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## **Nitric oxide donors enhance the frequency-dependence of dopamine release in nucleus accumbens**

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**Abstract**

Dopamine (DA) neurotransmission in the nucleus accumbens (NAc) is critically involved in normal as well as maladaptive motivated behaviours including drug addiction. Whether the striatal neuromodulator nitric oxide (NO) influences DA release in NAc is unknown. We investigated whether exogenous NO modulates DA transmission in NAc core and how this interaction varies depending on frequency of presynaptic activation. We detected DA with cyclic voltammetry at carbon-fiber microelectrodes in mouse NAc in slices following stimuli spanning a full range of DA neuron firing frequencies (1-100 Hz).

NO donors SIN-1 (3-morpholiniosydnonimine hydrochloride) or PAPA/NONOate (z-1-[N-(3-ammoniopropyl)-N-(n-propyl)amino]diazene-1-ium-1,2-diolate) enhanced DA release with increasing stimulus frequency. This NO-mediated enhancement of frequency sensitivity of DA release was not prevented by inhibition of soluble guanylyl cyclase (sGC), dopamine transporters, or large conductance  $\text{Ca}^{2+}$ -activated  $\text{K}^{+}$  (BK) channels, and did not require glutamatergic or GABAergic input. However, experiments to identify whether frequency-dependent NO effects were mediated via changes in powerful acetylcholine-DA interactions revealed multiple components to NO modulation of DA release. In the presence of a nicotinic receptor antagonist (dihydro- $\beta$ -erythroidine, DH $\beta$ E), NO donors increased DA release in a frequency-independent manner.

These data suggest that NO in the NAc can modulate DA release through multiple GC-independent neuronal mechanisms whose net outcome varies depending on activity in DA neurons and accumbal cholinergic interneurons. In the presence of accumbal acetylcholine, NO promotes the sensitivity of DA release to presynaptic activation, but

with reduced acetylcholine input, NO will promote DA release in an activity-independent manner through a direct action on dopaminergic terminals.

## Introduction

The nucleus accumbens (NAc), the major part of the limbic ventral striatum, plays a key role in normal goal-directed or motivated behaviours as well as in maladaptive states including drug addiction and schizophrenia. Here, inputs from major limbic-associated brain regions like the medial prefrontal cortex, basolateral amygdala and ventral subiculum of the hippocampus converge (Finch, 1996; French and Totterdell, 2002, 2003; Groenewegen *et al*, 1987, 1999; Mulder *et al*, 1998; O'Donnell *et al*, 1999; Sesack and Grace, 2010; Wright and Groenewegen, 1995), are integrated with thalamic inputs (Berendse and Groenewegen, 1990; Smith *et al*, 2004) and interface with motor loops of the basal ganglia (Groenewegen and Trimble, 2007; Groenewegen *et al*, 1996; Mogenson *et al*, 1980; Sesack and Grace, 2010; Zahm, 2000). These interactions are powerfully modulated by dopaminergic innervation from the ventral tegmental area (Ikemoto, 2007; Voorn *et al*, 1986). Dopaminergic neurons signal unpredicted rewards or other salient contextual stimuli and their conditioned cues by a shift in firing rates from tonic low frequencies to brief bursts at high frequency (Matsumoto and Hikosaka, 2009; Schultz, 1986, 2002), and corresponding accumbal DA release influences accumbal output and long term plasticity (Morris *et al*, 2010; Reynolds and Wickens, 2002; Schultz, 2010).

How the release of DA reflects dynamic changes in activity in dopaminergic neurons is governed by the local regulation of release probability within the NAc (Cragg, 2003, 2006). For example, DA release is powerfully modulated by acetylcholine (ACh) arising from intrinsic cholinergic interneurons (ChIs) (Cragg, 2006; Exley *et al*, 2008; Rice and Cragg, 2004; Threlfell *et al*, 2010). Nitric oxide (NO) is another potent neuromodulator that is thought to be produced locally by NOS-containing accumbal interneurons (French

*et al*, 2005; Hidaka and Totterdell, 2001; Kraus and Prast, 2001), but how NO influences the dynamic signalling of activity by DA in NAc is currently unknown.

Interactions between NO and DA have been extensively studied in the dorsal striatum where NO modulates the excitability of striatal GABAergic projection neurons (West and Grace, 2004), corticostriatal synaptic plasticity (Calabresi *et al*, 1999a, b) and the release of various neurotransmitters including glutamate, GABA, ACh, serotonin and DA (Prast *et al*, 1995, 1998; Prast and Philippu, 2001; Trabace and Kendrick, 2000; West and Galloway, 1996, 1997a, b, 1998). However, NO in dorsal striatum is reported either to facilitate (Black *et al*, 1994; Buyukuysal, 1997; Irvani *et al*, 1998; Liang and Kaufman, 1998; Lonart *et al*, 1993; Stewart *et al*, 1996; Trabace and Kendrick, 2000; West and Galloway, 1996, 1997a, b, 1998; Zhu and Luo, 1992) or inhibit DA release (Guevara-Guzman *et al*, 1994; Segovia and Mora, 1998; Silva *et al*, 1995, 2003). The effector mechanisms have been suggested to include inhibition of DA transporters (for review see Kiss and Vizi, 2001) as well as indirect mechanisms that involve NO-activation of soluble guanylyl cyclase (sGC) in striatal projection neurons, which through their projections to the substantia nigra subsequently modify the activity of DA neurons (West and Grace, 2000) and NO-mediated increases in local glutamate levels (Bogdanov and Wurtman, 1997; Guevara-Guzman *et al*, 1994; Trabace and Kendrick, 2000; West and Galloway, 1997a, b) which might subsequently modify DA release (for review see David *et al*, 2005).

We used fast-scan cyclic voltammetry at carbon-fiber microelectrodes in striatal slices to identify how exogenous NO modulates endogenous DA release in NAc core during a range of evoked activity that spans the frequencies seen for dopaminergic

neurons *in vivo*. We show that the outcome of NO on DA release in NAc varies depending on activity in DA axons as well as in other accumbal neurons.

## **Materials and Methods**

### **Brain slice preparation and voltammetry**

Coronal striatal slices (300  $\mu\text{m}$ ) containing the NAc were prepared from brains of 26-35 g CD-1 male mice (Harlan, UK). The sections were cut on a Vibratome (Leica) in ice-cold oxygenated HEPES buffered artificial cerebrospinal fluid (HEPES-aCSF) containing NaCl (120 mM),  $\text{NaHCO}_3$  (20 mM), D-glucose (10 mM), HEPES acid (6.7 mM), HEPES salt (3.3 mM), KCl (5 mM),  $\text{CaCl}_2$  (2.4 mM),  $\text{KH}_2\text{PO}_4$  (1.25 mM) and  $\text{MgSO}_4$  (2 mM) saturated with 95%  $\text{O}_2$  / 5%  $\text{CO}_2$ . After maintaining slices for at least an hour in HEPES-aCSF at room temperature, they were transferred to the recording chamber and allowed to equilibrate for another hour with the superfusion medium of the recording chamber, namely bicarbonate-buffered aCSF containing NaCl (125 mM),  $\text{NaHCO}_3$  (26 mM), D-glucose (10 mM), KCl (3.8 mM),  $\text{CaCl}_2$  (2.4 mM),  $\text{KH}_2\text{PO}_4$  (1.2 mM) and  $\text{MgSO}_4$  (1.3 mM) and saturated with 95 %  $\text{O}_2$  / 5 %  $\text{CO}_2$ . Recordings were done in aCSF at a flow rate of 1.3 ml/min and a bath temperature of 32-33  $^\circ\text{C}$ .

Extracellular DA concentration ( $[\text{DA}]_o$ ) was monitored and quantified using fast-scan cyclic voltammetry (FCV) as described previously (Cragg, 2003; Rice and Cragg, 2004; Threlfell *et al*, 2010). Briefly, recordings were made with 7-10  $\mu\text{m}$ -diameter carbon fibre microelectrodes of tip lengths  $\sim$  50-100  $\mu\text{m}$  that were fabricated in-house. The carbon-fibre electrode tip was inserted 100  $\mu\text{m}$  into tissue in the NAc core, ventral to the

anterior commissure and voltammetry was performed using a Millar Voltammeter (P.D. Systems, UK). The applied voltage was a triangular waveform, with a voltage range of -0.7 V to +1.3 V and back versus an Ag/AgCl reference electrode at a scan rate of 800 V/sec and a sampling frequency of 8 Hz.

All evoked currents were recorded in the faradaic mode, showing currents after an electronic subtraction of background currents. These background-subtracted currents were monitored and recorded on a computer for analysis using Strathclyde Whole Cell Program (University of Strathclyde). The evoked current profiles were attributed to DA by comparison of their potentials for peak oxidation and reduction currents with those of DA in calibration media (500-600 and -200 mV vs. Ag/AgCl respectively). Profiles of  $[DA]_o$  versus time were obtained by sampling the current at the DA oxidation peak.

### **Electrode calibrations**

Electrode sensitivity to DA (nA/ $\mu$ M) in the presence of each added drug compound was determined from standard curves for DA oxidation current versus applied DA concentration for a physiological range of DA concentrations (1-3  $\mu$ M) in aCSF in the presence of all experimental drugs, singly and in combination as used experimentally. Some of the applied drugs decreased absolute electrode sensitivity to DA e.g. electrode sensitivity to DA was decreased in the presence of SIN-1 (500  $\mu$ M) by ~ 63 %, and in the presence of PAPA/NONOate (300  $\mu$ M) by ~ 74 %, and in the presence of ODQ (100  $\mu$ M) by ~40%. We used appropriately modified calibration factors determined from each and every drug condition to calibrate the electrodes. Note, we also confirmed that these effects of the parent compound on DA sensitivity occurred when electrodes were situated in the tissue environment by performing additional calibrations in tissue (data not

illustrated; required higher applied DA concentrations and inclusion of a DA uptake inhibitor (cocaine) to allow applied DA in striatum to reach levels approaching low micromolar). Importantly, the relationship between  $[DA]_o$  and oxidation current remained linear in all drugs used for the range of  $[DA]_o$  seen *in situ* (data not illustrated).

