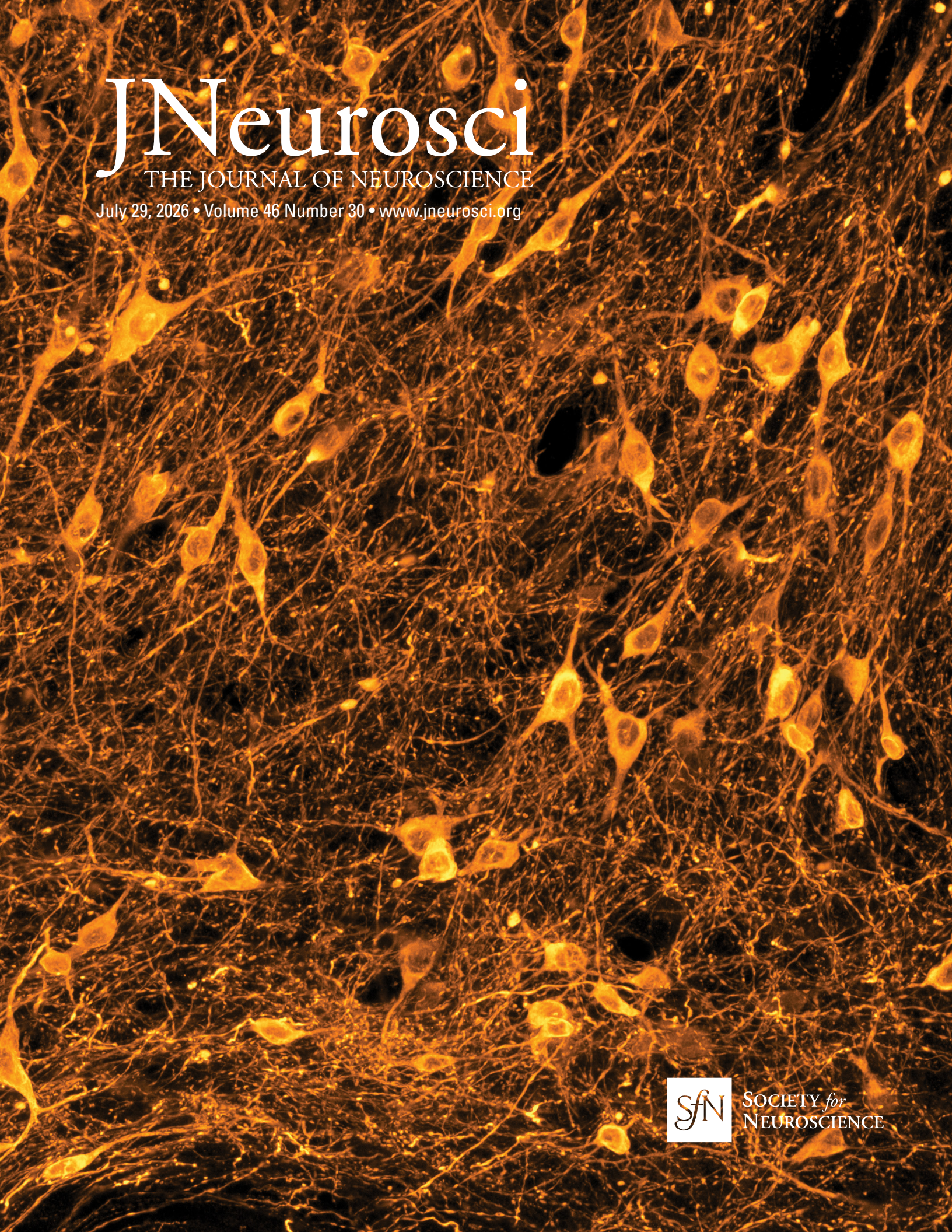




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The Synaptic Vesicle Priming Protein Munc13 Mediates Evoked Somatodendritic Dopamine Release

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Midbrain dopamine neurons release dopamine not only from their axons but also from their somata and dendrites. Shared and distinct properties have been proposed for somatodendritic and axonal release, but the mechanisms of somatodendritic release remain unclear. We here used gene knock-out, electrophysiology, and imaging to define roles of the synaptic vesicle priming protein Munc13 in somatodendritic dopamine release in comparison with axonal secretion. We characterized mice of either sex and found that Munc13 ablation decreased evoked but not spontaneous somatodendritic dopamine transmission measured as D2 receptor-mediated currents. Imaging with a fluorescent sensor confirmed the importance of Munc13 in evoked somatodendritic and axonal dopamine secretion. Pharmacological experiments revealed a modest contribution of release from norepinephrine axons to D2 receptor-mediated currents, and the relative contribution was enhanced after Munc13 knock-out. Altogether, these data establish important roles of Munc13 in evoked somatodendritic release. These roles are similar to Munc13 functions in axonal dopamine release and at fast synapses. Spontaneous midbrain dopamine release was not impaired by Munc13 ablation from dopamine neurons and may rely on a release pathway that is independent of the prototypical release machinery employed at synapses.

Key words: active zone; dopamine; Munc13; secretion; somatodendritic release; spontaneous release

Significance Statement

Neurons release neurotransmitter with high spatiotemporal precision from axonal nerve terminals. In addition, neurons also secrete transmitters such as monoamines, neurotrophins, and neuropeptides from their somata and dendrites. While axonal release has been well characterized, less is known about mechanisms of somatodendritic secretion. The presented data establish that the synaptic vesicle priming protein Munc13 is important for evoked somatodendritic dopamine release. In contrast, spontaneous somatodendritic dopamine release is not affected by Munc13 ablation from dopamine neurons in the tested mouse mutants. The Munc13 dependence of evoked somatodendritic secretion supports the model of spatiotemporally precise signaling for this form of transmission. Spontaneous dopamine release may rely on a pathway that is independent of the classical neurotransmitter release machinery.

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Introduction

Neurotransmitter release from axon terminals is mediated by specialized secretory machinery that enables spatiotemporally precise vesicle fusion (Südhof, 2012; Kaeser and Regehr, 2014). Most neurons also release neurotransmitters from the somatodendritic compartment, and both synaptic point-to-point transmission and volume transmission that lacks synaptic structure may mediate this dendro-dendritic signaling (Kennedy and Ehlers, 2011). Midbrain dopamine neurons, for example, release dopamine from somata and dendrites, and D2 receptor activation inhibits their firing (Björklund and Lindvall, 1975; Geffen et al., 1976; Aghajanian and Bunney, 1977; Araneda and Bustos, 1989; Pucak and Grace, 1994).

Recent work identified molecular machinery for somatodendritic dopamine release. First studies established that release is vesicular and relies on SNARE proteins for exocytosis (Bergquist et al., 2002; Beckstead et al., 2004; Fortin et al.,

2006). Evoked somatodendritic release is Ca^{2+} -dependent and utilizes Synaptotagmin-1 as a Ca^{2+} sensor (Beckstead et al., 2004; Lebowitz et al., 2023), and additional Ca^{2+} sensors might regulate it (Mendez et al., 2011; Delignat-Lavaud et al., 2022; Hikima et al., 2022; Lebowitz et al., 2024). Voltage-gated Ca^{2+} channels are a Ca^{2+} source, though the identity of the channels remains uncertain (Beckstead et al., 2004; Mendez et al., 2011). Furthermore, the active-zone protein RIM is important for stimulus-induced somatodendritic dopamine release, suggesting that preorganized protein complexes tethered by RIM mediate these fusion events similar to axonal dopamine secretion (Liu et al., 2018; Robinson et al., 2019). In aggregate, these studies revealed that the molecular machinery for somatodendritic dopamine release resembles that of classical synaptic release, supporting the model that small, clear vesicles might be the fusing membrane compartment (Liu et al., 2021; Özçete et al., 2024).

Spontaneous somatodendritic dopamine release occurs in the absence of action potentials, is Ca^{2+} -independent, and does not necessitate RIM or Synaptotagmin-1 in dopamine neurons (Gantz et al., 2013; Robinson et al., 2019; Lebowitz et al., 2023). While detected in somatodendritic regions as miniature events recorded as D2 receptor-mediated inhibitory postsynaptic currents (D2-IPSCs), it has remained uncertain whether this form of release is also present in axons. Ablating RIM from dopamine neurons does not remove all axonal release, striatal microdialysis detects activity-independent extracellular dopamine, and nanofilm sensors also detect activity-independent axonal release in cultured dopamine neurons (Liu et al., 2018; Banerjee et al., 2020; Bulumulla et al., 2022). This suggests that spontaneous dopamine release occurs in axons. Altogether, evoked and spontaneous dopamine release from axons, somata, and dendrites may all contribute to dopamine function and to dopamine-associated disorders (Gantz et al., 2013; Rice and Patel, 2015; Robinson et al., 2019; González-Rodríguez et al., 2021; Liu et al., 2021; Cai et al., 2024; Salvatore, 2024).

Here, we assessed roles of Munc13 in somatodendritic dopamine release. Munc13 is important for axonal release across species and cell types through its activities as a synaptic vesicle priming protein (Aravamudan et al., 1999; Augustin et al., 1999; Richmond et al., 1999; Varoquaux et al., 2002; Chen et al., 2013; Imig et al., 2014; Banerjee et al., 2022; Elizarova et al., 2022; Tan et al., 2022a,b). In mice with Munc13 ablation in dopamine neurons, we found a strong reduction in evoked somatodendritic dopamine release measured as D2-IPSCs. Dopamine release examined with a fluorescent sensor revealed a similar disruption when comparing somatodendritic release in the midbrain with axonal release in the striatum. Spontaneous dopamine transmission in the midbrain was unaffected by Munc13 ablation, bolstering the model of a separate secretory pathway. Finally, a small component of the midbrain D2-IPSC was due to norepinephrine innervation, reminiscent of the observation that D2 receptor-based dopamine sensors can report norepinephrine (López et al., 2026). The relative norepinephrine contribution to D2-IPSCs was increased after Munc13 ablation. Overall, these findings establish that Munc13 mediates evoked somatodendritic dopamine release. Our work supports that mechanisms for evoked somatodendritic release resemble those for axonal transmitter secretion and is suggestive of preassembled release sites for efficient and temporally precise dopamine signaling.

Materials and Methods

Mice. Munc13 cKO^{DA} mice were crossed as described before (Banerjee et al., 2022). Conditional Munc13-1 floxed mice

(MGI:7276178; Banerjee et al., 2022) were crossed to constitutive Munc13-2 knock-out mice (MGI:2449706; Varoquaux et al., 2002), constitutive Munc13-3 knock-out mice (MGI:2449467; Augustin et al., 2001), and DAT^{IRES-cre} mice (JAX: 006660; Backman et al., 2006). Munc13 cKO^{DA} mice were homozygote for Munc13-1 floxed, Munc13-2 null, and Munc13-3 null and heterozygote for DAT^{IRES-cre}. Munc13 control mice were heterozygote for Munc13-1 floxed and Munc13-2 null, homozygote for Munc13-3 null, and heterozygote for DAT^{IRES-cre}. We used littermate or age-matched mice of either sex from the same breeding colony for experiments. Experiments in Figures S1 and S2 were conducted in mice of either sex heterozygote for DAT^{IRES-cre}. Mice were genotyped in the lab or by Transnetex. Experiments were performed in accordance with protocols approved by Animal Care and Use Committees at Harvard Medical School and Oregon Health and Science University.

