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The impact of a parkinsonian lesion on dynamic striatal dopamine transmission depends on nicotinic receptor activation

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ABSTRACT

Dopamine function is disturbed in Parkinson's disease (PD), but whether and how release of dopamine from surviving neurons is altered has long been debated. Nicotinic acetylcholine receptors (nAChRs) on dopamine axons powerfully govern dopamine release and could be critical contributing factors. We revisited whether fundamental properties of dopamine transmission are changed in a parkinsonian brain and tested the potentially profound masking effects of nAChRs. Using real-time detection of dopamine in mouse striatum after a partial 6-hydroxydopamine lesion and under nAChR inhibition, we reveal that dopamine signals show diminished sensitivity to presynaptic activity. This effect manifested as diminished contrast between DA release evoked by the lowest versus highest frequencies. This reduced activity-dependence was underpinned by loss of short-term facilitation of dopamine release, consistent with an increase in release probability (P_r). With nAChRs active, the reduced activity-dependence of dopamine release after a parkinsonian lesion was masked. Consequently, moment-by-moment variation in activity of nAChRs may lead to dynamic co-variation in dopamine signal impairments in PD.

Introduction

In PD, degeneration of midbrain dopamine (DA) neurons causes progressive loss of striatal DA. Motor deficits occur when neurodegeneration exceeds ~60% suggesting that compensatory mechanisms maintain DA function (Zigmond et al., 1990). Whether changes in release or reuptake mediate this compensation has been debated. Early studies reported enhanced DA synthesis (tyrosine hydroxylase, TH activity) and turnover (altered brain DA metabolites) (Agid et al., 1973; Altar et al., 1987; Hornykiewicz and Kish, 1987; Stachowiak et al., 1987), hypothesising that increased release from the remaining release sites compensates for reduced axonal density (Garris et al., 1997; Zigmond et al., 1990). By contrast, voltammetry studies which can distinctly resolve release and reuptake find that release is unchanged in the parkinsonian rodent brain but that reduced uptake compensates for the diminished number of DA release sites through a process of 'passive stabilization' (Bergstrom and Garris, 2003; Bezard et al., 2000; Garris et al., 1997). Additionally, whilst direct evidence of increased release is lacking in voltammetry studies, one study has found that sustainability of DA release is enhanced after lesion (Bergstrom et al., 2011).

We have recently identified that striatal DA release is under potent control by striatal acetylcholine, which is primarily released from cholinergic interneurons (ChIs) and acts at nAChRs on DA axons. Compared to DA release when nAChRs are active, DA release when nAChRs are inactive shows a reduced probability of release and a stronger sensitivity to the firing frequency of DA neurons (Cragg, 2006; Rice and Cragg, 2004; Zhang and Sulzer, 2004). By contrast, upon activation of nAChRs, DA P_r is apparently increased, and sensitivity of DA release to DA neuron firing frequency is limited (Rice and Cragg, 2004; Cragg, 2006). Furthermore, DA release can even be driven directly by activation of ChIs, without the need for DA neuron activity (Threlfell et al., 2012). Therefore, the dynamic availability of extracellular DA is predicted to be highly variable depending on the activity of both DA neurons and ChIs (Cragg, 2006). In PD, altered DA release might be determined by adaptations in either of these two neuron types. Here, we identify novel adaptations to DA release after a parkinsonian lesion that are distinct from the potent influence of ChIs and which can be masked by nAChR activation.

Abbreviations: 6-OHDA, 6-hydroxydopamine; $[Ca^{2+}]_o$, extracellular calcium concentration; $[DA]_o$, extracellular dopamine concentration; ACh, acetylcholine; ChI, cholinergic interneuron; CFM, carbon-fibre microelectrode; DA, dopamine; DAT, dopamine transporter; DHβE, dihydro-*beta*-erythroidine; FCV, fast-scan cyclic voltammetry; nAChR, nicotinic acetylcholine receptor; P1 release, DA release at the first pulse; P2 release, DA release at the second pulse; PPR, paired-pulse ratio; P_r, release probability; PD, Parkinson's disease; IPI, interpulse interval; SNc, Substantia nigra pars compacta.