### **Local electrical stimulation**

Local stimulations used to evoke DA release were applied by a surface, bipolar concentric electrode (25  $\mu\text{m}$  diameter Pt/Ir; FHC, USA). Under a binocular microscope, the stimulating electrode was positioned flush with tissue at a distance of  $\sim 100 \mu\text{m}$  from the recording electrode. Stimulus pulses of 200  $\mu\text{s}$  duration were generated out-of-phase with FCV scans to prevent interference with the voltammetric current and applied at perimaximal currents (0.5-0.7 mA). Release evoked with stimulation used here (either a single pulse or brief 4-5 pulse trains) is inhibited by tetrodotoxin and is calcium-dependent (Cragg, 2003) but not modulated by ionotropic glutamate or GABA receptor activation (Cragg, 2003; Exley *et al*, 2008; Threlfell *et al*, 2010). However, DA release is controlled by ACh acting at presynaptic nicotinic acetylcholine receptors on DA axons (Exley *et al*, 2008; Rice and Cragg, 2004; Zhang and Sulzer, 2004; Zhou *et al*, 2001). This local cholinergic input results from activity of cholinergic interneurons which have been shown to be tonically active in slices as they are *in vivo* (Aosaki *et al*, 1994; Bennett and Wilson, 1999). ACh evoked by local electrical stimulation does not seem to add to already tonic levels generated by high tonic activity of ACh interneurons. DA release evoked by remote pathway stimulations was shown to be regulated by striatal ACh similarly to release evoked by local stimulations (Exley *et al*, 2008; Rice and Cragg, 2004).

## Experimental design and analysis

Stimulus protocols were repeated at a minimum of 2-minute intervals, which ensured stable, consistent release. To test the effect of frequency on DA release, a range of stimulations consisting of either a single pulse (1p) or 4 pulses at a range of frequencies spanning 5-100 Hz were applied in randomised order in triplicate at each given recording site. These stimulus frequencies include the full range of physiological DA neuron firing rates reported in *vivo*, consisting of tonic firing rates (<10 Hz) and phasic bursts (firing rates approx 15-25 Hz or higher) that accompany salient events (Bayer and Glimcher, 2005; Hyland *et al*, 2002; Schultz, 1986; Morris et al 2004), and also higher frequencies as used previously that are particularly useful for probing for changes in release probability. We have established that the peak value of  $[DA]_o$  for 1 Hz is indistinguishable from 1p (data not illustrated) and for simplicity we have used 1p data to represent 1 Hz outcome. Single-pulse stimulations were distributed regularly in time across each experiment (one 1p stimulation after three consecutive pulse train stimulations), to provide a reference value of  $[DA]_o$  against which  $[DA]_o$  evoked by other stimuli could be compared.

All data are means  $\pm$  standard error of the mean (SEM) and the sample size,  $n$  is number of observations. The number of animals in each data set is  $\geq 3$ . Data are expressed as  $[DA]_o$  normalized to release by a single pulse in control conditions. Mean value of mean peak  $[DA]_o$  for a single pulse across experiments were  $0.67 \pm 0.06 \mu\text{M}$  (range 0.39-1.7  $\mu\text{M}$ ). Comparisons for differences in means were assessed by Two-Way ANOVAs and post-hoc Bonferroni multiple comparison t-tests using GraphPad Prism.

## Drug application

NO donors of two different classes were used: 3-morpholinopyridone hydrochloride (SIN-1), purchased from Tocris Bioscience (UK); and (z)-1-[N-(3-Ammoniopropyl)-N-(n-propyl)amino] diazen-1-ium-1,2-diolate (PAPA/NONOate), purchased from Alexis Biochemical (UK). SIN-1 was prepared fresh immediately prior to use in aCSF and protected from light. SIN-1 spontaneously generates NO in aqueous solution by decomposition (Feelisch and Noack, 1987) at rates expected to be in the low micromolar range/min for 500  $\mu$ M SIN-1 as inferred by Feelisch *et al*, 1989 and Hogg *et al*, 1992. PAPA/NONOate was dissolved in 0.01 M NaOH to a stock concentration of 60 mM and diluted in aCSF prior to use to the desired final concentration. A concentration of 300  $\mu$ M PAPA/NONOate as used in this study is expected to generate NO bath concentrations in the low micromolar range as inferred by Garthwaite *et al*, 2002. Resulting tissue NO concentrations are likely to be several orders of magnitude lower than bath concentration (e.g. Garthwaite et al, 2002) owing to significant consumption of NO by tissue (Hall and Garthwaite, 2006).

D-AP5, bicuculline, dihydro- $\beta$ -erythroidine (DH $\beta$ E), GYKI-52466 hydrochloride, Iberiotoxin (IbTx), (S)-MCPG, 1H-[1,2,4]Oxadiazolo[4,3-a]quinoxalin-1-one (ODQ) and saclofen were purchased from Tocris Bioscience (UK) or Ascent Scientific (UK). Trolox<sup>®</sup> was purchased from Merck (UK) and nomifensine was purchased from Sigma Aldrich (UK). The drugs were dissolved in water, aqueous acid (GYKI-52466 hydrochloride, nomifensine), aqueous alkali ((S)-MCPG, saclofen), aqueous DMSO (ODQ) or ethanol (Trolox<sup>®</sup>) and were either prepared fresh or stored as stock aliquots of

500-2500 x final concentrations at -20 °C until required. Stock aliquots were diluted with oxygenated aCSF to final concentrations immediately before use.

Each drug condition involved drug application for approximately 60 minutes to include wash-on and a complete set of stimulations (40-45 minutes). Drug effects could be observed already 3-7 minutes after drug application and were maximal after 15 minutes. Thereafter drug effects remained constant for the whole course of frequency testing.

## Results

### **NO donors increase evoked DA release in a frequency-dependent manner**

We explored how NO modulates DA release evoked by a range of different frequencies (1-100 Hz, 4 pulses) which are in the range of firing rates that DA neurons display *in vivo* but also include higher frequencies as used previously that are particularly useful for probing for changes in release probability. Dopaminergic neurons respond to salient stimuli by shifting from tonic (approx. 0.5-10 Hz) frequencies to short phasic bursts of high frequency firing ( approx. 15-25 Hz or higher, durations <200 ms) (Hyland *et al*, 2002; Schultz, 1986).

In control conditions, evoked  $[DA]_o$  varied slightly but significantly with stimulus frequency in mouse NAc core (**Fig. 1**) by up to  $172 \pm 6$  % of release by a single pulse, according to an inverted U relationship, as described previously (Exley *et al*, 2008). Application of the NO donor SIN-1 (500  $\mu$ M) significantly increased the dependence of evoked  $[DA]_o$  on stimulus frequency (**Fig. 1a-b**; Two-way ANOVA, frequency:  $F_{4,133}=72.55$ ,  $P<0.001$ ; treatment:  $F_{2,133}=34.49$   $P<0.001$ ; interaction:  $F_{8,133}=10.19$ ,

$P < 0.001$ ). Release by lower frequencies ( $\leq 10$  Hz) remained unchanged but release by higher frequencies ( $\geq 25$  Hz) was significantly increased compared to control. Maximum  $[DA]_o$  were evoked by 100 Hz (4p;  $\sim 300$  % of release by a single pulse). Drug effects were reversible upon washout (**Suppl. Fig. S1a**) and were concentration-dependent (data not illustrated)

The effects of an alternative NO donor, PAPA/NONOate was also explored. PAPA/NONOate (300  $\mu$ M), like SIN-1, significantly increased  $[DA]_o$  in an activity-dependent manner (**Fig. 1c-d**; Two-way ANOVA, frequency:  $F_{4,92}=29.59$ ,  $P < 0.001$ ; treatment:  $F_{1,92}=89.09$ ,  $P < 0.001$ ; interaction:  $F_{4,92}=18.25$ ,  $P < 0.001$ ) which was reversible upon washout (**Suppl. Fig. S1b**). Release evoked by lower frequencies ( $\leq 5$  Hz) remained unchanged but release by higher frequencies ( $\geq 10$  Hz) was significantly increased compared to control. Greatest  $[DA]_o$  were evoked by 100 Hz bursts (4p; 364 % of release by a single pulse). The similar effects observed with two different NO donors suggests that these effects were due to NO rather than any non-specific effects of each donor or their different breakdown products. Thus in subsequent experiments, NO action was explored using a single example donor only, SIN-1.

To confirm that the effect of SIN-1 were not due to the concurrent release of superoxide anions, the subsequent formation of peroxynitrite and consequent modification of striatal DA release (Trabace and Kendrick, 2000), we identified whether the effects of SIN-1 were prevented by the peroxynitrite scavenger Trolox<sup>®</sup> (Edwards and Rickard, 2005; Halliwell *et al*, 1999; Regoli and Winston, 1999). Trolox<sup>®</sup> alone (200  $\mu$ M) did not significantly modify evoked  $[DA]_o$  compared to control conditions (**Fig. 1e-f**). Furthermore, Trolox<sup>®</sup> (200  $\mu$ M) did not prevent the subsequent effects of SIN-1 on

increasing the activity-dependence of evoked  $[DA]_o$  (**Fig. 1e-f**) (Two-way ANOVA, frequency:  $F_{4,134}=50.72$ ,  $P<0.001$ ; treatment:  $F_{2,134}=141.9$   $P<0.001$ ; interaction:  $F_{8,134}=15.74$ ,  $P<0.001$ ). These data suggest that peroxynitrite did not contribute to the effect of SIN-1 on evoked  $[DA]_o$ .