Stereotaxic surgeries. D2 receptor or GRAB_{DA} AAVs were delivered via stereotaxic injections of AAVs into the mouse brain. AAVs for D2 receptor expression were custom made as described (Lebowitz et al., 2023), and AAVs for GRAB_{DA} expression were purchased. For AAV9-GRAB_{DA2m} [also called AAV-hSyn-GRABDA2m, rAAV-hSyn-DA4.4; serotype, AAV2/9; Biohippo BHV12400441-9 (Sun et al., 2020)], 36–67-d-old mice were anesthetized with isoflurane and mounted on a stereotaxic frame. Anesthesia was maintained throughout surgery using isoflurane. After exposure of the skull, a burr hole was drilled for a unilateral AAV injection. Next, 1 μl of AAV ($1\text{--}2 \times 10^{12}$ viral genomic copies/ml) was injected into the dorsal striatum at a rate of 0.1 $\mu\text{l}/\text{min}$ using a microinjection syringe pump, coordinates were 1.0 mm anterior to the bregma, 2.0 mm lateral, and 2.5 mm below pia. Analgesia was provided following approved protocols. Experiments were conducted 48–60 d following injection. For AAV-GRAB-DA_{3m} [also called rAAV-hSyn-DA3m-WPRE-PA; serotype, AAV2/RETRO, BioHippo BHV12400547-12 (Zhuo et al., 2024)], 70–150-d-old mice were anesthetized with isoflurane and mounted on a stereotaxic frame. Anesthesia was maintained throughout surgery using isoflurane. After exposure of the skull, two burr holes were drilled for bilateral AAV injections. On both sides, 120 nl of AAV (titer, 5.0×10^{12} viral genomic copies/ml) were injected into the striatum at each of three locations along the dorsal/ventral axis. Virus was injected at a rate of 0.3 $\mu\text{l}/\text{min}$ using a microinjection syringe pump, coordinates were 1.25 mm anterior to the bregma, 1.50 mm lateral, and 3.40, 3.30, and 3.20 mm below pia. Analgesia was provided following approved protocols. Experiments were conducted 20–45 d following injection. For AAV-hD2Rs-P2A-GFP [custom made from pFB-AAV9-CAG-hD2Rshort-SNAP-p2A-GFP-WPRE-SV40pA, lab plasmid code p1038 (Lebowitz et al., 2023)], 90–140-d-old mice were anesthetized with isoflurane and mounted on a stereotaxic frame. Anesthesia was maintained throughout surgery using isoflurane. After exposure of the skull, two burr holes were drilled for bilateral AAV injections. On each side, 200 nl of AAV (titer, 2.15×10^{13} viral genomic copies/ml) were injected into the midbrain. Virus was injected at a rate of 0.3 $\mu\text{l}/\text{min}$ using a microinjection syringe pump. Coordinates were 2.3 mm posterior to the bregma, 1.3 mm lateral, and 4.5 mm below the pia. Postoperative analgesia was provided following approved protocols. Experiments were conducted 14–21 d following injection.

Brain slice preparation. For D2-IPSC recordings and GRAB_{DA3m} imaging, mice (110–209 d old) were deeply anesthetized with isoflurane and decapitated. The brain was dissected into warm (32–35°C) Krebs buffer containing the following (in mM): 126 NaCl, 2.5 KCl, 1.2 MgCl_2 , 2.4 CaCl_2 , 1.4 NaH_2PO_4 , 25 NaHCO_3 , and 11 dextrose. Krebs buffer was bubbled with 95% O_2 /5% CO_2 , pH 7.4, prior to decapitation and continuously throughout cutting and recovery and contained 10 μM MK-801. Horizontal brain slices containing the ventral midbrain were prepared at a thickness of 222 μm on a vibrating microtome and allowed to recover for at least 30 min at 30–32°C prior to recording or imaging. For GRAB_{DA2m} imaging, mice (84–127 d old) were deeply anesthetized with isoflurane and decapitated. Brains were dissected out, and 250- μm -thick parasagittal sections of the striatum were cut on a vibrating microtome in ice-cold cutting solution containing the

following (in mM): 7.5 MgSO₄, 75 sucrose, 75 NaCl, 1 NaH₂PO₄, 12 glucose, 26.2 NaHCO₃, 2.5 KCl, 1 sodium ascorbate, 1 myo-inositol, and 3 sodium pyruvate bubbled with 95% O₂/5% CO₂, pH 7.4, 300–305 mOsm. Slices were incubated in recovery solution for at least 1 h at room temperature (21–25°C) containing the following (in mM): 126 NaCl, 1 NaH₂PO₄, 2.5 KCl, 2 CaCl₂, 12 glucose, 1.3 MgSO₄, 12 glucose, 26.2 NaHCO₃, 1 sodium ascorbate, 1 myo-inositol, and 3 sodium pyruvate, bubbled with 95% O₂/5% CO₂, pH 7.4, 300–305 mOsm.

Electrophysiology. Experiments were performed using previously established methodology (Beckstead et al., 2004; Gantz et al., 2013; Robinson et al., 2019; Lebowitz et al., 2023). Slices were bisected along the midline, and experiments were carried out in hemisections placed in a recording chamber under continuous perfusion with Krebs buffer (2–3 ml/min) at 34°C bubbled with 95% O₂/5% CO₂, pH 7.4. Krebs buffer for recording also contained NBQX (600 nM), CGP55845 (300 nM), and picrotoxin (100 μM). In hemisected horizontal midbrain slices, dopamine neurons in the substantia nigra pars compacta (SNc) were identified by size, morphology, and position lateral to the medial terminal nucleus of the accessory optic system. Recordings were made using glass pipettes (initial resistance, 1.2–1.6 MΩ) filled with an internal solution containing the following (in mM): 100 K-methanesulfonate, 20 NaCl, 1.5 MgCl₂, 10 HEPES (K), 2 ATP, 0.3 GTP, 10 phosphocreatine, and 10 BAPTA (4 K), pH 7.4, 270–290 mOsm. Firing rates were recorded for ≥1 min in cell-attached mode prior to break-in, and action potentials were identified using an amplitude threshold. The holding potential was set to −55 mV prior to break-in, and cell capacitance, membrane resistance, and series resistance were measured with a 5 mV test pulse immediately after break-in. The uncompensated series resistance was monitored, and cells with an initial series resistance ≥10 MΩ were excluded from experiments. The *I_h* current was measured as the average amplitude over the last 25 ms of a 2 s, −50 mV hyperpolarizing voltage step. D2-IPSCs were recorded no sooner than 5 min after break-in. A monopolar electrode was positioned ~75–100 μm caudal to the recorded cell, and D2-IPSCs were induced with a train of five stimuli at 40 Hz using 50, 150, 250, and 350 μA stimulus intensities. The order of stimulation (ascending or descending) was alternated between cells. Each intensity was tested with three to five consecutive sweeps with an intersweep interval of 1 min, and the average amplitude of those sweeps was quantified at each stimulus intensity. Series resistance was monitored between different stimulus intensities and cells with a series resistance that exceeded 10 MΩ were discarded. The sensitivity to voltage-gated Na⁺ channel blockade was examined by comparing the D2-IPSC amplitude evoked with a 350 μA stimulus before and after the addition of 1 μM tetrodotoxin (TTX). Spontaneous D2-IPSCs were measured over a 5 min period starting no sooner than 10 min after break-in and detected automatically using a sliding template in Axograph (RRID:SCR_014284). In a second line of analyses, manual identification of spontaneous D2-IPSCs by an experimenter blind to genotype yielded results that were similar to those measured with the template function and shown in Figure 4, C and D. The sensitivity of D2-IPSCs to D2 receptor or voltage-gated Ca²⁺ channel blockade was assessed before and after addition of 1 μM sulpiride (Fig. S1) or 300 μM Cd²⁺ (Fig. S2), respectively; the sliding template method was used for detection of spontaneous events. If no IPSCs were detected, the frequency was considered 0 Hz. Occasionally, for the last cell of a given slice for the experiments in Figure 4, sulpiride (1 μM) was added at the end of the recording to qualitatively evaluate the dependence of spontaneous D2-IPSCs on D2 receptor activation and sulpiride blocked spontaneous events. D2 receptor activation by exogenous dopamine was measured by superfusing dopamine (1 or 10 μM) and is reported as the maximum change in current density. First, 1 μM dopamine was superfused until a peak current was attained (for ~2 min) and then removed. After the current returned to baseline, 10 μM dopamine was superfused until a new peak was attained (for ~2 min). Current density was calculated by dividing the amplitude of the change in holding current by the recorded cell's capacitance. Norepinephrine axon contribution was estimated by sequential perfusion of UK14,304 (1 μM) followed by idazoxan (1 μM). Effects were calculated using the final three sweeps (at 3–5 min) during an 8 min perfusion for each condition.

Representative traces of evoked D2-IPSCs are the average of three consecutive sweeps. Representative traces of pacemaker firing, dopamine superfusion, and spontaneous D2-IPSCs are from single sweeps.

Widefield imaging in the striatum. GRAB_{DA2m} imaging in parasagittal slices containing the dorsal striatum was performed following established methodology (Liu et al., 2022; Cai et al., 2024) with modifications. Images were acquired with a widefield fluorescence microscope (Olympus BX51) with a 470 nm excitation LED, a 4× objective, and a scientific metal–oxide–semiconductor camera (Hamamatsu ORCA-Flash4.0) in artificial cerebrospinal fluid (ACSF) containing the following (in mM): 126 NaCl, 26.2 NaHCO₃, 2.5 KCl, 1.3 MgSO₄, 2 CaCl₂, 1 NaH₂PO₄, and 12 glucose. The ACSF was heated to 34–36°C, and slices were perfused with a flow rate of 2–3 ml/min, and 1 μM DhβE was included in the bath. Solutions were continuously bubbled with 95% O₂/5% CO₂, and recordings were completed within 5 h of slicing. Electrical stimulation was delivered with an intensity of 90 μA (biphasic wave, 0.25 ms in each phase) and with a linear stimulus isolator (A395, World Precision Instruments) through a unipolar glass pipette (tip diameter of 2–4 μm) filled with ACSF, using single pulses or 10 pulse stimulus trains at 10 Hz. Image acquisition, LED illumination, and electrical stimulation were controlled with a digitizer (Molecular Devices, Digidata 1440A). LED power was set to 10% across genotypes and repeats. Images were acquired at 512 × 512 pixels per frame, with a sampling frequency of 50 fps and an exposure time of 20 ms per frame. Fields of view were selected based on the presence of similar visible baseline GRAB_{DA} fluorescence prior to stimulation. Three sweeps were performed and averaged for each area for single stimuli and for 10 stimuli at 10 Hz. A single pixel represents a striatal area of 6.4 × 6.4 μm². For analyses, ImageJ/Fiji (RRID:SCR_002285; Schindelin et al., 2012) was used, and background fluorescence was estimated from regions of no sensor expression and subtracted from each image frame. Pixels with intensity values in the top 50% of the intensity histogram of the image stack generated from background subtracted images were used for plotting the ΔF/F₀ time course. F₀ was calculated as the average GRAB_{DA2m} signal over 0.5 s (25 frames) immediately before stimulation (F₀ as mean ± SEM in Munc13 control, 696.2 ± 92.7 arbitrary fluorescence units; 12 images of 12 slices from 3 mice; Munc13 cKO^{DA}, 974.2 ± 131.7; 12/12/3; *t*₍₂₂₎ = 1.726; *p* = 0.09; unpaired *t* test). ΔF/F₀ was calculated for each pixel and quantified. For the peak plots, the maximum value of ΔF/F₀ within 0.7 or 1.1 s for single or train stimuli, respectively, was plotted. Sample heatmaps show the peak ΔF/F₀ image frame after background subtraction and with brightness and contrast adjustments identical across genotypes; the display color range was set to illustrate the full range of the ΔF/F₀ signal, and the underlying raw ΔF/F₀ was not saturated.

Confocal imaging in the striatum and midbrain. GRAB_{DA3m} imaging on hemisected horizontal slices was carried following methodology described before (Rodriguez-Contreras et al., 2025) but with an upright microscope (Olympus BX51 WI), a 60× objective (Olympus LUMPlanFL 60X/1.00W), and a Yokogawa CSU10-B-F300-E-B488 spinning-disc confocal unit. Excitation was at 488 nm with a Cobolt 06-MLD laser controlled by a NEOS AOTF module. Fluorescence was detected and recorded at 10 Hz with a Photometrics Prime 95B camera. Hardware and acquisition settings were controlled by μManager2.0 (RRID:SCR_000415). Stimulation was carried out using Axograph and triggered by μManager2.0 at the start of each video to synchronize acquisition and stimulus timing. Stimulation was delivered with a monopolar electrode as described for evoked D2-IPSCs at 250 μA stimulation intensity, and five pulses at 40 Hz were applied. Fields of view were selected based on the presence of similar visible baseline GRAB_{DA} fluorescence prior to stimulation. Each field was imaged once, and 3–7 images were captured from each slice. The duration between stimuli was ≥5 min. For analyses, a maximum intensity projection image was generated, and the pixels with intensities in the top 75% in the intensity histogram of the image stack were used to generate a region of interest (ROI) in ImageJ/Fiji. GRAB_{DA3m} intensity was measured as the average intensity in the ROI. F₀ was defined as the average fluorescence over 2 s (20 frames)

preceding stimulation [F_0 as mean $\pm$ SEM in striatum; Munc13 control, 126.3 ± 9.7 arbitrary fluorescence units; six fields of view of four slices from four mice; Munc13 cKO^{DA}, 111.4 ± 0.3 ; 7/3/3; $U(n_1 = 6; n_2 = 7) = 11$; $p = 0.18$; Mann–Whitney rank sum test; F_0 in midbrain; Munc13 control, 114.3 ± 0.6 ; 26/6/3; Munc13 cKO^{DA}, 124.9 ± 2.2 ; 14/6/4; $U(n_1 = 26; n_2 = 14) = 57$; $p < 0.01$; Mann–Whitney rank sum test] and used to determine $\Delta F/F_0$. The peak fluorescence value was extracted as the maximum change in $\Delta F/F_0$ over the same ROI from a single image frame from the time series. Sample heatmaps show the peak $\Delta F/F_0$ image frame with brightness and contrast adjustments identical across genotypes and brain regions; the display color range was set to illustrate the full range of $\Delta F/F_0$ signal, and the underlying raw $\Delta F/F_0$ was not saturated.