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Materials and methods

Animals and surgery

Male C57BL6J mice (8–18 weeks, Charles River, UK) were kept on a 12 h light:dark cycle (lights on at 7 am) and had free access to lab chow, water and nesting materials. All animal procedures were carried out in accordance with the Animals (Scientific Procedures) Act (1986). Mice were anaesthetised with isoflurane (4 then 1.6–2.5%) and local anaesthesia was administered at the incision site (bupivacaine 0.1 mg/kg s.c.). Unilateral craniotomy was performed and 6-hydroxydopamine solution was injected into the SNc using a graduated micropipette (1 μl of a 0.9% saline solution containing 4 μg/μl 6-OHDA [Sigma-Aldrich] and 0.02% acetic acid over 5–20 min; AP −3.5; ML ±1.0 from Bregma, DV −4.1 mm from brain surface). The pipette was withdrawn after a further 10 min to minimise backflow up the injection tract. Palatable food (forage mix, jelly) and heat were provided routinely during the first 48 h, and as necessary during the remainder of the time after surgery. No gross behavioural changes were observed after 6-OHDA lesion. All further reference of lesion refers to this 6-OHDA-induced lesion.

Slice preparation and fast scan cyclic voltammetry (FCV)

At 21 ± 5 days post-surgery, mice were killed by cervical dislocation and the brain dissected and sliced (300 μm) in ice-cold HEPES ringer (in mM: 120 NaCl, 5 KCl, 20 $NaHCO_3$, 6.7 HEPES acid, 3.3 HEPES salt, 2 $CaCl_2$, 2 $MgSO_4$, 1.2 KH_2PO_4 and 10 glucose saturated with 95% O_2/5% CO_2) using a Leica VT1000S vibratome. After ≥1 h at room temperature, slices were transferred to a recording chamber and superfused with bicarbonate-buffered artificial CSF (aCSF) at ~1.5 ml/min and 30–32 °C (in mM: 124 NaCl, 3.7 KCl, 26 $NaHCO_3$, 2.4 $CaCl_2$, 1.3 $MgSO_4$, 1.3 KH_2PO_4, and 10 glucose saturated with 95% O_2/5% CO_2).

All DA recordings were made using a Millar Voltammeter (Julian Millar, Barts and the London School of Medicine and Dentistry, UK) as described previously (e.g., Threlfell et al., 2012). Voltammograms were obtained by scanning with a triangular waveform (−0.7 to +1.3 V to −0.7 V versus Ag/AgCl switching to 0 V between scans; 800 V/s; 8 Hz). Evoked extracellular DA concentration ($[DA]_o$) was measured using FCV with carbon fibre microelectrodes (CFMs) constructed in-house (fibre tip diameter, ~7 μm; exposed fibre length, 50–100 μm) and positioned ~100 μm into the tissue. Electrode calibrations were performed after recordings for each electrode using 2 μM DA in Ca^{2+}-appropriate aCSF.

Experiments were designed so that lesioned and non-lesioned experiments were carried out in the same animal (except in $[Ca^{2+}]$ response experiments). Each non-lesioned recording was carried out in the equivalent anatomical location as a paired lesioned recording from that animal.

Electrical stimulation

A concentric bipolar stimulating electrode (FHC, Bowdoinham, ME) was used to electrically evoke DA release in the striatum. The stimulating electrode was positioned on the tissue surface ~50–100 μm from the CFM. Monophasic stimulus pulses (0.2 ms duration; 0.65 mA) were applied out-of-phase with FCV scans. Stimuli were repeated at intervals of 2.5 min, as single pulses and 5-pulse trains at 5, 10, 25 and 100 Hz. Stimulation at 5–25 Hz was chosen to encompass the physiological firing frequencies commonly reported in DA neurons. Stimulation at 100 Hz was chosen as a 'release-limited frequency' specifically for probing the short-term plasticity of DA release. This method has previously been published for DA (Cragg, 2003; Rice and Cragg, 2004) and the approach is a well-established method for interrogating short-term plasticity mechanisms at glutamate and GABA synapses (e.g., Regehr, 2012; Thomson, 2000, 2003).