### **NO donors regulate DA transmission via guanylyl cyclase-independent mechanism**

NO is reported to act through a variety of effector mechanisms. One major target of NO is sGC (Bellamy *et al*, 2002; Garthwaite and Boulton, 1995). NO activation of sGC generates cyclic guanosine monophosphate (cGMP) which has a variety of downstream targets e.g. ion channels, phosphodiesterases, and protein kinases (Garthwaite and Boulton, 1995). However, the involvement of sGC in reported facilitatory effects of NO on DA levels in the striatum remains controversial. This facilitatory effect of NO on DA levels has been shown to be both sGC-dependent (Guevara-Guzman *et al*, 1994; Trabace and Kendrick, 2000) and sGC-independent (Buyukuysal, 1997; Rocchitta *et al*, 2004; Stewart *et al*, 1996; West and Galloway, 1996). We explored whether the effect of NO donors on the frequency-dependent control of DA release identified here in NAc was sGC-dependent or -independent.

The sGC inhibitor ODQ (100  $\mu$ M) alone did not significantly change evoked  $[DA]_o$  (**Fig. 2a-c**), and furthermore, ODQ did not prevent the subsequent effects of SIN-1 (**Fig. 2a-c**). SIN-1 significantly increased evoked  $[DA]_o$  in a frequency-dependent manner (Two-way ANOVA, frequency:  $F_{4,130}=44.09$ ,  $P<0.001$ ; treatment:  $F_{2,130}=42.98$ ,  $P<0.001$ ; interaction:  $F_{8,130}=7.71$ ,  $P<0.001$ ), which was not different to the effect of SIN-1 alone (Two-way ANOVA, treatment:  $F_{1,84}=2.22$ ,  $P>0.05$ ; interaction:  $F_{4,84}=1.32$ ,  $P>0.05$ ).

These data suggest that NO-mediated effects on  $[DA]_o$  observed here were sGC-independent.

Another frequently reported action of NO is S-nitrosylation of proteins such as ion channels. A commonly used approach to explore whether this mechanism underlies actions of NO is to block S-nitrosylation with N-ethylmaleimide (NEM). However, in pilot studies, NEM (2 mM) induced stimulus-independent continuous release of DA that prevented subsequent evoked DA release (data not illustrated), presumably via disruption of the SNARE complex. Thus, NEM is unsuitable as a tool to explore the role of S-nitrosylation in these experiments.

#### **Effect of NO on evoked DA release is not mediated by modulation of DA reuptake**

Previous studies have reported that NO-mediated enhancement of extracellular DA levels occurs by inhibiting DA reuptake via blockade of the dopamine transporter (DAT) *in vitro* (Buyukuysal, 1997; Lonart and Johnson, 1994; Pogun *et al*, 1994) and *in vivo* (Kiss *et al*, 1999; Lin *et al*, 1995). Our data has sufficiently high temporal resolution to enable changes in re-uptake rates to be indicated by changes in the time course of disappearance of the evoked extracellular DA signal. We compared the falling phases of the DA transients evoked by 100 Hz pulse trains in control conditions versus those obtained during application of SIN-1. However, SIN-1 did not modify the decay of the DA signal (**Fig. 2d**; contrast with **Fig. 2e**, the change in decay of the DA signal seen after re-uptake blockade). Comparisons of the time required for peak evoked  $[DA]_o$  to decay by 50% ( $t_{50}$ ) following 100 Hz pulse trains in control versus during application of SIN-1 did not reveal significant differences (control:  $t_{50} = 0.61 \pm 0.03$  s; SIN-1:  $t_{50} = 0.62 \pm 0.03$  s, paired t-test,  $P > 0.05$ ,  $n=9$ ). Furthermore, to ensure that modulation of the function of the DAT

was not responsible for the SIN-1-induced changes in the activity-dependence of evoked  $[DA]_o$ , we explored the effect of SIN-1 in the presence of DAT inhibition. Application of the DAT inhibitor nomifensine alone (10  $\mu$ M) enhanced peak evoked  $[DA]_o$  and significantly prolonged the extracellular lifetime of  $[DA]_o$  (**Fig. 2f-g**) as previously shown (Jones *et al*, 1995a, b, 1996; Schmitz *et al*, 2002). Subsequent application of SIN-1 significantly modified  $[DA]_o$  in an activity dependent manner, (**Fig. 2f-h**; Two-way ANOVA, frequency:  $F_{4,181}=16.50$ ,  $P<0.001$ ; treatment:  $F_{2,181}=140.5$   $P<0.001$ ; interaction:  $F_{8,181}=5.75$ ,  $P<0.001$ ) not different to the effect of SIN-1 alone (**see Fig. 2h**) suggesting that modulation of DA re-uptake is not responsible for these NO-mediated effects on  $[DA]_o$ .

### **Major component of effect of NO on DA release is BK channel-independent**

One candidate sGC-independent mechanism through which NO has been reported to influence cellular excitability is via modulation of large conductance  $Ca^{2+}$ -activated  $K^+$  (BK) channels. The BK current has been shown to be directly modulated by NO through S-nitrosylation of cysteine residues (and indirectly by activation of sGC depending on local NO concentrations) (Ahern *et al*, 2002). We explored whether BK channels might mediate NO effects on DA transmission. Blockade of BK channels with Iberitoxin (100 nM, IbTx) significantly increased the inverted U-dependence of the relationship between evoked  $[DA]_o$  and frequency. IbTx significantly increased  $[DA]_o$  evoked by 10 and 25 Hz compared to control (**Fig. 2i-j**) (Two-way ANOVA, post-hoc Bonferroni t-tests, 10 Hz:  $P<0.05$ ,  $n=9$ ; 25 Hz:  $P<0.01$ ,  $n=9$ ). The presence of IbTx however, did not prevent SIN-1 effects. SIN-1 (500  $\mu$ M) significantly increased evoked  $[DA]_o$  in an activity-dependent manner with greatest effect at highest frequencies (**Fig. 2i-k**) (Two-way ANOVA,

frequency:  $F_{4,119}=208.1$ ,  $P<0.001$ ; treatment:  $F_{2,119}=312.0$ ,  $P<0.001$ ; interaction:  $F_{8,119}=22.29$ ,  $P<0.001$ ). IbTx did however slightly change the effect of SIN-1. In the presence of IbTx, SIN-1 appeared to more generally increase evoked  $[DA]_o$  throughout the range of stimulation frequencies applied, compared to the effects of SIN-1 in the absence of IbTx (**Fig. 2k**; compare solid versus dotted line). This apparent shift in the actions of SIN-1 may be due to a small component of NO action being via, or being shunted due to, a change in BK channel function. Nonetheless, a major activity-dependent component of NO action was independent of BK channels.

### **NO modulation of DA release is multifactorial**

We explored whether the effects of NO on DA release reported here are mediated directly by an action on DA axons, or indirectly via regulation of an intermediary neurotransmitter(s). Accumbal glutamate and GABA do not normally play significant roles in regulating DA release probability during discrete subsecond stimuli (Cragg, 2003; Exley *et al*, 2008; Threlfell *et al*, 2010). However, since GABAergic and glutamatergic transmission can be modulated by NO *in vivo* (Bogdanov and Wurtman, 1997; Guevara-Guzman *et al*, 1994; Trabace and Kendrick, 2000; West and Galloway, 1997a) we investigated whether a potential change of the local glutamatergic or GABAergic tone by NO contributed to the SIN-1 effect on DA release. Application of a cocktail of antagonists for glutamate (NMDA: D-AP5, 50  $\mu$ M; AMPA: GYKI-52466, 10  $\mu$ M; mGluR: (S)-MCPG, 200  $\mu$ M) and GABA receptors (GABA<sub>A</sub>: bicuculline, 10  $\mu$ M; GABA<sub>B</sub>: saclofen, 50  $\mu$ M) did not significantly modulate evoked  $[DA]_o$  at any frequency applied compared to control (**Fig. 3a-c**) as shown previously (Cragg, 2003; Exley *et al*, 2008; Threlfell *et al*, 2010). Furthermore, glutamate and GABA receptor blockade did

not prevent the activity-dependent effect of subsequent SIN-1 application (**Fig. 3a-c**, Two-way ANOVA, frequency:  $F_{4,136}=35.22$ ,  $P<0.001$ ; treatment:  $F_{2,136}=68.30$ ,  $P<0.001$ ; interaction:  $F_{8,136}=9.72$ ,  $P<0.001$ ) suggesting that the effect of NO on  $[DA]_o$  is not via modulation of local glutamatergic or GABAergic tone.

Accumbal nicotinic acetylcholine receptors (nAChRs) on dopaminergic terminals have a powerful control over DA release in the NAc (Exley *et al*, 2008; Rice and Cragg, 2004). Normally, endogenous accumbal ACh released by tonically active cholinergic interneurons (ChIs) maintains ACh tone at accumbal nAChRs located on DA axons. This ACh tone ensures that initial DA release probability by a single stimulus pulse is high (Rice and Cragg, 2004; Zhou *et al*, 2001), that short-term depression of DA re-release at subsequent stimuli is prominent, and that sensitivity of DA release to frequency of activation is consequently limited (Cragg, 2003; Rice and Cragg, 2004). Changes in nAChR activation in turn modify the frequency sensitivity of DA transmission. We explored whether NO effects on DA transmission were mediated wholly or in part via an action involving ChIs/ACh. Application of the nicotinic receptor antagonist, DH $\beta$ E (1  $\mu$ M) to block cholinergic input significantly modified evoked DA release in an activity-dependent manner (**Fig. 3d-e**, Two-way ANOVA, frequency:  $F_{4,134}=463.9$ ,  $P<0.001$ ; treatment:  $F_{2,134}=602.4$ ,  $P<0.001$ ; interaction:  $F_{8,134}=127.1$ ,  $P<0.001$ ), by reducing release by lower frequencies and enhancing release by higher frequencies as shown previously (Exley *et al*, 2008; Rice and Cragg, 2004). In the presence of DH $\beta$ E, subsequent application of SIN-1 (500  $\mu$ M) only slightly modified further the activity-dependence of evoked  $[DA]_o$  (**Fig. 3f**, Two-way ANOVA, frequency:  $F_{4,134}=463.9$ ,  $P<0.001$ ; treatment:  $F_{2,134}=602.4$ ,  $P<0.001$ ; interaction:  $F_{8,134}=127.1$ ,  $P<0.001$ ), but moreover, resulted in a

significant increase (63-89%) in  $[DA]_o$  evoked by all frequencies compared to DH $\beta$ E alone (**Fig. 3f**, Two-way ANOVA, frequency:  $F_{4,134}=463.9$ ,  $P<0.001$ ; treatment:  $F_{2,134}=602.4$ ,  $P<0.001$ ; interaction:  $F_{8,134}=127.1$ ,  $P<0.001$ ).