Experimental design and statistical analyses. Summary data are presented as mean $\pm$ SEM with individual values shown as circles. Statistical significance is denoted as * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ in each figure. Statistical testing was done in GraphPad Prism 10 (RRID: SCR_002798). The number of observations is described in each figure legend. Numerical values for the data presented in figures were deposited at 10.5281/zenodo.20067463. For comparison of two groups, datasets were tested for normality with a Shapiro–Wilk test. Normally distributed data were assessed with paired or unpaired Student's t tests, and non-normally distributed data with Mann–Whitney (unpaired) or Wilcoxon matched-pairs (paired) rank sum tests. For comparisons of multiple groups and variables, two-way ANOVA was used with individual comparisons made using the post hoc test listed in the corresponding figure legend. Experiments with genotype comparisons were performed by an experimenter blind to genotype during data acquisition and analyses.

Results

Munc13 ablation disrupts evoked somatodendritic dopamine release

Roles of Munc13 in somatodendritic dopamine release were examined using whole-cell recordings from SNc dopamine neurons in horizontal slices of mice in which Munc13 proteins were genetically ablated. We pursued a strategy previously used to study axonal dopamine release (Banerjee et al., 2022) with dopamine neuron-specific ablation of Munc13-1 and constitutive knock-out of Munc13-2 and Munc13-3 (Munc13 cKO^{DA}; Fig. 1A). In whole-cell recordings from dopamine neurons (Fig. 1B) of Munc13 cKO^{DA} and Munc13 control mice, the spontaneous firing rates and inward currents induced by a hyperpolarizing step (I_H) were similar (Fig. 1C–F). The input resistance (R_M) and cell capacitance of these neurons were also within expected ranges, but small magnitude decreases in R_M and increases in capacitance were detected in slices of Munc13 cKO^{DA} mice (Fig. 1G,H). This is consistent with the modestly altered axon structure in the striatum of these mice (Banerjee et al., 2022). Midbrain dopamine transmission was measured via D2-IPSCs recorded in the SNc evoked by extracellular electrical stimulation (5 stimuli, 40 Hz) at varying intensities (Fig. 2A–C). At the lowest intensity (50 μ A), neither Munc13 cKO^{DA} nor Munc13 control cells exhibited an appreciable D2-IPSC. As stimulus intensity increased, D2-IPSCs were induced in dopamine neurons of Munc13 control and Munc13 cKO^{DA} mice. D2-IPSC amplitudes in Munc13 cKO^{DA} slices were reduced by 73% at 150 μ A stimulation intensity, 74% at 250 μ A, and 67% at 350 μ A. In both genotypes, D2-IPSCs were mediated by action potentials as they were eliminated by acute bath application of the Na⁺ channel blocker TTX (Fig. 2D,E).

We next tested whether the genetic manipulations might alter D2 receptor activation. We measured D2 receptor-mediated currents induced in response to perfusion of 1 or 10 μ M exogenous dopamine (Fig. 2F,G). Quantification of the current density did

not reveal a difference between Munc13 cKO^{DA} and control neurons. This establishes that somatodendritic D2 receptors are present in Munc13 cKO^{DA} neurons and that these receptors detect exogenous dopamine similar to the control condition. These observations support that the reduced D2-IPSC amplitudes are a result of decreased release from dopamine neurons following ablation of Munc13.

Munc13 ablation similarly disrupts axonal and somatodendritic release

A more direct measure of dopamine release was next obtained by imaging fluorescence changes of GPCR-activation based dopamine (GRAB_{DA}) sensors. In a first set of imaging experiments, we used widefield microscopy as established before to assess axonal dopamine release in the striatum with the D2 receptor-based dopamine sensor GRAB_{DA2m} (Sun et al., 2020; Cai et al., 2024; Fig. 3A). GRAB_{DA} was expressed with AAVs injected into the striatum. Acute brain slices were prepared several weeks after injection, and GRAB_{DA} fluorescence changes were measured and quantified as $\Delta F/F_0$ in a large field of view. GRAB_{DA} fluorescence transients in response to either single stimuli or trains of 10 stimuli at 10 Hz (Fig. 3B–G) were strongly reduced in Munc13 cKO^{DA} slices. This confirms previous work that defined Munc13 proteins as important mediators of evoked axonal dopamine release with carbon fiber amperometry in acute striatal brain slices (Banerjee et al., 2022).

In a next set of experiments, we used a retrograde AAV strategy expressing GRAB_{DA3m}, a D1 receptor-based dopamine sensor with an increased signal-to-noise ratio compared with GRAB_{DA2m} (Zhuo et al., 2024). We injected AAV-GRAB_{DA} into the striatum to transduce striatal neurons and neurons with afferent projections into the striatum. This strategy transduces dopamine neurons via their striatal axons. GRAB_{DA} fluorescence was then imaged using spinning-disc confocal microscopy in smaller fields of view compared with the widefield imaging experiments described above and with a stimulus protocol (5 stimuli, 40 Hz) well suited for midbrain measurements. We used this approach in parallel in the striatum and the midbrain to assess dopamine release in both areas (Fig. 3H,L). Similar to the widefield measurements (Fig. 3A–G), there was a robust impairment of dopamine release in Munc13 cKO^{DA} slices in the striatum (Fig. 3H–K). The assessment of stimulus-induced GRAB_{DA} transients in the ventral midbrain of Munc13 cKO^{DA} and Munc13 control slices revealed a strong reduction in somatodendritic dopamine release (Fig. 3L–O). Overall, the reduction in somatodendritic dopamine release monitored with GRAB_{DA} was similar in magnitude to effects observed in striatal GRAB_{DA} experiments (Fig. 3A–K) and in midbrain D2-IPSC recordings (Fig. 2A–E). Hence, axonal and somatodendritic dopamine release have a comparable dependency on Munc13. These findings are reminiscent of previous work that established shared dependency of axonal and somatodendritic dopamine release on RIM active-zone scaffolds and on the Ca²⁺ sensor Synaptotagmin-1 (Liu et al., 2018; Robinson et al., 2019; Banerjee et al., 2020, 2022; Delignat-Lavaud et al., 2023; Lebowitz et al., 2023).

Spontaneous somatodendritic dopamine transmission persists after Munc13 ablation from dopamine neurons

Unlike evoked release, spontaneous somatodendritic dopamine release is insensitive to blockade of voltage-gated Na⁺ channels, voltage-gated Ca²⁺ channels, lowering of extracellular Ca²⁺, or genetic ablation of RIM or Synaptotagmin-1 from dopamine

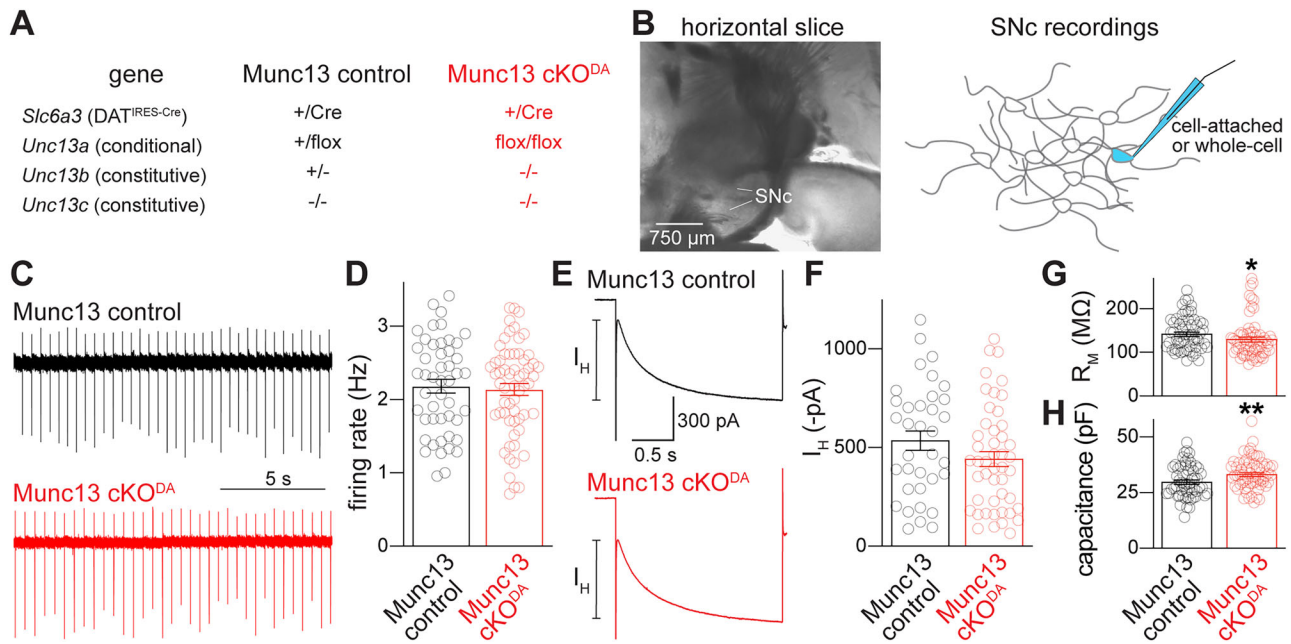


Figure 1. Electrical properties of dopamine neurons after Munc13 ablation. **A**, Genetic strategy for Munc13 ablation as established before (Banerjee et al., 2022); Munc13-1 ablation is dopamine neuron-specific; Munc13-2 and Munc13-3 ablations are constitutive. **B**, Bright-field image of a horizontal brain slice and a schematic of the recordings in the SNc. **C**, **D**, Example traces (**C**) and quantification of rates (**D**) of spontaneous firing of dopamine neurons, measured cell-attached over 2–4 min prior to break-in; Munc13 control 48 cells from 34 slices of 18 mice, Munc13 cKO^{DA} 60/40/21. **E**, **F**, Example traces (**E**) and quantification of the amplitude (**F**) of HCN-mediated inward currents (I_H) in response to a -50 mV, 2 s hyperpolarizing step; Munc13 control 35/25/15, Munc13 cKO^{DA} 47/32/15. **G**, **H**, Quantification of the input resistance (**G**) and cell capacitance (**H**) measured after break-in; Munc13 control 57/33/16, Munc13 cKO^{DA} 64/45/15. Data are shown as mean $\pm$ SEM; * $p < 0.05$; ** $p < 0.01$; assessed by unpaired t tests (**D**, $t_{(106)} = 0.3527$; $p = 0.725$; **H**, $t_{(119)} = 2.76$; $p = 0.0067$) or Mann–Whitney rank sum tests (**F**, $U(n_1 = 35; n_2 = 47) = 666$; $p = 0.1443$; **G**, $U(n_1 = 57; n_2 = 64) = 1387$; $p = 0.0228$).