Paired pulse experiments

In a subset of experiments, paired-pulse release was explored to assess release probability (P_r). Because release summates during two-pulse trains, release at the second pulse (P2 release) was calculated by subtracting single pulse release (P1 release) from paired-pulse release. P2 release was then divided by P1 release to obtain the paired-pulse ratio (PPR). Previous studies of glutamate neurons have demonstrated that as initial P_r increases (i.e., P1 release), P2 release and PPR decrease. Conversely, when initial P_r is low, P2 release increases and can show facilitation such that P2 release > P1 release and PPR > 1. Note that PPR is here expressed as a percentage to maintain consistency throughout the figures.

Drug application

Experimental drugs were made up in stock solutions of 1000–5000× final concentration in water and stored in aliquots at −20 °C until use. Just prior to use, aliquots were diluted into O_2/CO_2-saturated aCSF and bath-applied. In experiments where extracellular $[Ca^{2+}]$ was varied, measurements were taken in standard aCSF (2.4 mM Ca^{2+}), before switching to the lowest $[Ca^{2+}]$ solution and working up through the concentrations.

High performance liquid chromatography

Tissue punches (1 mm diameter) were taken from the striatum of lesioned and non-lesioned hemispheres of acute slices prepared from 6-OHDA treated mice. Punches were placed into ice-cold perchloric acid (0.1 M) and stored at −80 °C until analysis. Tissue was homogenised using a sonicating probe (pulsed for ~10 s), centrifuged (25000 ×*g*; 15 min) and supernatant removed for analysis. Dopamine was separated on a ChromSep C_{18} reverse-phase column (5 μm; 4.6 × 250 mm; Varian) and detected (Decade SDC; Antec Leyden) using a glassy carbon working electrode (VT03 Antec Leyden; +0.7 V vs Ag/AgCl reference). Mobile phase consisted of 13% methanol, 0.12 M NaH_2PO_4, 0.8 mM EDTA, 2.0 mM octane sulphonic acid, pH 3.2. Dopamine concentrations were interpolated from known concentrations of dopamine run alongside samples and normalised to protein content.

Immunohistochemistry

After FCV, acute slices were fixed in 4% paraformaldehyde for several days before re-sectioning (40 μm) and processing for immunohistochemistry. Sections were incubated in 0.5% triton in phosphate buffered saline (PBS) for 30 min at room temperature, washed (3 × 10 min PBS), blocked for 30 min (PBS with 0.5% triton, 10% normal goat serum [NGS], 10% foetal calf serum [FCS]) and incubated overnight in primary antibody (1:1000; rabbit anti-tyrosine hydroxylase, T8700, Sigma; in PBS with 0.5% triton, 1% NGS, 1% FCS). The following day, sections were washed (3 × 10 min PBS), and incubated in secondary antibody (1:500 goat anti-rabbit, PK6101, Vector Labs; in PBS with 0.5% Triton, 1% NGS, 1% FCS) and visualised using the ABC system (PK6101, Vector Labs) and VIP peroxidase substrate (SK4600; 3–4 min incubation). Sections were then mounted on glass slides, dehydrated and delipidated in ascending ethanol concentrations (1–2 min each) followed by xylene (30 min) and glass coverslips were mounted using DePex.

Data analysis and statistics

FCV data were acquired and analysed using Strathclyde Whole Cell Program (University of Strathclyde, Glasgow, Scotland, UK) and Microsoft Excel. DA oxidation currents were measured from background-subtracted voltammograms, converted to concentration using the calibration factor for that CFM in each solution, and plotted