These data suggest a combination of both direct and indirect effects of NO on DA release. In the absence of cholinergic input to DA terminals (i.e. in the presence of nAChR antagonist DH $\beta$ E), NO seems to act directly at the level of DA terminals to enhance release in a manner independent of stimulation frequency. However, in the presence of cholinergic input (i.e. in the absence of nAChR antagonist DH $\beta$ E), these direct effects of NO at the level of the dopamine terminal to increase release at all frequencies appear to be set against an indirect action via the cholinergic system, with a net outcome to increase the sensitivity of DA release to frequency (**Fig. 4**).

## Discussion

This study reveals that exogenous NO can powerfully and variably modulate DA release in the NAc core, and enhance the frequency-dependence of DA release. These NO-mediated effects are independent of sGC activation, and largely independent of two other candidate NO targets, DATs and BK channels. Furthermore, this frequency-dependent modulation of DA release by NO appears to be multifactorial, involving an indirect action via (or interaction with) ACh released from ChIs, as well as a direct action on DA axons. These data reveal a variable neuromodulatory influence of local NO on DA in the NAc that depends on activity in DA neurons as well as local accumbal circuits. Given the central role of DA neurotransmission in the NAc on motivated behaviours, interactions

between NO, ACh and DA may be important for regulating these behaviours in normal as well as pathological states.

**NO increases the contrast of DA signals released by phasic versus tonic frequencies of activity via GC-independent mechanism(s)**

Two separate NO donors, SIN-1 and PAPA/NONOate increased evoked  $[DA]_o$  and enhanced the frequency sensitivity of DA release. Thus NO donors enhanced the contrast between  $[DA]_o$  evoked by phasic versus tonic frequencies of activation. Donors were used at concentrations that are those typically used to produce effects of NO that are thought to be physiologically relevant (Bon and Garthwaite, 2001; East *et al*, 1991; Garthwaite *et al*, 2002; Luchowski and Urbanska, 2007; Yang and Cox, 2008). Here, the similar effects on DA release of these two different NO donors suggests that their outcomes are due to their common property to generate NO with physiological consequences rather than any non-specific effects or other breakdown products of each compound.

The concentrations of NO that are physiological are still debated (Hall and Garthwaite, 2009). Current estimates of NO concentrations found during normal tissue functioning are in the range of hundreds of picomolar to low nanomolar (i.e.  $10^{-10}$  -  $10^{-8}$  M) (Hall and Garthwaite, 2009; Sammut *et al*, 2006) and are a function of the rates of NO production, diffusion and consumption. Tissue concentrations of NO that result from the NO donors applied here, will depend on the NO concentrations generated in solution (100- to 1000-fold lower than the donor itself, e.g. Feelisch *et al*, 1989; Garthwaite *et al*, 2002; Hogg *et al*, 1999) and also on tissue penetration by NO. The high rate of consumption of NO by

tissue is thought to result in a substantial concentration difference between NO applied in solution and the limited NO reaching tissue (estimated to be 1,000-10,000-fold lower in tissue; Hall and Garthwaite, 2006, 2009). Thus, taking these different ‘dilution’ factors into account in the current study, the NO concentrations in tissue resulting from the donor concentrations applied in solution ( $10^{-4}$  M) may be between  $10^5$  and  $10^7$ -times lower, i.e. in the range of  $10^{-9}$  to  $10^{-11}$  M. These picomolar to low nanomolar estimates are very similar to estimates of NO concentrations found physiologically. Indeed, the effects here were consistent with physiological and not pathological effects because they were completely reversible within minutes of washout. Furthermore, previous studies using isolated rat optic nerve preparations found no signs of nervous tissue damage after 2-hour exposure to 300  $\mu$ M PAPA/NONOate or a 4-hour exposure to concentrations of SIN-1 (2 mM) an order of magnitude higher than those used here (Garthwaite *et al*, 2002). In addition, NO donor effects persisted in the presence of the peroxynitrite scavenger Trolox<sup>®</sup>, indicating that they did not depend on a pathological conversion to peroxynitrite.

The enzyme sGC is an effector mechanism for some actions of NO. In dorsal striatum, the sGC-dependence of facilitatory effects of NO on DA levels remains debated and has been shown to be sGC-dependent *in vivo* (Guevara-Guzman *et al*, 1994; Trabace and Kendrick, 2000) but also sGC independent both *in vitro* (Buyukuysal, 1997; Stewart *et al*, 1996) and *in vivo* (Rocchitta *et al*, 2004; West and Galloway, 1996). In our study in NAc, the effects of NO on dynamic DA signalling were not prevented by an inhibitor of sGC, indicating that they are sGC-independent. This is in line with previous *in vitro*

studies in dorsal striatum revealing sGC-independent effects of NO on DA release (Buyukuysal, 1997; Stewart *et al*, 1996).

Many target molecules have been identified in various systems to mediate the many physiological functions of NO. It has been suggested that NO might increase extracellular striatal DA levels via inhibition of DATs in some studies (Buyukuysal, 1997; Lonart *et al*, 1994; Pogun *et al*, 1994). However, NO donors modified DA transmission in the current study via a mechanism that did not involve any modulation of, or dependence on, DA uptake via DAT.

The conductance of BK channels (amongst other K<sup>+</sup>-channels) has also been reported to be modulated by NO, via both sGC-dependent and -independent mechanisms (Ahern *et al*, 1999; Klyachko *et al*, 2001). In posterior pituitary nerve terminals, NO has been reported to increase BK channel conductance therefore promoting spike afterhyperpolarization and Na<sup>+</sup>-channel recovery from inactivation, and thus reducing action potential failures during spike trains (Klyachko *et al*, 2001). Such a mechanism would be expected to give rise to a short-term, frequency-dependent enhancement of transmitter release, and was thus an attractive mechanism to explain NO effects on [DA]<sub>o</sub> described here. While BK channel expression/function has to date not been reported in DA neurons or axons, BK channels regulate neurotransmitter release from some other central neurons (e.g. Xu *et al*, 2005) and are also present in striatum, e.g. in dorsal striatal ChIs where they contribute to action potential repolarization (Bennett *et al*, 2000). Since striatal ACh potently regulates DA transmission in a manner that varies with presynaptic activity (Cragg, 2006; Rice and Cragg, 2004; Zhang and Sulzer, 2004), these channels on ChIs might in turn modulate DA release. However, while IbTx, a blocker of BK channels,

slightly modified evoked [DA]<sub>o</sub>, it did not prevent significant frequency-dependent effects of SIN-1 on DA release in NAc. Together these data suggest that the sGC-independent effector mechanisms involved in NO-mediated modulation of DA release do not require either the DAT or BK channels, and must involve an alternate target(s), of which there are numerous candidates e.g. Na<sup>+</sup> channels (Hammarstrom and Gage, 1999), the ryanodine receptor (Sun *et al*, 2001; Xu *et al*, 1998), L-type Ca<sup>2+</sup> channel (Campbell *et al*, 1996; Summers *et al*, 1999), cyclic nucleotide-gated channels (Broillet, 2000; Broillet and Firestein, 1996).

#### **NO modulates DA release via an indirect ACh-dependent mechanism and via direct actions on dopaminergic terminals**

To identify which accumbal neuron type(s) mediate NO regulation of DA transmission, we explored whether these effects required local accumbal glutamatergic, GABAergic or cholinergic inputs (e.g. see Bogdanov and Wurtman, 1997; Guevara-Guzman *et al*, 1994; Trabace and Kendrick, 2000; West and Galloway, 1997a). NO-mediated modulation of evoked DA release was independent of glutamate and GABA inputs, consistent with previous studies showing that neither glutamate nor GABA modulate DA release evoked by single pulses and brief 4-5 pulse trains (Cragg, 2003; Exley *et al*, 2008; Threlfell *et al*, 2010).

NO has been shown to powerfully modulate the activity of ChIs in dorsal striatum (Centonze *et al*, 2001) as well as release of ACh in dorsal and ventral striatum (Guevara-Guzman *et al*, 1994; Prast *et al*, 1995, 1998; Prast and Philippu, 2001; Trabace and Kendrick, 2000). Notably, acetylcholine at nAChRs on DA axons plays a major role in

governing the frequency-sensitivity of DA release (Exley *et al*, 2008; Rice and Cragg, 2004; Zhang and Sulzer, 2004). In the current study, we reveal that when ACh action at nAChRs in NAc is prevented, the frequency-dependent effects of NO on DA transmission are also prevented. Without nAChR activity, NO then increases DA release independently of frequency of activation. The simplest explanation for these data is that NO operates two partly opposing mechanisms that control DA release. One mechanism is indirect, involving regulation of ACh input to nAChRs akin to switching nAChRs off. Switching nAChRs off is expected to decrease DA release at low frequencies but enhance frequency sensitivity of DA release ultimately enabling enhanced DA release at high frequencies, and can result from either a decrease in ACh release, or an increase sufficiently large to cause nAChR desensitization as seen with nicotine (Rice and Cragg, 2004; Zhang and Sulzer, 2004). The second mechanism, revealed in the absence of nAChR activation, appears to be directly located to DA axons, and increases evoked  $[DA]_o$  uniformly regardless of stimulus frequency. When nAChR tone is intact, these two mechanisms acting in concert would be expected at low frequencies to oppose each other resulting in no net effect. By contrast, at high frequencies, they would be expected to boost DA signals. These outcomes are indeed those observed here, and are summarized in a cartoon representation of individual and net effects (**Fig. 4**).