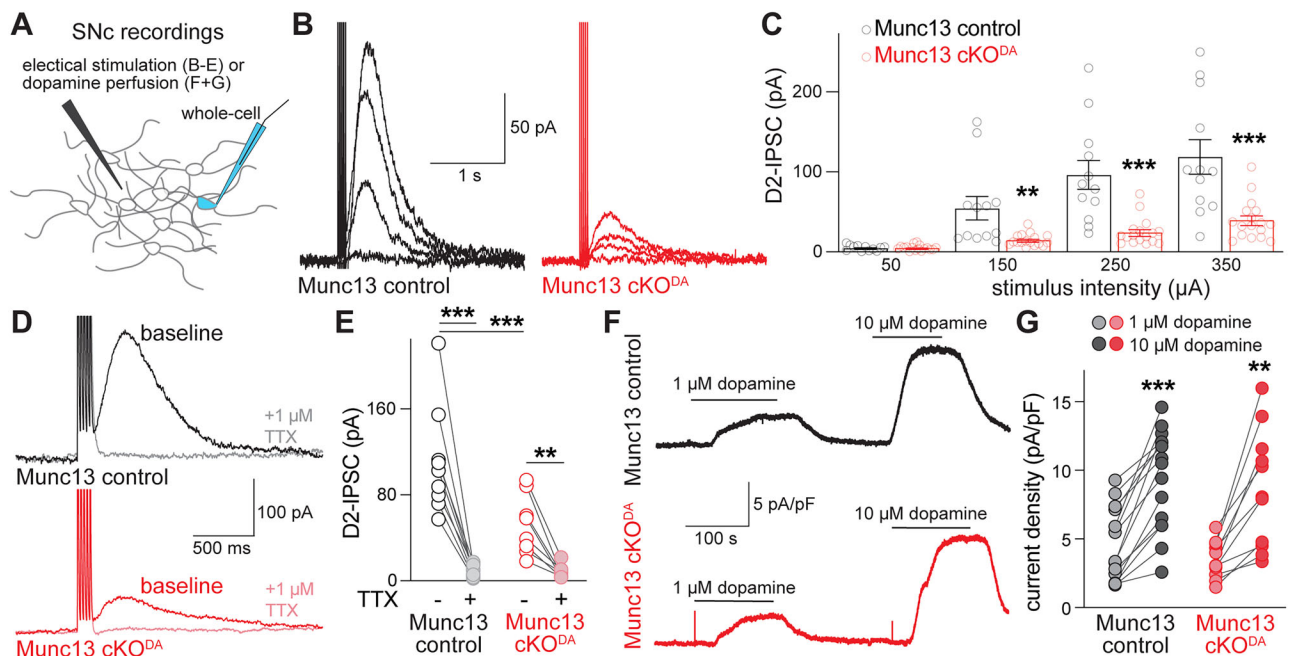


Figure 2. Munc13 ablation impairs dopamine release in the SNc. **A**, Schematic of recordings in the SNc. **B**, **C**, Example traces (**B**) and quantification of peak amplitudes (**C**) of D2-IPSCs induced by five stimuli at 40 Hz at 50, 150, 250, or 350 μ A stimulation intensity; Munc13 control 12 cells from 10 slices of six mice, Munc13 cKO^{DA} 17/15/7. **D**, **E**, Example traces (**D**) and quantification of peak amplitudes (**E**) of D2-IPSCs induced by five stimuli at 40 Hz at 350 μ A stimulation intensity before or after perfusion of the voltage-gated Na^+ channel blocker TTX (1 μ M); Munc13 control 9/9/6, Munc13 cKO^{DA} 8/8/7. **F**, **G**, Example traces (**F**) and quantification (**G**) of current density induced by 1 or 10 μ M dopamine perfusion for ~ 2 min until a peak current was achieved (calculated as the change in holding current divided by cell capacitance); Munc13 control 15/15/7, Munc13 cKO^{DA} 11/11/9. Data are shown as mean $\pm$ SEM; ** $p < 0.01$; *** $p < 0.001$; assessed by two-way ANOVA in **C** (genotype, ***; intensity, ***; interaction, ***; $F_{\text{Genotype}(1,27)} = 16.68$; $p = 0.0004$; $F_{\text{Intensity}(3,81)} = 53.11$; $p < 0.0001$; $F_{\text{Interaction}(3,81)} = 16.67$; $p < 0.0001$), **E** (genotype, *; TTX, ***; interaction, **; $F_{\text{Genotype}(1,15)} = 7.691$; $p = 0.0142$; $F_{\text{TTX}(1,15)} = 59.01$; $p < 0.0001$; $F_{\text{Interaction}(1,15)} = 9.201$; $p = 0.0084$), and **G** (genotype, ns; concentration, ***; interaction, ns; $F_{\text{Genotype}(1,48)} = 1.001$; $p = 0.3222$; $F_{\text{Concentration}(1,48)} = 31.81$; $p < 0.0001$; $F_{\text{Interaction}(1,48)} = 0.025$; $p = 0.8748$) followed by Sidak's multiple-comparison tests (**C**, **E**, **G**, significance indicated in panels).

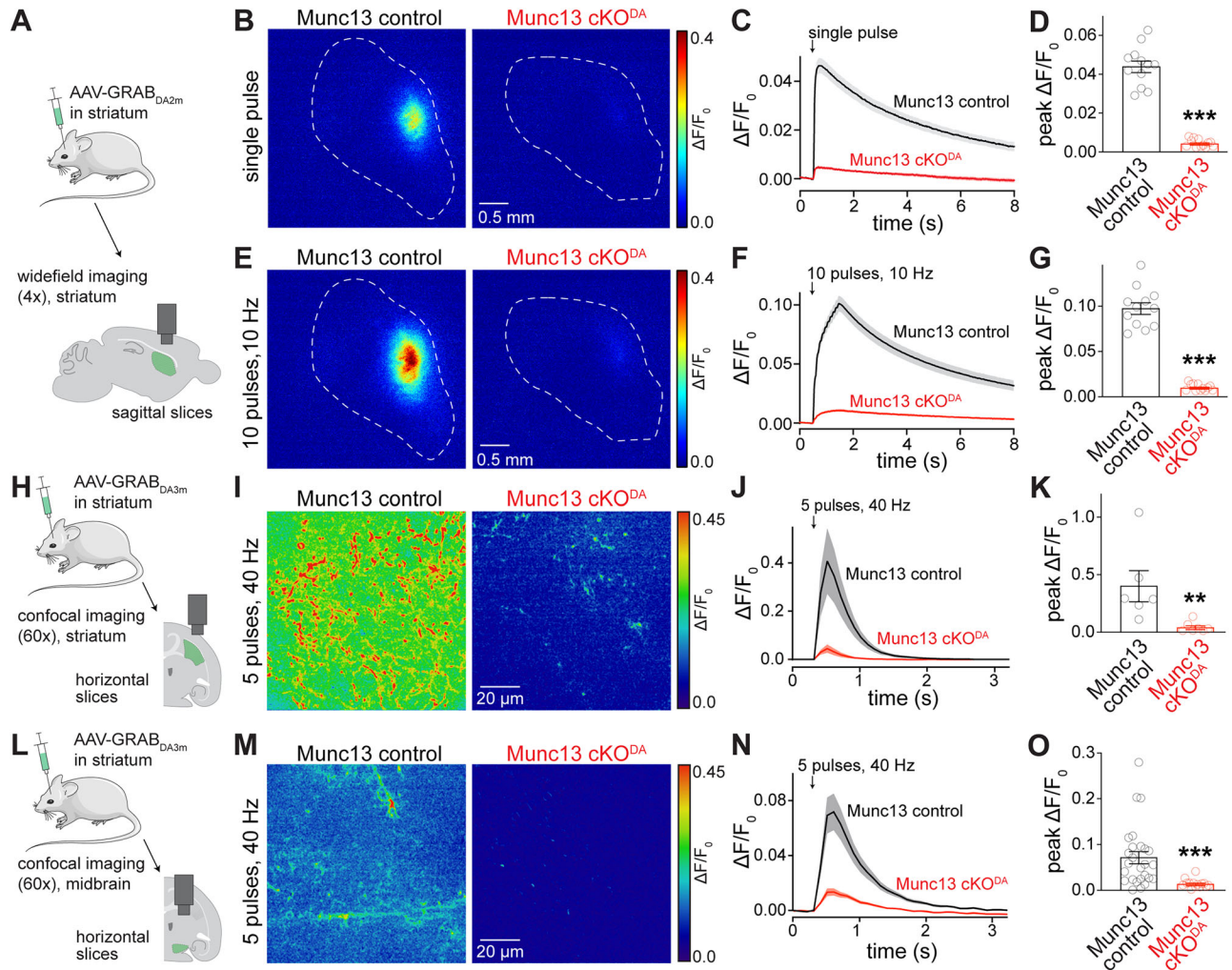


Figure 3. Munc13 ablation disrupts striatal and midbrain dopamine release monitored with GRAB_{DA}. **A**, Schematic of striatal unilateral AAV injections followed by widefield imaging in parasagittal striatal slices. **B–D**, Example $\Delta F/F_0$ images (**B**) and quantification of the $\Delta F/F_0$ time course (**C**) and peak amplitude (**D**) during single extracellular electrical stimuli; Munc13 control 12 images of 12 slices from three mice, Munc13 cKO^{DA} 12/12/3. **E–G**, As in **B–D**, but for 10 stimuli at 10 Hz; Munc13 control 12/12/3, Munc13 cKO^{DA} 12/12/3. **H**, Schematic of bilateral striatal AAV injections followed by confocal imaging in hemisected horizontal striatal slices. **I–K**, Example $\Delta F/F_0$ images (**I**) and quantification of the $\Delta F/F_0$ time course (**J**) and peak amplitude (**K**) during five electrical stimuli at 40 Hz and 250 μ A stimulation intensity; Munc13 control 6/4/4, Munc13 cKO^{DA} 7/3/3. **L**, Schematic of bilateral striatal AAV injections followed by confocal imaging in hemisected horizontal midbrain slices. **M–O**, As in **I–K**, but for horizontal midbrain slices; Munc13 control 26/6/3, Munc13 cKO^{DA} 14/6/4. Data are shown as mean $\pm$ SEM; *** p < 0.001; assessed by unpaired t tests (**D**, $t_{(22)} = 13.17$; p < 0.0001; **G**, $t_{(22)} = 13.56$; p < 0.0001) or Mann–Whitney rank sum test [**K**, $U(n_1 = 6; n_2 = 7) = 1$; $p = 0.0023$; **O**, $U(n_1 = 26; n_2 = 14) = 47$; p < 0.0001].

neurons (Beckstead et al., 2004; Gantz et al., 2013; Robinson et al., 2019; Lebowitz et al., 2023). Spontaneous glutamate or GABA release at fast synapses is greatly reduced when Munc13 is ablated (Varoqueaux et al., 2002; Tan et al., 2022a,b). We set out to examine whether spontaneous dopamine release shows a similar dependency on Munc13. We used a previously employed strategy to facilitate spontaneous D2-IPSC detection by exogenous expression of D2 receptors via AAVs injected into the midbrain (Fig. 4A; Lebowitz et al., 2023). We previously established that lowering extracellular Ca^{2+} or blockade of action potential firing with TTX does not decrease the frequency of the spontaneous release events in these experiments (Lebowitz et al., 2023). Here, we further established this methodology by assessing the sensitivity of spontaneous D2-IPSCs to D2 receptor blockade with sulpiride and voltage-gated Ca^{2+} channel blockade with Cd^{2+} . In acute brain slices prepared ~ 2 weeks after AAV transduction, bath addition of sulpiride abolished spontaneous and evoked D2-IPSCs (Fig. S1), establishing their D2 receptor

dependence. In additional experiments, Cd^{2+} did not detectably inhibit spontaneous D2-IPSCs, supporting their independence of voltage-gated Ca^{2+} channels, but impaired evoked D2-IPSCs (Fig. S2). These results further establish the strategy of assessing spontaneous somatodendritic dopamine transmission in mice with AAV-mediated D2 receptor expression.

We then assessed spontaneous somatodendritic dopamine release using this experimental strategy in slices of Munc13 cKO^{DA} and Munc13 control mice (Fig. 4A). Neither the amplitude nor the frequency of spontaneous D2-IPSCs were changed in Munc13 cKO^{DA} slices (Fig. 4B–D). To assess whether the D2 receptor overexpression strategy altered the phenotypes observed in response to electrical stimulation, evoked D2-IPSCs were measured. Similar to the D2-IPSCs mediated by endogenous receptors (Fig. 2), the current amplitudes were strongly reduced after ablation of Munc13 in slices expressing exogenous D2 receptors (Fig. 4E,F). Hence, expression of exogenous D2 receptors did not alter the impairment in stimulated

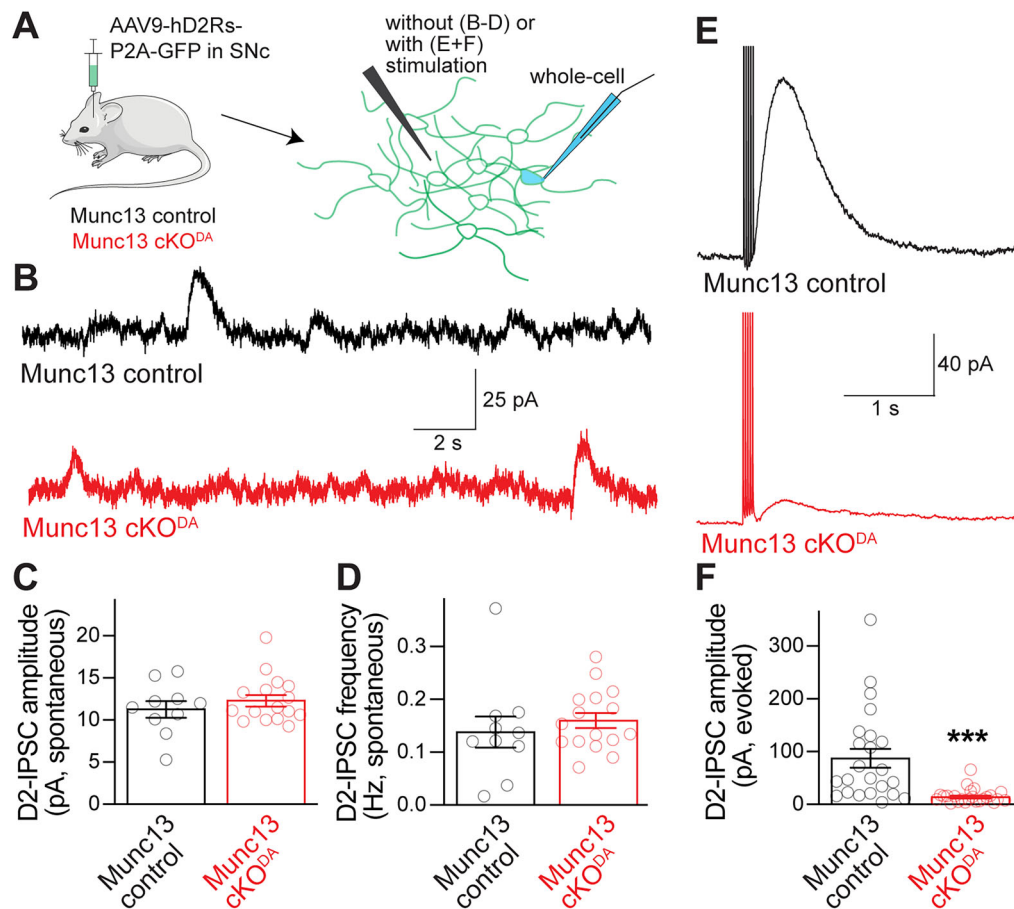


Figure 4. Spontaneous dopamine release in the SNc persists after Munc13 ablation. **A**, Schematic of AAV-mediated D2 receptor expression followed by whole-cell recordings of SNc dopamine neurons. **B–D**, Example traces (**B**) and quantification of amplitude (**C**) and frequency (**D**) of spontaneous D2-IPSCs; Munc13 control 10 cells from four slices of four mice, Munc13 cKO^{DA} 16/8/4. **E, F**, Example traces (**E**) and quantification of peak amplitudes (**F**) of D2-IPSCs induced by five stimuli at 40 Hz and 150 μ A stimulation intensity; Munc13 control 23/17/4, Munc13 cKO^{DA} 24/13/4. Data are shown as mean $\pm$ SEM, *** $p < 0.001$; assessed by Mann–Whitney rank sum tests (**C**: $U(n_1 = 10, n_2 = 16) = 71, p = 0.6599$, **F**: $U(n_1 = 23, n_2 = 24) = 69, p < 0.0001$) or an unpaired t test (**D**: $t_{(24)} = 0.6937, p = 0.4945$). For sensitivity of D2-IPSCs to sulpiride or Cd^{2+} , see Figs. S1, S2.

somatodendritic dopamine release. Previous work describing the independence of spontaneous release on RIM or Synaptotagmin-1, or on Ca^{2+} entry, is consistent with a model that spontaneous release in the midbrain occurs through a secretory pathway that is distinct from that for evoked release (Gantz et al., 2013; Robinson et al., 2019; Lebowitz et al., 2023). The lack of reliance on Munc13 further supports the model of a separate release pathway for spontaneous release in the midbrain.

Norepinephrine innervation modestly contributes to D2-IPSCs measured in the midbrain

The residual component of dopamine release in Munc13 cKO^{DA} mice could be explained by Munc13 independence, by the presence of a small amount of a left-over Munc13 protein, or by neurotransmitters other than dopamine that might activate D2 receptors or dopamine sensors (Banerjee et al., 2022; Tan et al., 2022a,b; López et al., 2026). The substantia nigra receives norepinephrine innervation, and neurotransmitter released from these axons might act on D2 receptors (Mejías-Aponte et al., 2009; Agster et al., 2013; Sánchez-Soto et al., 2016; López et al., 2026). To examine this possibility, D2-IPSCs were measured before and after inhibition of release from norepinephrine axons by the selective α_2 -adrenergic receptor agonist UK14,304 (Fig. 5A). Activation of this G_i -coupled autoreceptor is known to inhibit stimulation-evoked norepinephrine release (Hu and

Fredholm, 1989; Rump et al., 1991; Bücheler et al., 2002). D2-IPSCs recorded in control slices showed a modest (17%) but statistically significant decrease in amplitude after bath addition of UK14,304, which was reversed by the α_2 adrenergic receptor antagonist idazoxan (Fig. 5B–E). In neurons recorded in Munc13 cKO^{DA} slices, the remaining D2-IPSC was reduced by a similar absolute magnitude in response to bath application of UK14,304, though the smaller initial D2-IPSC amplitude before application of UK14,304 resulted in a larger relative inhibition (47%), which was also reversed by idazoxan (Fig. 5B–E). We conclude that only a small component of the D2-IPSC is mediated by norepinephrine axons. While this mechanism contributes significantly to the measured D2-IPSC that remains after Munc13 ablation, it does not fully account for it, and a small amount of somatodendritic dopamine release persists after the genetic ablation of Munc13 tested here.

Discussion

The D2-IPSC and dopamine sensor measurements establish that the vesicle priming protein Munc13 is important for evoked somatodendritic dopamine release. Effect magnitudes of Munc13 ablation are similar for axonal and somatodendritic dopamine release. There is a small midbrain D2-IPSC remaining after Munc13 ablation, and about half of it is accounted for by release from norepinephrine axons. Contrasting evoked

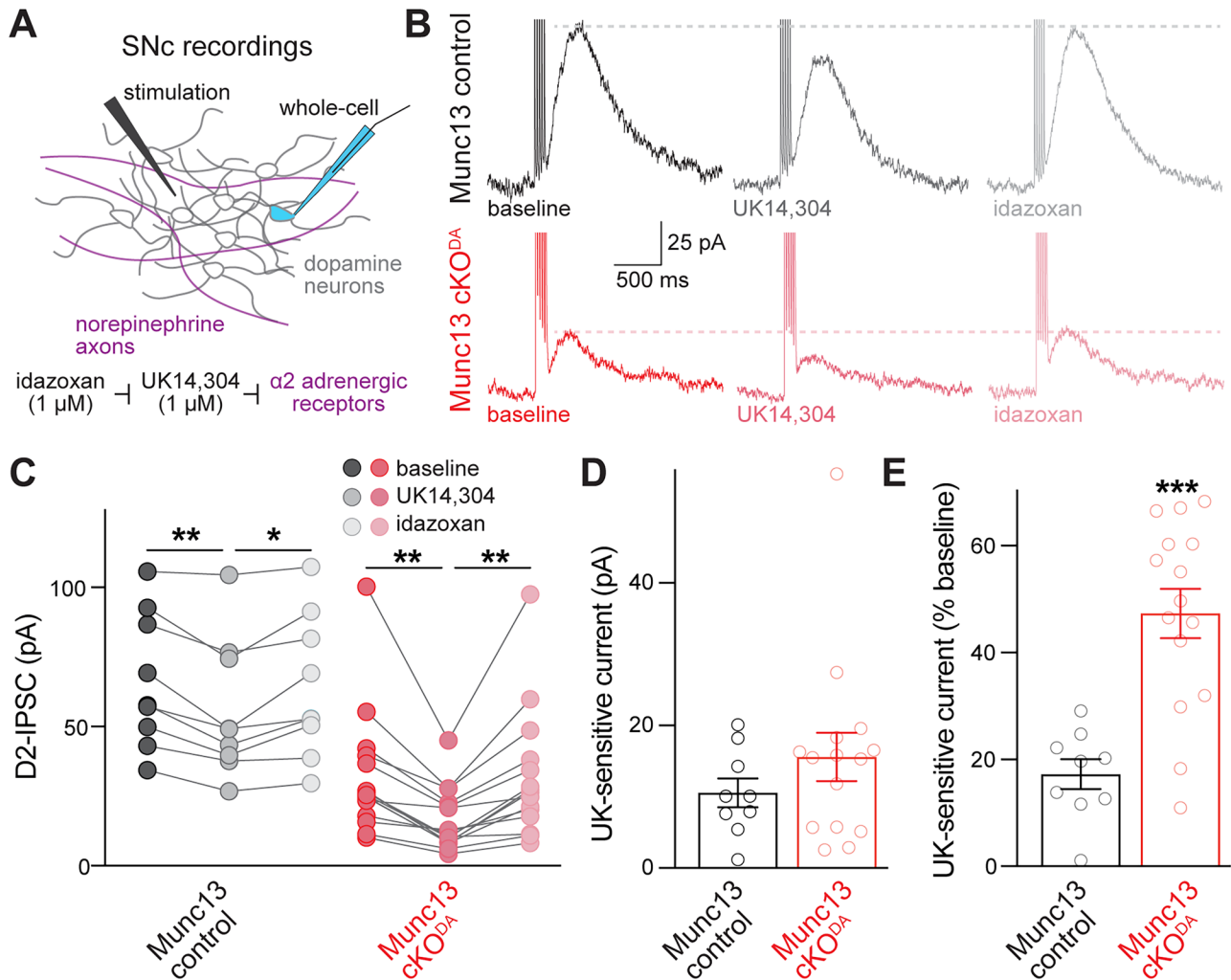


Figure 5. Contribution of release from norepinephrine axons to D2-IPSCs in the SNc. **A**, Schematic and pharmacological strategy of D2-IPSC recordings in the SNc. **B–E**, Example traces (**B**) and quantification of peak amplitudes (**C**) of D2-IPSCs induced by five stimuli at 40 Hz and 250 μ A stimulation intensity, and quantification of absolute (**D**) and normalized (**E**) UK14,304 sensitivity in an experiment with sequential addition of UK14,304 and idazoxan; the dashed line in **B** marks baseline peak amplitude; Munc13 control nine cells from nine slices of four mice, Munc13 cKO DA 15/15/6. Data are shown as individual cells (**C**) or as mean $\pm$ SEM; * p < 0.05; ** p < 0.01; *** p < 0.001; assessed by a two-way ANOVA (**C**; genotype, ***; drug, ***; interaction, ns; $F_{\text{Genotype}(1,22)} = 15.95$; $p = 0.0006$; $F_{\text{Drug}(1,251,27.51)} = 25.55$; $p < 0.0001$; $F_{\text{Interaction}(1,251,27.51)} = 1.698$; $p = 0.2053$) followed by Tukey's post-tests (indicated in the figure), a Mann–Whitney rank sum test (**D**; $U(n_1 = 9; n_2 = 15) = 53$; $p = 0.4115$), or an unpaired t test (**E**; $t_{(22)} = 4.734$; $p < 0.0001$).

somatodendritic release, spontaneous D2-IPSCs are independent of Munc13 in dopamine neurons. Overall, our work is consistent with the model of preorganized sites for spatiotemporally precise somatodendritic dopamine release.

Are there sites to prime vesicles for somatodendritic dopamine release?

For fast, action potential-induced secretion, fusion-ready vesicles must be pre-tethered at release sites (Neher, 2015; Kaeser and Regehr, 2017). In addition, voltage-gated Ca^{2+} channels need to be concentrated near the vesicular Ca^{2+} sensors (Eggermann et al., 2011; Kaeser and Regehr, 2014; Chin and Kaeser, 2024). At conventional synapses and in dopamine axons, active-zone proteins orchestrate these processes (Südhof, 2013; Liu and Kaeser, 2019).

Whether similar protein assemblies control somatodendritic neurotransmitter secretion has remained an open question. Munc13's importance provides molecular evidence for vesicle priming in midbrain dopamine secretion. Munc13 is the principal priming protein at synapses, where it mediates the assembly of SNARE complexes (Augustin et al., 1999; Varoqueaux et al., 2002; Dulubova et al., 2005; Ma et al., 2011; Lai et al., 2017)

and supports the tight docking of synaptic vesicles (Siksou et al., 2009; Imig et al., 2014; Tan et al., 2022a,b). Central to these roles is the recruitment and activation of Munc13 by RIM (Betz et al., 2001; Dulubova et al., 2005; Deng et al., 2011; Camacho et al., 2017; Tan et al., 2022a,b).