Mechanistically the increase in DA release by both direct and indirect actions of NO could be explained by an increase in vesicle fusion events as suggested for hippocampal synaptosomes by Meffert *et al*, (1996, 1994). NO may increase the docking and fusion of dopaminergic vesicles at dopaminergic terminals leading to an increase in release independent of frequency. In addition, NO may also increase the docking and

fusion of cholinergic vesicles at cholinergic terminals leading to an increase in ACh release that, as described above, could desensitize nAChRs with the effect of increasing DA release at high frequencies.

An action of NO at multiple neuronal sites with variable outcome would certainly be in keeping with the body of literature to date indicating that NO that has diverse target molecules and proposed effector mechanisms and various reported outcomes on DA release (in dorsal striatum). However, these data do not preclude an alternative explanation that NO regulation of DA transmission is via action at a single site, through a single mechanism that is in some way shunted at low frequencies in the presence of nAChR tone.

### **Summary and Concluding Remarks**

Whether there are single or multiple effector mechanisms, these effects of NO donors suggest that the action of endogenous NO on accumbal DA signaling may be highly dynamic, depending on DA axon activity and also on the state of the local ventral striatal network, especially ChIs. Our data suggest that during ChI and nAChR activation, accumbal NO might enhance how DA release conveys high frequencies of activation. This postulated action for NO would be in opposition to those of ACh which limits the frequency-dependence of DA signaling (Cragg, 2006). However, in the absence of nAChR activation by ACh, when the frequency-dependence of DA signaling is great, NO might boost this outcome by promoting all DA signals uniformly. We speculate that NO might co-operate in outcome with the pauses in ChIs that signal motivationally

significant stimuli (Aosaki *et al*, 1994; Apicella, 2002; Morris *et al*, 2004; Ravel *et al*, 2001; Shimo and Hikosaka, 2001), when nAChR activation will be minimal.

The neurons that are the most likely source of endogenous accumbal NO are nNOS-expressing, GABAergic interneurons that also contain somatostatin and neuropeptide Y (Beal *et al*, 1986; French *et al*, 2005; Smith and Parent, 1986) and are highly interconnected to form a local nNOS-containing interneuron network (French *et al*, 2005). Knowledge of their functions within the accumbal network is limited but our data suggest that they might play a role in promoting transmission by DA of phasic versus tonic activity in DA neurons. The outcome of endogenous NO on accumbal DA function might vary dynamically with activity within the accumbal neuron network and might also impact significantly on the behavioral outcome of activation of limbic basal ganglia loops.

### **Disclosure/ Conflict of Interest**

The author(s) declare that, except for income received from my primary employer, no financial support or compensation has been received from any individual or corporate entity over the past three years for research or professional service and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest.

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**FIGURE LEGENDS****Figure 1. NO donors increase evoked DA release in a frequency-dependent manner.**

**(a, c, e)** Profiles of mean  $[DA]_o \pm SEM$  versus time following stimuli (*arrows*) of either a single pulse (p) or 4p (5-100 Hz) in **(a)** control conditions (*left*) and NO donor SIN-1 (500  $\mu M$ ) (*right*), **(c)** control conditions (*left*) and NO donor PAPA/NONOate (300  $\mu M$ ) (*right*) or **(e)** control conditions (*left*), Trolox<sup>®</sup> (200  $\mu M$ ) (*center*) and Trolox<sup>®</sup>+SIN-1 (*right*). Data are normalised to peak  $[DA]_o$  evoked by 1p in controls. **(b, d, f)** Mean peak  $[DA]_o \pm SEM$  versus frequency at 1p or 4p (5-100 Hz) in **(b)** control conditions (*filled circles*) and NO donor SIN-1 (*unfilled*) ( $n=9-15$ ), **(d)** control conditions (*filled circles*) and NO donor PAPA/NONOate (*unfilled*), ( $n=9-17$ ) or **(f)** control conditions (*filled circles*), Trolox<sup>®</sup> (*unfilled*) and Trolox<sup>®</sup>+SIN-1 (*gray fill*) ( $n=9-14$ ). Data are normalized to peak  $[DA]_o$  evoked by 1p in controls. Asterisks indicate significance level in post-hoc Bonferroni t-test for drug treatment versus controls, \* $P < 0.05$ , \*\*\* $P < 0.001$ . Crucifixes indicate significance level in Bonferroni post-hoc t-tests for Trolox versus Trolox + SIN-1, † $P < 0.05$ , †† $P < 0.001$ .

**Figure 2. The effect of NO on evoked DA release is independent of sGC or DA re-uptake modulation and only a small component is dependent on BK channels.**

**(a, f, i)** Profiles of mean  $[DA]_o \pm SEM$  versus time following stimuli (*arrows*) of either 1p or 4p (5-100 Hz) in control conditions (*left*), various antagonists (*center*) (**a**, ODQ, 100  $\mu M$ ; **f**, nomifensine, 10  $\mu M$ ; **i**, IbTx, 100 nM) and antagonist + SIN-1 (500  $\mu M$ ) (*right*) normalised to peak  $[DA]_o$  evoked by 1p in controls. **(b, g, j)** Mean peak  $[DA]_o \pm$

SEM versus frequency during 1p or 4p (5-100 Hz) in control conditions (*filled circles*), antagonist (*unfilled*) (**b**, ODQ ( $n= 8-15$ ); **g**, nomifensine ( $n = 12-20$ ); **j**, IbTx ( $n=9$ )) and antagonist + SIN-1 (*gray fill*) normalized to peak  $[DA]_o$  evoked by 1p in controls. Asterisks indicate significance level in post-hoc Bonferroni t-test versus controls,  $*P<0.05$ ,  $**P<0.01$ . Crucifixes indicate significance level in Bonferroni post-hoc t-tests for antagonist versus antagonist + SIN-1,  $\dagger\dagger P<0.01$ ,  $\dagger\dagger\dagger P<0.001$ . (**c**, **h**, **k**) Mean peak  $[DA]_o$  versus frequency expressed as % of peak  $[DA]_o$  evoked at that frequency prior to SIN-1 cocktail application, to compare the effect of SIN-1 on  $[DA]_o$  at each applied frequency in the presence of (**c**) ODQ (**h**) nomifensine or (**k**) IbTx versus SIN-1 alone (*dashed line*; determined from data in **Fig. 1b**). Error bars are also percentage of pre-SIN-1 levels. (**k**) Although IbTx does not prevent the activity-dependent increase in DA release (Two-way ANOVA,  $P<0.001$ ,  $n=9$ ) which is still prominent at 100 Hz, the effects of SIN-1 may be slightly modified towards a general frequency-independent increase in DA release. (**d**, **e**) Falling phases of mean  $[DA]_o \pm$  SEM profiles versus time released by high-frequency bursts (4 p/ 100 Hz) in control (*straight line*) and (**d**) SIN-1 or (**e**) nomifensine (*dashed line*) normalised to peak  $[DA]_o$  evoked by 1p in controls.

**Figure 3. The effect of NO on DA release is independent of striatal glutamate or GABA input but varies with cholinergic input.**

(**a**, **d**) Profiles of mean  $[DA]_o \pm$  SEM versus time following stimuli (*arrows*) of either 1p or 4p (5-100 Hz) in control conditions (*left*), antagonists (*center*) (**a**, glu/GABA antagonist cocktail (10  $\mu$ M bicuculline, 50  $\mu$ M saclofen, 50  $\mu$ M D-AP5, 10  $\mu$ M GYKI-52466, 200  $\mu$ M (S)-MCPG); **d**, DH $\beta$ E, 1  $\mu$ M) and antagonists + SIN-1 (500  $\mu$ M) (*right*)

normalised to peak  $[DA]_o$  evoked by 1p in controls. **(b, e)** Mean peak  $[DA]_o \pm$  SEM versus frequency during 1p or 4p (5-100 Hz) in control conditions (*filled circles*), antagonists (*unfilled*) **(b, glu/ GABA antags,  $n=9-16$ ; e, DH $\beta$ E,  $n=9-16$ )** and antagonists + SIN-1 (*gray fill*) normalized to peak  $[DA]_o$  evoked by 1p in controls. Asterisks indicate significance level in post-hoc Bonferroni t-test versus controls, \*\*\*  $P<0.001$ . Crucifixes indicate significance level in Bonferroni post-hoc t-tests for antagonist versus antagonist + SIN-1,  $\dagger P<0.05$ ,  $\dagger\dagger\dagger P<0.001$ . **(c, f)** Mean peak  $[DA]_o$  versus frequency expressed as % of peak  $[DA]_o$  evoked at that frequency prior to SIN-1 cocktail application, to compare the effect of SIN-1 on  $[DA]_o$  at each applied frequency in the presence of **(c)** Glu/GABA antagonists or **(f)**, DH $\beta$ E, versus SIN-1 alone (*dashed line*; determined from data in **Fig. 1b**). Error bars are also percentage of pre-SIN-1 levels.

**Figure 4. Direct and indirect effects of NO on DA release supplement each other at high frequency DA neuron activity but cancel out at low frequency.**

Cartoon to explain net outcome of the ‘direct’ and ‘indirect’ effects of NO on DA release evoked by varying frequencies of stimulation. ‘Indirect’ modulation of DA release by NO involving a net reduction of nAChR control of DA would be expected to reduce DA release at low frequencies, but consequently enhance frequency sensitivity and then even enhance DA release at high frequencies (see Cragg, 2006; Rice and Cragg, 2004). ‘Direct’ modulation of DA release by NO (seen in absence of nAChR activation) increases  $[DA]_o$  uniformly regardless of stimulus frequency. When nAChR tone is intact, these two mechanisms acting in concert will enhance  $[DA]_o$  at high frequencies but cancel out at low frequencies.