The findings that Munc13 (Figs. 2, 3) and RIM (Robinson et al., 2019) are important for stimulus-induced somatodendritic dopamine release support that the synaptic vesicle priming mechanism operates in somatodendritic dopamine secretion. More broadly, the dependence on Munc13, RIM (Robinson et al., 2019), and the vesicular Ca^{2+} sensor Synaptotagmin-1 (Lebowitz et al., 2023) suggests that somatodendritic secretion occurs at preassembled exocytic sites at the plasma membrane of somata and/or dendrites and imply that the vesicles are docked before fusion. While it has remained difficult to morphologically define somatodendritic release sites, the data presented here and in previous studies provide a foundation to identify them via the presence of RIM, Munc13, and Synaptotagmin-1. This is a critical goal, because axon collaterals might contribute to midbrain dopamine release, though their abundance in the substantia nigra is likely low (Wassef et al., 1981; Groves and Linder, 1983).

Because RIM, Munc13, and Synaptotagmin-1 typically mediate the fusion of synaptic vesicles (Südhof, 2013; Kaeser and Regehr, 2014; Biederer et al., 2017), their roles in midbrain dopamine transmission also suggest that somatodendritic release is mediated by small, clear vesicles. This model differs from early studies that suggested fusion of tubulovesicular structures for somatodendritic release and is more consistent with reports on the presence of small, pleiomorphic vesicles (Hajdu et al., 1973; Wilson et al., 1977; Groves and Linder, 1983; Nirenberg et al., 1996). Overall, this and previous work identifies molecules important for midbrain dopamine release.

Organization of somatodendritic transmission beyond vesicle priming

Many features of somatodendritic transmission remain understudied. Previous work on evoked release indicates tight coupling of Ca^{2+} entry and secretion with a high initial release probability, and complete Cd^{2+} sensitivity established the dependence on voltage-gated Ca^{2+} channels (Beckstead et al., 2004, 2007; Ford et al., 2010; Chen et al., 2011). The exact Ca^{2+} sources and mechanisms for their targeting to release sites, however, remain uncertain, and blocking L-, N- or P/Q-type channels inhibited somatodendritic only release partially (Beckstead et al., 2004; Chen et al., 2006; Liu et al., 2021). Deletion of the fast Ca^{2+} sensor Synaptotagmin-1 strongly impairs single pulse D2-IPSCs, but stimulus trains evoke significant somatodendritic release in Synaptotagmin-1 knock-out dopamine neurons (Lebowitz et al., 2023). Knockdown, knock-out, and antibody application experiments have also implicated Synaptotagmin-4 and Synaptotagmin-7 (Mendez et al., 2011; Delignat-Lavaud et al., 2022; Hikima et al., 2022; Lebowitz et al., 2024). While vertebrate Synaptotagmin-4 is unlikely to mediate Ca^{2+} sensing (Dai et al., 2004), effects of Synaptotagmin-7 ablation or inhibition are generally mild. Overall, the full complement of Ca^{2+} sensors for somatodendritic dopamine release remains unknown.

A determinant for efficacy of signaling is the positioning of receptors relative to release sites. At synapses, tight coupling at tens of nanometers controls receptor activation (Biederer et al., 2017). For somatodendritic dopamine transmission, release-receptor organization remains unclear. Morphological studies have identified discrete D2 receptor puncta (Gantz et al., 2015; Robinson et al., 2017; Lebowitz et al., 2022), but it has not been possible to assess their proximity to release sites. Functional imaging has established that somatodendritic dopamine release generates hotspots and that uncaging a D2 receptor antagonist inhibits receptor activation within the first ~100 ms of stimulation, but not at later time points (Condon et al., 2021). In addition, D2-IPSC induction relies on a high dopamine concentration, 30–100 μM (Ford et al., 2009). These studies suggest that dopamine acts rapidly and in a contained space. It is noteworthy, though, that dopamine diffusion is fast, and receptors several micrometers away from release sites might be reached within 100 ms (Rice and Cragg, 2008; Yee et al., 2025). Future studies should assess release-receptor organization in the midbrain dopamine system to define dopamine's signaling mechanisms.

Norepinephrine contribution to somatodendritic dopamine signaling

Norepinephrine neurons innervate the midbrain (Liprando et al., 2004; Mejías-Aponte et al., 2009; Mejías-Aponte, 2016), and $\alpha 1$ adrenoreceptors inhibit dopamine neuron activity (Paladini and Williams, 2004). Norepinephrine may also act as an agonist of D2 and D2-like receptors (White and Wang, 1984; Sánchez-

Soto et al., 2016), and it activates D2 receptor-based dopamine sensors (López et al., 2026). Here, we find that norepinephrine innervation can contribute to D2-IPSCs. In control mice, the contribution is small (Fig. 5). In Munc13 cKO^{DA} mice, norepinephrine innervation accounts for half of the remaining D2-IPSC. In our experiments, whether released norepinephrine activates D2 receptors or whether norepinephrine neurons corelease dopamine before its conversion to norepinephrine (Weinshilboum et al., 1971; Stewart and Klinman, 1988; Özçete et al., 2024) remains unclear.

Spontaneous somatodendritic dopamine release

Dopamine is associated with movement initiation and vigor, though its mechanisms remain debated (Carlsson, 1960; Panigrahi et al., 2015; Howe and Dombeck, 2016; Berke, 2018; da Silva et al., 2018; Cai et al., 2024; Liu et al., 2025). Recent studies suggest that spontaneous dopamine release suffices to mediate movement. In mice with disrupted evoked dopamine release and in vivo dopamine dynamics (via RIM ablation and/or pharmacological manipulations), movement initiations persist (Cai et al., 2024). Spontaneous somatodendritic dopamine release is unaffected by RIM ablation (Robinson et al., 2019). Our findings bolster the model that evoked dopamine release is dispensable for movement. Munc13 cKO^{DA} mice have a large reduction in evoked dopamine release (Figs. 2, 3). While their body weight at weaning is reduced, they survive, move, and eat (Banerjee et al., 2022).

The cellular pathway for spontaneous somatodendritic dopamine release remains to be defined. Previous work indicates that it is vesicular and reveals independence of voltage-gated Na^{+} channels, voltage-gated Ca^{2+} channels, extracellular Ca^{2+} , intracellular Ca^{2+} stores, and Synaptotagmin-1 and RIM expression in dopamine neurons (Gantz et al., 2013; Robinson et al., 2019; Lebowitz et al., 2023). Here, we show that spontaneous somatodendritic dopamine transmission persists after Munc13 ablation (Fig. 4). The release pathway may not only be different from that of evoked release, but it might also differ from that of spontaneous release at synapses, where ablations of RIM, Munc13, or Synaptotagmin-1 alter event frequencies across synapses, cell types, and species (Varoqueaux et al., 2002; Yoshihara and Littleton, 2002; Xu et al., 2009; Deng et al., 2011; Zhou et al., 2015; Tan et al., 2022a,b). What could those pathways be? One possibility is that distinct SNARE proteins or their regulators may control spontaneous dopamine release. For example, Vt1a, Vamp7, and Doc2 have been implicated in spontaneous fusion at synapses (Groffen et al., 2010; Hua et al., 2011; Ramirez et al., 2012; Kaeser and Regehr, 2014), and they may have roles in controlling spontaneous fusion events in dopamine neurons. Furthermore, recent work has identified adaptor complexes for vesicles released during high-frequency firing of dopamine neurons (Jain et al., 2023). Similar mechanisms could help generate a pool of vesicles for spontaneous release. Alternatively, spontaneous dopamine release may be mediated by a different secretory pathway, by different cells, or by a small amount of Munc13 present in the tested mutants (Banerjee et al., 2022). Regarding an alternate pathway, the exocyst is a candidate that consists of conserved secretory machinery, though it typically participates in neuronal development but not transmitter exocytosis (Murthy et al., 2003; He and Guo, 2009).

Overall, our studies provide a molecular handle on the identity of the sites that underlie evoked release in the midbrain, as they indicate that Munc13 might be a marker similar to synaptic release sites (Reddy-Alla et al., 2017; Sakamoto et al., 2018). The alternate

pathways described above should be systematically investigated for a role in spontaneous somatodendritic dopamine release.