## References

- Ahern GP, Hsu SF, Jackson MB (1999). Direct actions of nitric oxide on rat neurohypophysial K<sup>+</sup> channels. *J Physiol* **520 Pt 1**: 165-176.
- Ahern GP, Klyachko VA, Jackson MB (2002). cGMP and S-nitrosylation: two routes for modulation of neuronal excitability by NO. *Trends Neurosci* **25**(10): 510-517.
- Aosaki T, Tsubokawa H, Ishida A, Watanabe K, Graybiel AM, Kimura M (1994). Responses of tonically active neurons in the primate's striatum undergo systematic changes during behavioral sensorimotor conditioning. *J Neurosci* **14**(6): 3969-3984.
- Apicella P (2002). Tonically active neurons in the primate striatum and their role in the processing of information about motivationally relevant events. *Eur J Neurosci* **16**(11): 2017-2026.
- Bayer HM, Glimcher PW (2005). Midbrain dopamine neurons encode a quantitative reward prediction error signal. *Neuron* **47**(1): 129-141.
- Beal MF, Chattha GK, Martin JB (1986). A comparison of regional somatostatin and neuropeptide Y distribution in rat striatum and brain. *Brain Res* **377**(2): 240-245.
- Bellamy TC, Wood J, Garthwaite J (2002). On the activation of soluble guanylyl cyclase by nitric oxide. *Proc Natl Acad Sci U S A* **99**(1): 507-510.
- Bennett BD, Callaway JC, Wilson CJ (2000). Intrinsic membrane properties underlying spontaneous tonic firing in neostriatal cholinergic interneurons. *J Neurosci* **20**(22): 8493-8503.
- Bennett BD, Wilson CJ (1999). Spontaneous activity of neostriatal cholinergic interneurons in vitro. *J Neurosci* **19**(13): 5586-5596.
- Berendse HW, Groenewegen HJ (1990). Organization of the thalamostriatal projections in the rat, with special emphasis on the ventral striatum. *J Comp Neurol* **299**(2): 187-228.
- Black MD, Matthews EK, Humphrey PP (1994). The effects of a photosensitive nitric oxide donor on basal and electrically-stimulated dopamine efflux from the rat striatum in vitro. *Neuropharmacology* **33**(11): 1357-1365.
- Bogdanov MB, Wurtman RJ (1997). Possible involvement of nitric oxide in NMDA-induced glutamate release in the rat striatum: an in vivo microdialysis study. *Neurosci Lett* **221**(2-3): 197-201.
- Bon CL, Garthwaite J (2001). Nitric oxide-induced potentiation of CA1 hippocampal synaptic transmission during baseline stimulation is strictly frequency-dependent. *Neuropharmacology* **40**(4): 501-507.

Broillet MC (2000). A single intracellular cysteine residue is responsible for the activation of the olfactory cyclic nucleotide-gated channel by NO. *J Biol Chem* **275**(20): 15135-15141.

Broillet MC, Firestein S (1996). Direct activation of the olfactory cyclic nucleotide-gated channel through modification of sulfhydryl groups by NO compounds. *Neuron* **16**(2): 377-385.

Buyukuysal RL (1997). Effect of nitric oxide donors on endogenous dopamine release from rat striatal slices. II: The role of voltage-dependent sodium channels, calcium channel activation, reverse transport mechanism, guanylate cyclase and endogenous glutamate. *Fundam Clin Pharmacol* **11**(6): 528-536.

Calabresi P, Centonze D, Gubellini P, Marfia GA, Bernardi G (1999a). Glutamate-triggered events inducing corticostriatal long-term depression. *J Neurosci* **19**(14): 6102-6110.

Calabresi P, Gubellini P, Centonze D, Sancesario G, Morello M, Giorgi M, Pisani A, Bernardi G (1999b). A critical role of the nitric oxide/cGMP pathway in corticostriatal long-term depression. *J Neurosci* **19**(7): 2489-2499.

Campbell DL, Stamler JS, Strauss HC (1996). Redox modulation of L-type calcium channels in ferret ventricular myocytes. Dual mechanism regulation by nitric oxide and S-nitrosothiols. *J Gen Physiol* **108**(4): 277-293.

Centonze D, Pisani A, Bonsi P, Giacomini P, Bernardi G, Calabresi P (2001). Stimulation of nitric oxide-cGMP pathway excites striatal cholinergic interneurons via protein kinase G activation. *J Neurosci* **21**(4): 1393-1400.

Cragg SJ (2003). Variable dopamine release probability and short-term plasticity between functional domains of the primate striatum. *J Neurosci* **23**(10): 4378-4385.

Cragg SJ (2006). Meaningful silences: how dopamine listens to the ACh pause. *Trends Neurosci* **29**(3): 125-131.

David HN, Ansseau M, Abbraini JH (2005). Dopamine-glutamate reciprocal modulation of release and motor responses in the rat caudate-putamen and nucleus accumbens of "intact" animals. *Brain Res Brain Res Rev* **50**(2): 336-360.

East SJ, Batchelor AM, Garthwaite J (1991). Selective blockade of N-methyl-D-aspartate receptor function by the nitric oxide donor, nitroprusside. *Eur J Pharmacol* **209**(1-2): 119-121.

Edwards TM, Rickard NS (2005). A scavenger of peroxynitrite prevents long-term memory formation using a single trial passive avoidance task for the day-old chick. *Neurobiol Learn Mem* **83**(2): 163-167.

Exley R, Clements MA, Hartung H, McIntosh JM, Cragg SJ (2008). Alpha6-containing nicotinic acetylcholine receptors dominate the nicotine control of dopamine neurotransmission in nucleus accumbens. *Neuropsychopharmacology* **33**(9): 2158-2166.

Feelisch M, Noack EA (1987). Correlation between nitric oxide formation during degradation of organic nitrates and activation of guanylate cyclase. *European Journal of Pharmacology* **139**(1): 19.

Feelisch M, Ostrowski J, Noack E (1989). On the mechanism of NO release from sydnonimines. *J Cardiovasc Pharmacol* **14 Suppl 11**: S13-22.

Finch DM (1996). Neurophysiology of converging synaptic inputs from the rat prefrontal cortex, amygdala, midline thalamus, and hippocampal formation onto single neurons of the caudate/putamen and nucleus accumbens. *Hippocampus* **6**(5): 495-512.

French SJ, Ritson GP, Hidaka S, Totterdell S (2005). Nucleus accumbens nitric oxide immunoreactive interneurons receive nitric oxide and ventral subicular afferents in rats. *Neuroscience* **135**(1): 121.

French SJ, Totterdell S (2002). Hippocampal and prefrontal cortical inputs monosynaptically converge with individual projection neurons of the nucleus accumbens. *The Journal of Comparative Neurology* **446**(2): 151-165.

French SJ, Totterdell S (2003). Individual nucleus accumbens-projection neurons receive both basolateral amygdala and ventral subicular afferents in rats. *Neuroscience* **119**(1): 19-31.

Garthwaite G, Goodwin DA, Batchelor AM, Leeming K, Garthwaite J (2002). Nitric oxide toxicity in CNS white matter: an in vitro study using rat optic nerve. *Neuroscience* **109**(1): 145-155.

Garthwaite J, Boulton CL (1995). Nitric oxide signaling in the central nervous system. *Annu Rev Physiol* **57**: 683-706.

Groenewegen HJ, Trimble M (2007). The ventral striatum as an interface between the limbic and motor systems. *CNS Spectr* **12**(12): 887-892.

Groenewegen HJ, Vermeulen-Van der Zee E, te Kortschot A, Witter MP (1987). Organization of the projections from the subiculum to the ventral striatum in the rat. A study using anterograde transport of Phaseolus vulgaris leucoagglutinin. *Neuroscience* **23**(1): 103-120.

- Groenewegen HJ, Wright CI, Beijer AV (1996). The nucleus accumbens: gateway for limbic structures to reach the motor system? *Prog Brain Res* **107**: 485-511.
- Groenewegen HJ, Wright CI, Beijer AV, Voorn P (1999). Convergence and segregation of ventral striatal inputs and outputs. *Ann N Y Acad Sci* **877**: 49-63.
- Guevara-Guzman R, Emson PC, Kendrick KM (1994). Modulation of in vivo striatal transmitter release by nitric oxide and cyclic GMP. *J Neurochem* **62**(2): 807-810.
- Hall CN, Garthwaite J (2006). Inactivation of nitric oxide by rat cerebellar slices. *J Physiol* **577**(Pt 2): 549-567.
- Hall CN, Garthwaite J (2009). What is the real physiological NO concentration in vivo? *Nitric Oxide* **21**(2): 92-103.
- Halliwell B, Evans P, Whiteman M (1999). Assessment of peroxynitrite scavengers in vitro. *Methods Enzymol* **301**: 333-342.
- Hammarstrom AK, Gage PW (1999). Nitric oxide increases persistent sodium current in rat hippocampal neurons. *J Physiol* **520 Pt 2**: 451-461.
- Hidaka S, Totterdell S (2001). Ultrastructural features of the nitric oxide synthase-containing interneurons in the nucleus accumbens and their relationship with tyrosine hydroxylase-containing terminals. *J Comp Neurol* **431**(2): 139-154.
- Hogg N, Darley-Usmar VM, Wilson MT, Moncada S (1992). Production of hydroxyl radicals from the simultaneous generation of superoxide and nitric oxide. *Biochem J* **281 ( Pt 2)**: 419-424.
- Hyland BI, Reynolds JN, Hay J, Perk CG, Miller R (2002). Firing modes of midbrain dopamine cells in the freely moving rat. *Neuroscience* **114**(2): 475-492.
- Ikemoto S (2007). Dopamine reward circuitry: two projection systems from the ventral midbrain to the nucleus accumbens-olfactory tubercle complex. *Brain Res Rev* **56**(1): 27-78.
- Iravani MM, Millar J, Kruk ZL (1998). Differential release of dopamine by nitric oxide in subregions of rat caudate putamen slices. *J Neurochem* **71**(5): 1969-1977.
- Jones SR, Garris PA, Kilts CD, Wightman RM (1995a). Comparison of Dopamine Uptake in the Basolateral Amygdaloid Nucleus, Caudate-Putamen, and Nucleus Accumbens of the Rat. *Journal of Neurochemistry* **64**(6): 2581-2589.
- Jones SR, Garris PA, Wightman RM (1995b). Different effects of cocaine and nomifensine on dopamine uptake in the caudate-putamen and nucleus accumbens. *J Pharmacol Exp Ther* **274**(1): 396-403.

Jones SR, O'Dell SJ, Marshall JF, Wightman RM (1996). Functional and anatomical evidence for different dopamine dynamics in the core and shell of the nucleus accumbens in slices of rat brain. *Synapse* **23**(3): 224-231.

Kiss JP, Hennings EC, Zsilla G, Vizi ES (1999). A possible role of nitric oxide in the regulation of dopamine transporter function in the striatum. *Neurochem Int* **34**(4): 345-350.

Kiss JP, Vizi ES (2001). Nitric oxide: a novel link between synaptic and nonsynaptic transmission. *Trends in Neurosciences* **24**(4): 211.

Klyachko VA, Ahern GP, Jackson MB (2001). cGMP-mediated facilitation in nerve terminals by enhancement of the spike afterhyperpolarization. *Neuron* **31**(6): 1015-1025.

Kraus MM, Prast H (2001). The nitric oxide system modulates the in vivo release of acetylcholine in the nucleus accumbens induced by stimulation of the hippocampal fornix/fimbria-projection. *Eur J Neurosci* **14**(7): 1105-1112.

Liang LP, Kaufman S (1998). The regulation of dopamine release from striatum slices by tetrahydrobiopterin and L-arginine-derived nitric oxide. *Brain Res* **800**(2): 181-186.

Lin AM, Kao LS, Chai CY (1995). Involvement of nitric oxide in dopaminergic transmission in rat striatum: an in vivo electrochemical study. *J Neurochem* **65**(5): 2043-2049.

Lonart G, Cassels KL, Johnson KM (1993). Nitric oxide induces calcium-dependent [3H]dopamine release from striatal slices. *J Neurosci Res* **35**(2): 192-198.

Lonart G, Johnson KM (1994). Inhibitory effects of nitric oxide on the uptake of [3H]dopamine and [3H]glutamate by striatal synaptosomes. *J Neurochem* **63**(6): 2108-2117.

Luchowski P, Urbanska EM (2007). SNAP and SIN-1 increase brain production of kynurenic acid. *Eur J Pharmacol* **563**(1-3): 130-133.

Matsumoto M, Hikosaka O (2009). Two types of dopamine neuron distinctly convey positive and negative motivational signals. *Nature* **459**(7248): 837-841.

Meffert MK, Calakos NC, Scheller RH, Schulman H (1996). Nitric oxide modulates synaptic vesicle docking fusion reactions. *Neuron* **16**(6): 1229-1236.

Meffert MK, Premack BA, Schulman H (1994). Nitric oxide stimulates Ca<sup>2+</sup>-independent synaptic vesicle release. *Neuron* **12**(6): 1235-1244.

- Mogenson GJ, Jones DL, Yim CY (1980). From motivation to action: functional interface between the limbic system and the motor system. *Prog Neurobiol* **14**(2-3): 69-97.
- Morris G, Arkadir D, Nevet A, Vaadia E, Bergman H (2004). Coincident but distinct messages of midbrain dopamine and striatal tonically active neurons. *Neuron* **43**(1): 133-143.
- Morris G, Schmidt R, Bergman H (2010). Striatal action-learning based on dopamine concentration. *Exp Brain Res* **200**(3-4): 307-317.
- Mulder AB, Hodenpijl MG, Lopes da Silva FH (1998). Electrophysiology of the hippocampal and amygdaloid projections to the nucleus accumbens of the rat: convergence, segregation, and interaction of inputs. *J Neurosci* **18**(13): 5095-5102.
- O'Donnell P, Greene J, Pabello N, Lewis BL, Grace AA (1999). Modulation of cell firing in the nucleus accumbens. *Ann N Y Acad Sci* **877**: 157-175.
- Pogun S, Baumann MH, Kuhar MJ (1994). Nitric oxide inhibits [3H]dopamine uptake. *Brain Res* **641**(1): 83-91.
- Prast H, Fischer H, Werner E, Werner-Felmayer G, Philippu A (1995). Nitric oxide modulates the release of acetylcholine in the ventral striatum of the freely moving rat. *Naunyn Schmiedebergs Arch Pharmacol* **352**(1): 67-73.
- Prast H, Philippu A (2001). Nitric oxide as modulator of neuronal function. *Prog Neurobiol* **64**(1): 51-68.
- Prast H, Tran MH, Fischer H, Philippu A (1998). Nitric oxide-induced release of acetylcholine in the nucleus accumbens: role of cyclic GMP, glutamate, and GABA. *J Neurochem* **71**(1): 266-273.
- Ravel S, Sardo P, Legallet E, Apicella P (2001). Reward unpredictability inside and outside of a task context as a determinant of the responses of tonically active neurons in the monkey striatum. *J Neurosci* **21**(15): 5730-5739.
- Regoli F, Winston GW (1999). Quantification of total oxidant scavenging capacity of antioxidants for peroxynitrite, peroxy radicals, and hydroxyl radicals. *Toxicol Appl Pharmacol* **156**(2): 96-105.
- Reynolds JN, Wickens JR (2002). Dopamine-dependent plasticity of corticostriatal synapses. *Neural Netw* **15**(4-6): 507-521.
- Rice ME, Cragg SJ (2004). Nicotine amplifies reward-related dopamine signals in striatum. *Nat Neurosci* **7**(6): 583-584.

- Rocchitta G, Migheli R, Mura MP, Esposito G, Desole MS, Miele E, Miele M, Serra PA (2004). Signalling pathways in the nitric oxide donor-induced dopamine release in the striatum of freely moving rats: evidence that exogenous nitric oxide promotes Ca<sup>2+</sup> entry through store-operated channels. *Brain Res* **1023**(2): 243-252.
- Sammut S, Dec A, Mitchell D, Linardakis J, Ortiguera M, West AR (2006). Phasic dopaminergic transmission increases NO efflux in the rat dorsal striatum via a neuronal NOS and a dopamine D(1/5) receptor-dependent mechanism. *Neuropsychopharmacology* **31**(3): 493-505
- Schmitz Y, Schmauss C, Sulzer D (2002). Altered dopamine release and uptake kinetics in mice lacking D2 receptors. *J Neurosci* **22**(18): 8002-8009.
- Schultz W (1986). Responses of midbrain dopamine neurons to behavioral trigger stimuli in the monkey. *J Neurophysiol* **56**(5): 1439-1461.
- Schultz W (2002). Getting formal with dopamine and reward. *Neuron* **36**(2): 241-263.
- Schultz W (2010). Dopamine signals for reward value and risk: basic and recent data. *Behav Brain Funct* **6**: 24.
- Segovia G, Mora F (1998). Role of nitric oxide in modulating the release of dopamine, glutamate, and GABA in striatum of the freely moving rat. *Brain Res Bull* **45**(3): 275-279.
- Sesack SR, Grace AA (2010). Cortico-Basal Ganglia reward network: microcircuitry. *Neuropsychopharmacology* **35**(1): 27-47.
- Shimo Y, Hikosaka O (2001). Role of tonically active neurons in primate caudate in reward-oriented saccadic eye movement. *J Neurosci* **21**(19): 7804-7814.
- Silva MT, Rose S, Hindmarsh JG, Aislaitner G, Gorrod JW, Moore PK, Jenner P, Marsden CD (1995). Increased striatal dopamine efflux in vivo following inhibition of cerebral nitric oxide synthase by the novel monosodium salt of 7-nitro indazole. *Br J Pharmacol* **114**(2): 257-258.
- Silva MT, Rose S, Hindmarsh JG, Jenner P (2003). Inhibition of neuronal nitric oxide synthase increases dopamine efflux from rat striatum. *J Neural Transm* **110**(4): 353-362.
- Smith Y, Parent A (1986). Neuropeptide Y-immunoreactive neurons in the striatum of cat and monkey: morphological characteristics, intrinsic organization and co-localization with somatostatin. *Brain Res* **372**(2): 241-252.
- Smith Y, Raju DV, Pare JF, Sidibe M (2004). The thalamostriatal system: a highly specific network of the basal ganglia circuitry. *Trends Neurosci* **27**(9): 520-527.

Stewart TL, Michel AD, Black MD, Humphrey PP (1996). Evidence that nitric oxide causes calcium-independent release of [3H] dopamine from rat striatum in vitro. *J Neurochem* **66**(1): 131-137.

Summers BA, Overholt JL, Prabhakar NR (1999). Nitric oxide inhibits L-type Ca<sup>2+</sup> current in glomus cells of the rabbit carotid body via a cGMP-independent mechanism. *J Neurophysiol* **81**(4): 1449-1457.

Sun J, Xin C, Eu JP, Stamler JS, Meissner G (2001). Cysteine-3635 is responsible for skeletal muscle ryanodine receptor modulation by NO. *Proc Natl Acad Sci U S A* **98**(20): 11158-11162.

Threlfell S, Clements MA, Khodai T, Pienaar IS, Exley R, Wess J, Cragg SJ (2010). Striatal muscarinic receptors promote activity dependence of dopamine transmission via distinct receptor subtypes on cholinergic interneurons in ventral versus dorsal striatum. *J Neurosci* **30**(9): 3398-3408.

Trabace L, Kendrick KM (2000). Nitric oxide can differentially modulate striatal neurotransmitter concentrations via soluble guanylate cyclase and peroxynitrite formation. *J Neurochem* **75**(4): 1664-1674.

Voorn P, Jorritsma-Byham B, Van Dijk C, Buijs RM (1986). The dopaminergic innervation of the ventral striatum in the rat: a light- and electron-microscopical study with antibodies against dopamine. *J Comp Neurol* **251**(1): 84-99.

West AR, Galloway MP (1996). Intra-striatal infusion of (+/-)-S-nitroso-N-acetylpenicillamine releases vesicular dopamine via an ionotropic glutamate receptor-mediated mechanism: an in vivo microdialysis study in chloral hydrate-anesthetized rats. *J Neurochem* **66**(5): 1971-1980.

West AR, Galloway MP (1997a). Endogenous nitric oxide facilitates striatal dopamine and glutamate efflux in vivo: role of ionotropic glutamate receptor-dependent mechanisms. *Neuropharmacology* **36**(11-12): 1571-1581.