References

- Aghajanian GK, Bunney BS (1977) Dopamine "autoreceptors": pharmacological characterization by microiontophoretic single cell recording studies. *Naunyn Schmiedeberg Arch Pharmacol* 297:1–7.
- Agster KL, Mejias-Aponte CA, Clark BD, Waterhouse BD (2013) Evidence for a regional specificity in the density and distribution of noradrenergic varicosities in rat cortex. *J Comp Neurol* 521:2195–2207.
- Araneda R, Bustos G (1989) Modulation of dendritic release of dopamine by N-methyl-D-aspartate receptors in rat substantia nigra. *J Neurochem* 52:962–970.
- Aravamudan B, Fergestad T, Davis WS, Rodesch CK, Broadie K (1999) *Drosophila* UNC-13 is essential for synaptic transmission. *Nat Neurosci* 2:965–971.
- Augustin I, Rosenmund C, Südhof TC, Brose N (1999) Munc13-1 is essential for fusion competence of glutamatergic synaptic vesicles. *Nature* 400:457–461.
- Augustin I, Korte S, Rickmann M, Kretschmar HA, Südhof TC, Herms JW, Brose N (2001) The cerebellum-specific Munc13 isoform Munc13-3 regulates cerebellar synaptic transmission and motor learning in mice. *J Neurosci* 21:10–17.
- Backman CM, Malik N, Zhang Y, Shan L, Grinberg A, Hoffer BJ, Westphal H, Tomac AC (2006) Characterization of a mouse strain expressing Cre recombinase from the 3' untranslated region of the dopamine transporter locus. *Genesis* 44:383–390.
- Banerjee A, Lee J, Nemcova P, Liu C, Kaeser PS (2020) Synaptotagmin-1 is the Ca²⁺ sensor for fast striatal dopamine release. *eLife* 9:e58359.
- Banerjee A, et al. (2022) Molecular and functional architecture of striatal dopamine release sites. *Neuron* 110:248–265.e9.
- Beckstead MJ, Grandy DK, Wickman K, Williams JT (2004) Vesicular dopamine release elicits an inhibitory postsynaptic current in midbrain dopamine neurons. *Neuron* 42:939–946.
- Beckstead MJ, Ford CP, Phillips PEM, Williams JT (2007) Presynaptic regulation of dendrodendritic dopamine transmission. *Eur J Neurosci* 26:1479–1488.
- Bergquist F, Niaz HS, Nissbrandt H (2002) Evidence for different exocytosis pathways in dendritic and terminal dopamine release in vivo. *Brain Res* 950:245–253.
- Berke JD (2018) What does dopamine mean? *Nat Neurosci* 21:787–793.
- Betz A, Thakur P, Junge HJ, Ashery U, Rhee JS, Scheuss V, Rosenmund C, Rettig J, Brose N (2001) Functional interaction of the active zone proteins Munc13-1 and RIM1 in synaptic vesicle priming. *Neuron* 30:183–196.
- Biederer T, Kaeser PS, Blanpied TA (2017) Transcellular nanoalignment of synaptic function. *Neuron* 96:680–696.
- Björklund A, Lindvall O (1975) Dopamine in dendrites of substantia nigra neurons: suggestions for a role in dendritic terminals. *Brain Res* 83:531–537.
- Bücheler MM, Hadamek K, Hein L (2002) Two alpha(2)-adrenergic receptor subtypes, alpha(2A) and alpha(2C), inhibit transmitter release in the brain of gene-targeted mice. *Neuroscience* 109:819–826.
- Bulumulla C, Krasley AT, Cristofori-Armstrong B, Valinsky WC, Walpita D, Ackerman D, Clapham DE, Beyene AG (2022) Visualizing synaptic dopamine efflux with a 2D composite nanofilm. *eLife* 11:e78773.
- Cai X, et al. (2024) Dopamine dynamics are dispensable for movement but promote reward responses. *Nature* 635:406–414.
- Camacho M, Basu J, Trimbuch T, Chang S, Pulido-Lozano C, Chang S-S, Duluvova I, Abo-Rady M, Rizo J, Rosenmund C (2017) Heterodimerization of Munc13 C2A domain with RIM regulates synaptic vesicle docking and priming. *Nat Commun* 8:15293.
- Carlsson A (1960) [On the problem of the mechanism of action of some psychopharmacol]. *Psychiatr Neurol (Basel)* 140:220–222.
- Chen BT, Moran KA, Avshalomov MV, Rice ME (2006) Limited regulation of somatodendritic dopamine release by voltage-sensitive Ca²⁺ channels contrasted with strong regulation of axonal dopamine release. *J Neurochem* 96:645–655.
- Chen BT, Patel JC, Moran KA, Rice ME (2011) Differential calcium dependence of axonal versus somatodendritic dopamine release, with characteristics of both in the ventral tegmental area. *Front Syst Neurosci* 5:39.
- Chen Z, Cooper B, Kalla S, Varoqueaux F, Young SM (2013) The Munc13 proteins differentially regulate readily releasable pool dynamics and calcium-dependent recovery at a central synapse. *J Neurosci* 33:8336–8351.
- Chin M, Kaeser PS (2024) On the targeting of voltage-gated calcium channels to neurotransmitter release sites. *Curr Opin Neurobiol* 89:102931.
- Condon AF, Robinson BG, Asad N, Dore TM, Tian L, Williams JT (2021) The residence of synaptically released dopamine on D2 autoreceptors. *Cell Rep* 36:109465.
- Dai H, Shin O-H, Machius M, Tomchick DR, Südhof TC, Rizo J (2004) Structural basis for the evolutionary inactivation of Ca²⁺ binding to synaptotagmin 4. *Nat Struct Mol Biol* 11:844–849.
- da Silva JA, Tecuapetla F, Paixao V, Costa RM (2018) Dopamine neuron activity before action initiation gates and invigorates future movements. *Nature* 554:244–248.
- Delignat-Lavaud B, Ducrot C, Kouwenhoven W, Feller N, Trudeau L-É (2022) Implication of synaptotagmins 4 and 7 in activity-dependent somatodendritic dopamine release in the ventral midbrain. *Open Biol* 12:210339.
- Delignat-Lavaud B, et al. (2023) Synaptotagmin-1-dependent phasic axonal dopamine release is dispensable for basic motor behaviors in mice. *Nat Commun* 14:4120.
- Deng L, Kaeser PS, Xu W, Südhof TC (2011) RIM proteins activate vesicle priming by reversing autoinhibitory homodimerization of Munc13. *Neuron* 69:317–331.
- Dulubova I, Lou X, Lu J, Huryeva I, Alam A, Schneggenburger R, Südhof TC, Rizo J (2005) A Munc13/RIM/Rab3 tripartite complex: from priming to plasticity? *EMBO J* 24:2839–2850.
- Eggermann E, Bucurenciu I, Goswami SP, Jonas P (2011) Nanodomain coupling between Ca²⁺ channels and sensors of exocytosis at fast mammalian synapses. *Nat Rev Neurosci* 13:7–21.
- Elizarova S, Chouaib AA, Shaib A, Hill B, Mann F, Brose N, Kruss S, Daniel JA (2022) A fluorescent nanosensor paint detects dopamine release at axonal varicosities with high spatiotemporal resolution. *Proc Natl Acad Sci U S A* 119:e2202842119.
- Ford CP, Phillips PEM, Williams JT (2009) The time course of dopamine transmission in the ventral tegmental area. *J Neurosci* 29:13344–13352.
- Ford CP, Gantz SC, Phillips PEM, Williams JT (2010) Control of extracellular dopamine at dendrite and axon terminals. *J Neurosci* 30:6975–6983.
- Fortin GD, Desrosiers CC, Yamaguchi N, Trudeau LE (2006) Basal somatodendritic dopamine release requires snare proteins. *J Neurochem* 96:1740–1749.
- Gantz SC, Bunzow JR, Williams JT (2013) Spontaneous inhibitory synaptic currents mediated by a G protein-coupled receptor. *Neuron* 78:807–812.
- Gantz SC, Robinson BG, Buck DC, Bunzow JR, Neve RL, Williams JT, Neve KA (2015) Distinct regulation of dopamine D2S and D2L autoreceptor signaling by calcium. *eLife* 4:e09358.
- Geffen LB, Jessell TM, Cuello AC, Iversen LL (1976) Release of dopamine from dendrites in rat substantia nigra. *Nature* 260:258–260.
- González-Rodríguez P, et al. (2021) Disruption of mitochondrial complex I induces progressive parkinsonism. *Nature* 599:650–656.
- Groffen AJ, et al. (2010) Doc2b is a high-affinity Ca²⁺ sensor for spontaneous neurotransmitter release. *Science* 327:1614–1618.
- Groves PM, Linder JC (1983) Dendro-dendritic synapses in substantia nigra: descriptions based on analysis of serial sections. *Exp Brain Res* 49:209–217.
- Hajdu F, Hassler R, Bak IJ (1973) Electron microscopic study of the substantia nigra and the strio-nigral projection in the rat. *Z Zellforsch Mikrosk Anat* 146:207–221.
- He B, Guo W (2009) The exocyst complex in polarized exocytosis. *Curr Opin Cell Biol* 21:537–542.
- Hikima T, Witkovsky P, Khatri L, Chao MV, Rice ME (2022) Synaptotagmins 1 and 7 play complementary roles in somatodendritic dopamine release. *J Neurosci* 42:3919–3930.
- Howe MW, Dombeck DA (2016) Rapid signalling in distinct dopaminergic axons during locomotion and reward. *Nature* 535:505–510.
- Hu PS, Fredholm BB (1989) Alpha 2-adrenoceptor agonist-mediated inhibition of [3H]noradrenaline release from rat hippocampus is reduced by 4-aminopyridine, but that caused by an adenosine analogue or omega-conotoxin is not. *Acta Physiol Scand* 136:347–353.
- Hua Z, Leal-Ortiz S, Foss SM, Waites CL, Garner CC, Voglmaier SM, Edwards RH (2011) V-SNARE composition distinguishes synaptic vesicle pools. *Neuron* 71:474–487.
- Imig C, Min SW, Krinner S, Arancillo M, Rosenmund C, Südhof TC, Rhee JS, Brose N, Cooper BH (2014) The morphological and molecular nature of synaptic vesicle priming at presynaptic active zones. *Neuron* 84:416–431.

- Jain S, et al. (2023) Adaptor protein-3 produces synaptic vesicles that release phasic dopamine. *Proc Natl Acad Sci U S A* 120:e2309843120.
- Kaeser PS, Regehr WG (2014) Molecular mechanisms for synchronous, asynchronous, and spontaneous neurotransmitter release. *Annu Rev Physiol* 76:333–363.
- Kaeser PS, Regehr WG (2017) The readily releasable pool of synaptic vesicles. *Curr Opin Neurobiol* 43:63–70.
- Kennedy MJ, Ehlers MD (2011) Mechanisms and function of dendritic exocytosis. *Neuron* 69:856–875.
- Lai Y, et al. (2017) Molecular mechanisms of synaptic vesicle priming by Munc13 and Munc18. *Neuron* 95:591–607.e10.
- Lebowitz JJ, Trinkle M, Bunzow JR, Balcita-Pedicino JJ, Hetelekides S, Robinson B, De La Torre S, Aicher SA, Sesack SR, Williams JT (2022) Subcellular localization of D2 receptors in the murine substantia nigra. *Brain Struct Funct* 227:925–941.
- Lebowitz JJ, Banerjee A, Qiao C, Bunzow JR, Williams JT, Kaeser PS (2023) Synaptotagmin-1 is a Ca²⁺ sensor for somatodendritic dopamine release. *Cell Rep* 42:111915.
- Lebowitz JJ, Kissiwa SA, Engeln KA, Bowman AM, Williams JT, Jackman SL (2024) Synaptotagmin-7 counteracts short-term depression during phasic dopamine release. *eNeuro* 11:ENEURO.0501-23.2024.
- Liprandi LA, Miner LH, Blakely RD, Lewis DA, Sesack SR (2004) Ultrastructural interactions between terminals expressing the norepinephrine transporter and dopamine neurons in the rat and monkey ventral tegmental area. *Synapse* 52:233–244.
- Liu C, Kaeser PS (2019) Mechanisms and regulation of dopamine release. *Curr Opin Neurobiol* 57:46–53.
- Liu C, Kershberg L, Wang J, Schneeberger S, Kaeser PS (2018) Dopamine secretion is mediated by sparse active zone-like release sites. *Cell* 172:706–718.e15.
- Liu C, Goel P, Kaeser PS (2021) Spatial and temporal scales of dopamine transmission. *Nat Rev Neurosci* 22:345–358.
- Liu C, Cai X, Ritzau-Jost A, Kramer PF, Li Y, Khaliq ZM, Hallermann S, Kaeser PS (2022) An action potential initiation mechanism in distal axons for the control of dopamine release. *Science* 375:1378–1385.
- Liu H, Melani R, Maltese M, Taniguchi J, Sankaramanchi A, Zeng R, Martin JR, Tritsch NX (2025) Subsecond dopamine fluctuations do not specify the vigor of ongoing actions. *Nat Neurosci* 28:2432–2438.
- López RC, Noble N, Özçete ÖD, Silenzi L, Cai X, Handy GE, Andersen JW, Patriarchi T, Li Y, Kaeser PS (2026) Norepinephrine and dopamine sensor crosstalk depends on local innervation density. *Nat Neurosci* [In press].
- Ma C, Li W, Xu Y, Rizo J (2011) Munc13 mediates the transition from the closed syntaxin-Munc18 complex to the SNARE complex. *Nat Struct Mol Biol* 18:542–549.
- Mejías-Aponte CA (2016) Specificity and impact of adrenergic projections to the midbrain dopamine system. *Brain Res* 1641:258–273.
- Mejías-Aponte CA, Drouin C, Aston-Jones G (2009) Adrenergic and noradrenergic innervation of the midbrain ventral tegmental area and retrorubral field: prominent inputs from medullary homeostatic centers. *J Neurosci* 29:3613–3626.
- Mendez JA, Bourque M-J, Fasano C, Kortleven C, Trudeau L-E (2011) Somatodendritic dopamine release requires synaptotagmin 4 and 7 and the participation of voltage-gated calcium channels. *J Biol Chem* 286:23928–23937.
- Murthy M, Garza D, Scheller RH, Schwarz TL (2003) Mutations in the exocyst component Sec5 disrupt neuronal membrane traffic, but neurotransmitter release persists. *Neuron* 37:433–447.
- Neher E (2015) Merits and limitations of vesicle pool models in view of heterogeneous populations of synaptic vesicles. *Neuron* 87:1131–1142.
- Nirenberg MJ, Chan J, Liu Y, Edwards RH, Pickel VM (1996) Ultrastructural localization of the vesicular monoamine transporter-2 in midbrain dopaminergic neurons: potential sites for somatodendritic storage and release of dopamine. *J Neurosci* 16:4135–4145.
- Özçete ÖD, Banerjee A, Kaeser PS (2024) Mechanisms of neuromodulatory volume transmission. *Mol Psychiatry* 193:73–88.
- Paladini CA, Williams JT (2004) Noradrenergic inhibition of midbrain dopamine neurons. *J Neurosci* 24:4568–4575.
- Panigrahi B, Martin KA, Li Y, Graves AR, Vollmer A, Olson L, Mensh BD, Karpova AY, Dudman JT (2015) Dopamine is required for the neural representation and control of movement vigor. *Cell* 162:1418–1430.
- Pucak ML, Grace AA (1994) Regulation of substantia nigra dopamine neurons. *Crit Rev Neurobiol* 9:67–89.
- Ramirez DMO, Khvotchev M, Trauterman B, Kavalali ET (2012) Vti1a identifies a vesicle pool that preferentially recycles at rest and maintains spontaneous neurotransmission. *Neuron* 73:121–134.
- Reddy-Alla S, et al. (2017) Stable positioning of Unc13 restricts synaptic vesicle fusion to defined release sites to promote synchronous neurotransmission. *Neuron* 95:1350–1364.e12.
- Rice ME, Cragg SJ (2008) Dopamine spillover after quantal release: rethinking dopamine transmission in the nigrostriatal pathway. *Brain Res Rev* 58:303–313.
- Rice ME, Patel JC (2015) Somatodendritic dopamine release: recent mechanistic insights. *Philos Trans R Soc Lond B Biol Sci* 370:20140185.
- Richmond JE, Davis WS, Jorgensen EM (1999) UNC-13 is required for synaptic vesicle fusion in *C. elegans*. *Nat Neurosci* 2:959–964.
- Robinson BG, Bunzow JR, Grimm JB, Lavis LD, Dudman JT, Brown J, Neve KA, Williams JT (2017) Desensitized D2 autoreceptors are resistant to trafficking. *Sci Rep* 7:4379.
- Robinson BG, Cai X, Wang J, Bunzow JR, Williams JT, Kaeser PS (2019) RIM is essential for stimulated but not spontaneous somatodendritic dopamine release in the midbrain. *eLife* 8:e47972.
- Rodriguez-Contreras D, et al. (2025) Mice carrying the human dopamine D2 receptor pathogenic mutation p.Met374Arg exhibit hyperactivity and aberrant D2 receptor function. *Mol Pharmacol* 107:100080.
- Rump LC, Ruff G, Wolk V, Schollmeyer P (1991) Alpha 2-adrenoceptor activation inhibits noradrenaline release in human and rabbit isolated renal arteries. *Eur J Pharmacol* 196:277–283.
- Sakamoto H, Ariyoshi T, Kimpura N, Sugao K, Taiko I, Takikawa K, Asanuma D, Namiki S, Hirose K (2018) Synaptic weight set by Munc13-1 supramolecular assemblies. *Nat Neurosci* 21:41–49.
- Salvatore MF (2024) Dopamine signaling in substantia nigra and its impact on locomotor function—not a new concept, but neglected reality. *Int J Mol Sci* 25:1131.
- Sánchez-Soto M, Bonifazi A, Cai NS, Ellenberger MP, Newman AH, Ferré S, Yano H (2016) Evidence for noncanonical neurotransmitter activation: norepinephrine as a dopamine D2-like receptor agonist. *Mol Pharmacol* 89:457–466.
- Schindelin J, et al. (2012) Fiji: an open-source platform for biological-image analysis. *Nat Methods* 9:676–682.
- Siksou L, Varoqueaux F, Pascual O, Triller A, Brose N, Marty S (2009) A common molecular basis for membrane docking and functional priming of synaptic vesicles. *Eur J Neurosci* 30:49–56.
- Stewart LC, Klinman JP (1988) Dopamine beta-hydroxylase of adrenal chromaffin granules: structure and function. *Annu Rev Biochem* 57:551–590.
- Südhof TC (2012) The presynaptic active zone. *Neuron* 75:11–25.
- Südhof TC (2013) Neurotransmitter release: the last millisecond in the life of a synaptic vesicle. *Neuron* 80:675–690.
- Sun F, et al. (2020) Next-generation GRAB sensors for monitoring dopaminergic activity in vivo. *Nat Methods* 17:1156–1166.
- Tan C, de Nola G, Qiao C, Imig C, Born RT, Brose N, Kaeser PS (2022a) Munc13 supports fusogenicity of non-docked vesicles at synapses with disrupted active zones. *eLife* 11:2022.04.01.486686.
- Tan C, Wang SSH, de Nola G, Kaeser PS (2022b) Rebuilding essential active zone functions within a synapse. *Neuron* 110:1498–1515.e8.
- Varoqueaux F, Sigler A, Rhee JS, Brose N, Enk C, Reim K, Rosenmund C (2002) Total arrest of spontaneous and evoked synaptic transmission but normal synaptogenesis in the absence of Munc13-mediated vesicle priming. *Proc Natl Acad Sci U S A* 99:9037–9042.
- Wassef M, Berod A, Sotelo C (1981) Dopaminergic dendrites in the pars reticulata of the rat substantia nigra and their striatal input. Combined immunocytochemical localization of tyrosine hydroxylase and anterograde degeneration. *Neuroscience* 6:2125–2139.
- Weinshilboum RM, Thoa NB, Johnson DG, Kopin IJ, Axelrod J (1971) Proportional release of norepinephrine and dopamine-beta-hydroxylase from sympathetic nerves. *Science* 174:1349–1351.
- White FJ, Wang RY (1984) Pharmacological characterization of dopamine autoreceptors in the rat ventral tegmental area: microiontophoretic studies. *J Pharmacol Exp Ther* 231:275–280.
- Wilson CJ, Groves PM, Fiková E (1977) Monoaminergic synapses, including dendro-dendritic synapses in the rat substantia nigra. *Exp Brain Res* 30:161–174.
- Xu J, Pang ZP, Shin O-H, Südhof TC (2009) Synaptotagmin-1 functions as a Ca²⁺ sensor for spontaneous release. *Nat Neurosci* 12:759–766.

Yee AG, Liao Y, Muntean BS, Ford CP (2025) Discrete spatiotemporal encoding of striatal dopamine transmission. *Science* 389:200–206.

Yoshihara M, Littleton JT (2002) Synaptotagmin I functions as a calcium sensor to synchronize neurotransmitter release. *Neuron* 36:897–908.

Zhou Q, et al. (2015) Architecture of the synaptotagmin–SNARE machinery for neuronal exocytosis. *Nature* 525:62–67.

Zhuo Y, et al. (2024) Improved green and red GRAB sensors for monitoring dopaminergic activity in vivo. *Nat Methods* 21:680–691.

Supplemental Material

Research Articles | Cellular/Molecular

The synaptic vesicle priming protein Munc13 mediates evoked somatodendritic dopamine release

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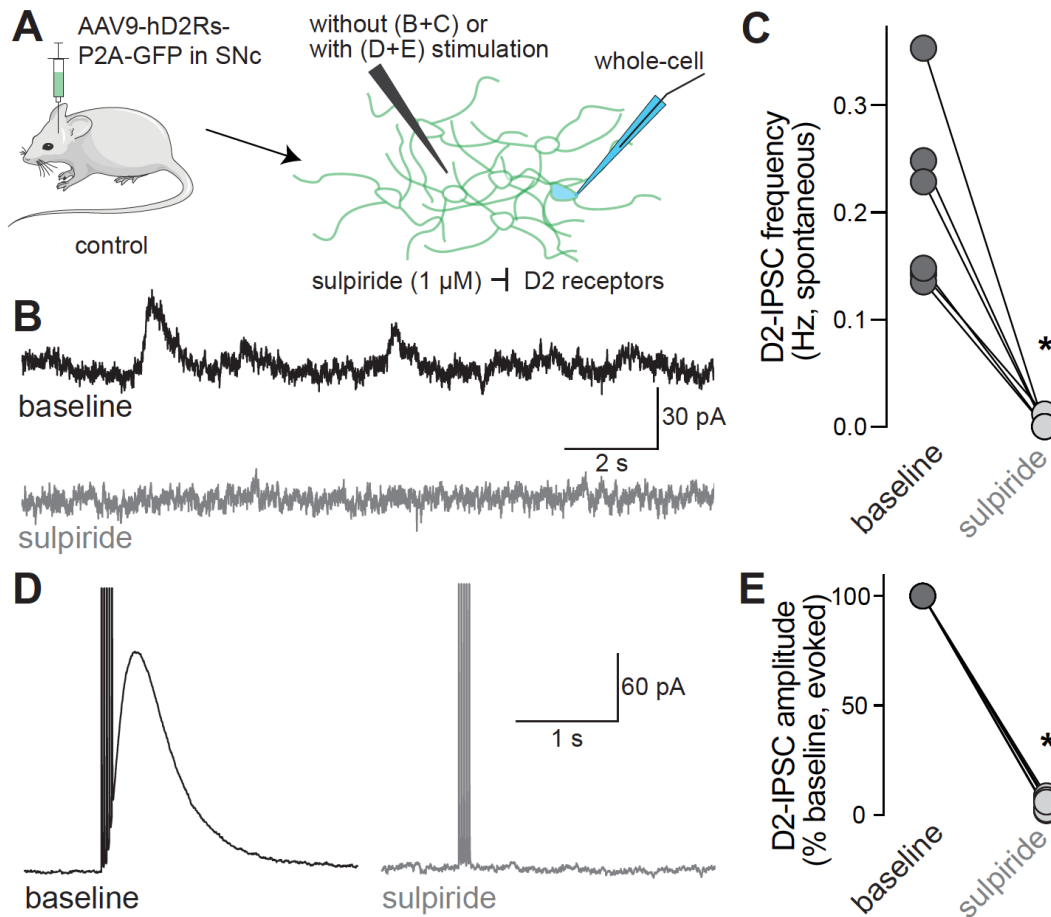


Figure S1. Blockade of D2 receptors ablates spontaneous and evoked D2-IPSCs

(A) Schematic of AAV-mediated D2 receptor expression followed by whole-cell recordings of SNc dopamine neurons before and after the addition of 1 μ M sulpiride.

(B, C) Example traces (B) and quantification of frequency (C) of spontaneous D2-IPSCs measured before and after blockade of D2 receptors with 1 μ M sulpiride; 6 cells from 6 slices of 3 mice.

(D, E) Example traces (D) and quantification of peak amplitudes (E) of D2-IPSCs induced by five stimuli at 40 Hz and 150 μ A stimulation intensity before and after blockade of D2 receptors with 1 μ M sulpiride; 6/6/3.

Individual cells are shown, * $p < 0.05$; assessed by Wilcoxon matched-pairs signed rank tests (C; $W = -21$, $p = 0.0312$, E; $W = -21$, $p = 0.0312$).

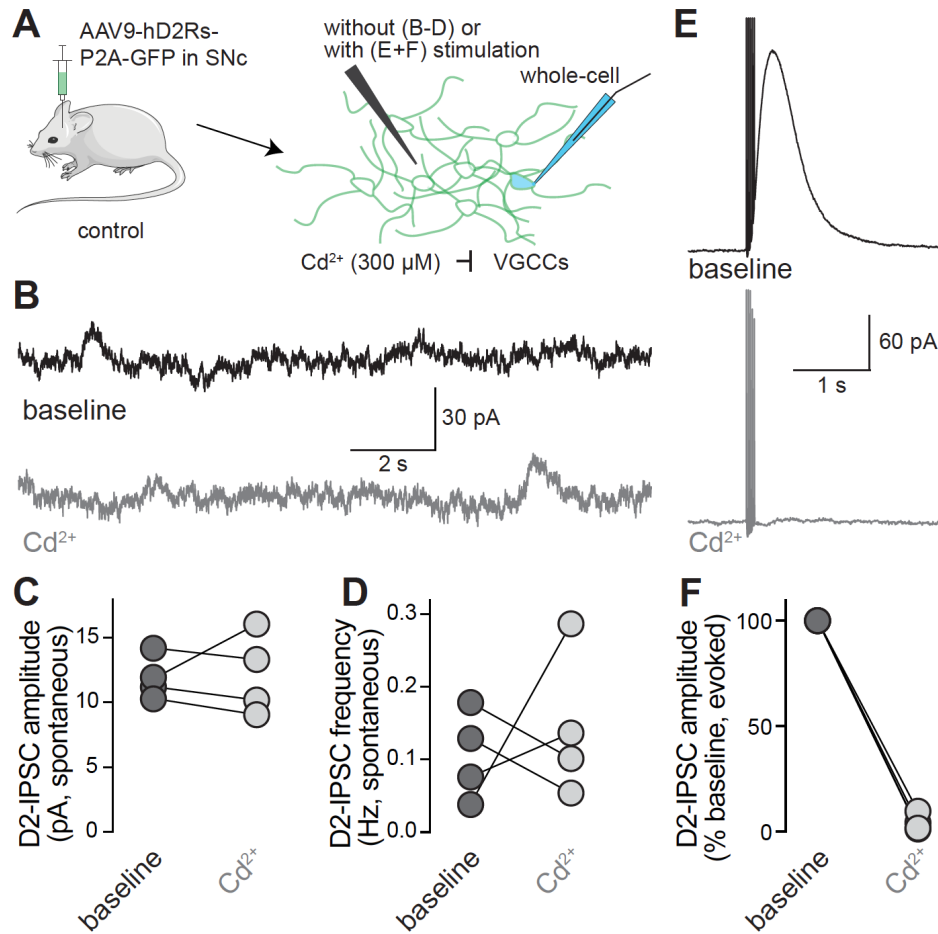


Figure S2. D2-IPSC measurements during blockade of voltage-gated Ca^{2+} channels

(A) Schematic of AAV-mediated D2 receptor expression followed by whole-cell recordings of SNc dopamine neurons before and after the addition of 300 μM Cd^{2+} .

(B-D) Example traces (B) and quantification of amplitude (C) and frequency (D) of spontaneous D2-IPSCs measured before and after blockade of voltage-gated Ca^{2+} channels with 300 μM Cd^{2+} ; 4 cells from 4 slices of 2 mice.

(E, F) Example traces (E) and quantification of peak amplitudes (F) of D2-IPSCs induced by five stimuli at 40 Hz and 150 μA stimulation intensity before and after blockade of voltage-gated Ca^{2+} channels with 300 μM Cd^{2+} ; 4/4/2.

Individual cells are shown, no statistics were performed due to the limited number of observations.