West AR, Galloway MP (1997b). Inhibition of glutamate reuptake potentiates endogenous nitric oxide-facilitated dopamine efflux in the rat striatum: an in vivo microdialysis study. *Neurosci Lett* **230**(1): 21-24.

West AR, Galloway MP (1998). Nitric oxide and potassium chloride-facilitated striatal dopamine efflux in vivo: role of calcium-dependent release mechanisms. *Neurochem Int* **33**(6): 493-501.

West AR, Grace AA (2000). Striatal nitric oxide signaling regulates the neuronal activity of midbrain dopamine neurons in vivo. *J Neurophysiol* **83**(4): 1796-1808.

West AR, Grace AA (2004). The nitric oxide-guanylyl cyclase signaling pathway modulates membrane activity States and electrophysiological properties of striatal medium spiny neurons recorded in vivo. *J Neurosci* **24**(8): 1924-1935.

Wright CI, Groenewegen HJ (1995). Patterns of convergence and segregation in the medial nucleus accumbens of the rat: relationships of prefrontal cortical, midline thalamic, and basal amygdaloid afferents. *J Comp Neurol* **361**(3): 383-403.

Xu JW, Hou M, Slaughter MM (2005). Photoreceptor encoding of supersaturating light stimuli in salamander retina. *J Physiol* **569**(Pt 2): 575-585.

Xu L, Eu JP, Meissner G, Stamler JS (1998). Activation of the cardiac calcium release channel (ryanodine receptor) by poly-S-nitrosylation. *Science* **279**(5348): 234-237.

Yang S, Cox CL (2008). Excitatory and anti-oscillatory actions of nitric oxide in thalamus. *J Physiol* **586**(Pt 15): 3617-3628.

Zahm DS (2000). An integrative neuroanatomical perspective on some subcortical substrates of adaptive responding with emphasis on the nucleus accumbens. *Neurosci Biobehav Rev* **24**(1): 85-105.

Zhang H, Sulzer D (2004). Frequency-dependent modulation of dopamine release by nicotine. *Nat Neurosci* **7**(6): 581-582.

Zhou FM, Liang Y, Dani JA (2001). Endogenous nicotinic cholinergic activity regulates dopamine release in the striatum. *Nat Neurosci* **4**(12): 1224-1229.

Zhu XZ, Luo LG (1992). Effect of nitroprusside (nitric oxide) on endogenous dopamine release from rat striatal slices. *J Neurochem* **59**(3): 932-935.

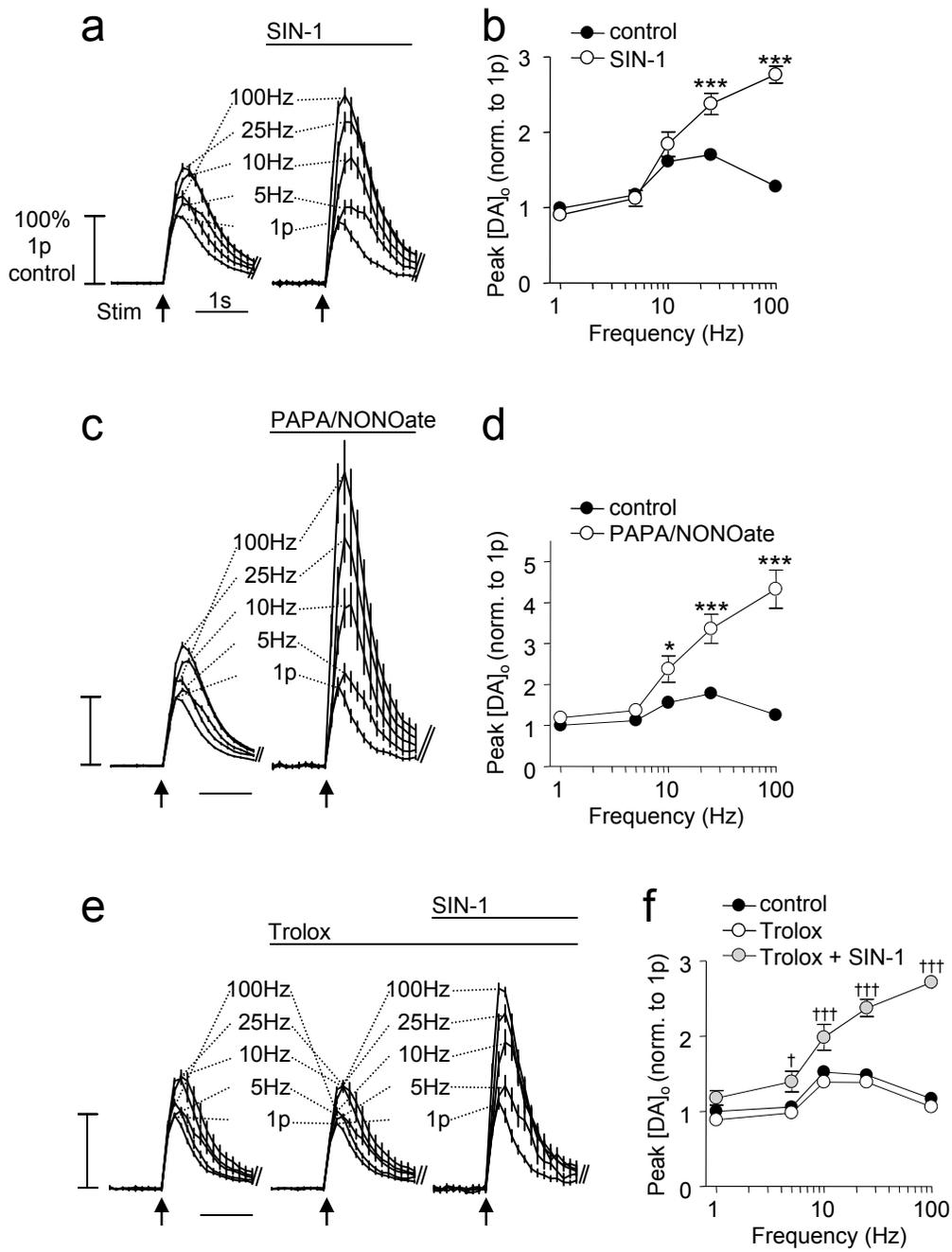


Figure 1

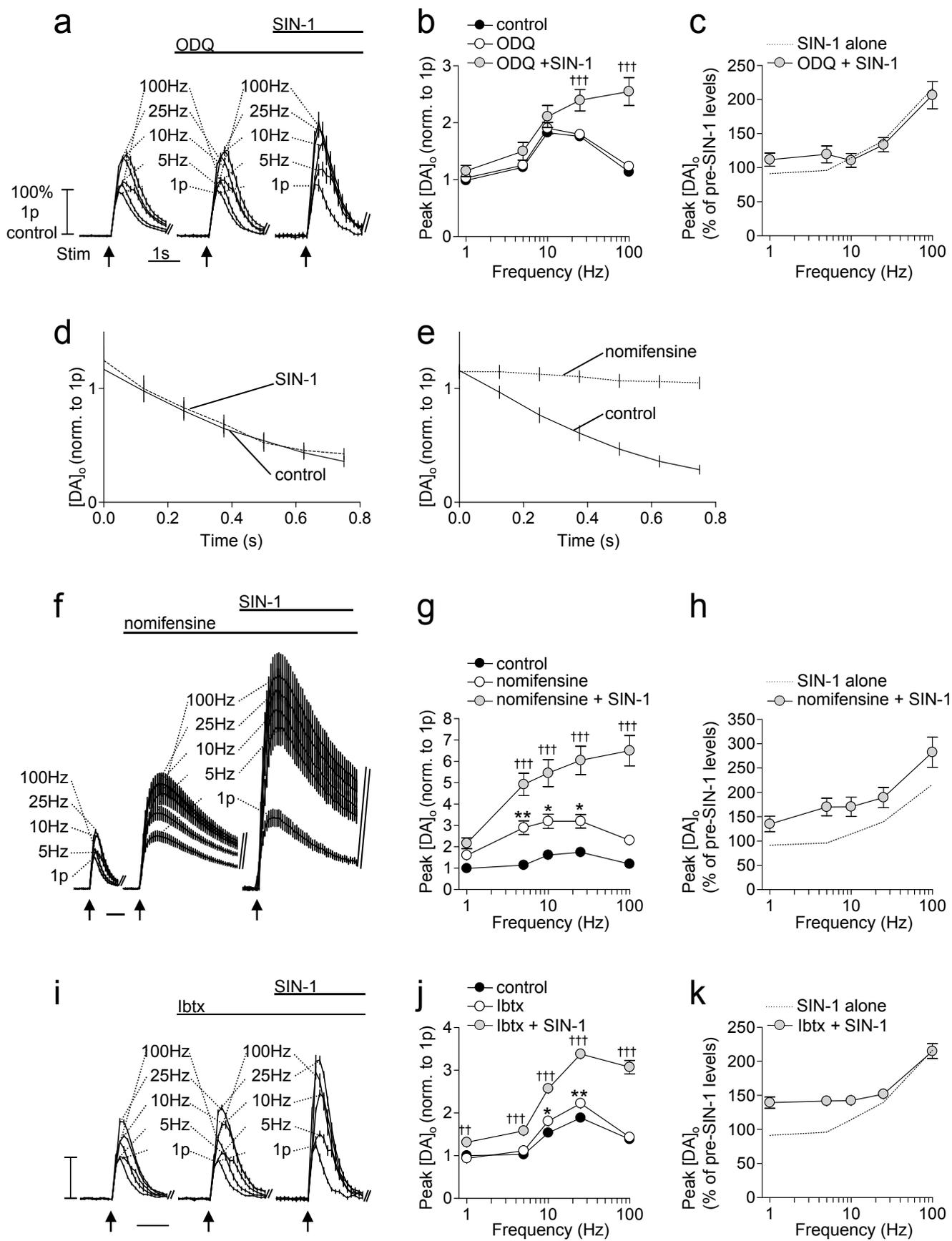


Figure 2

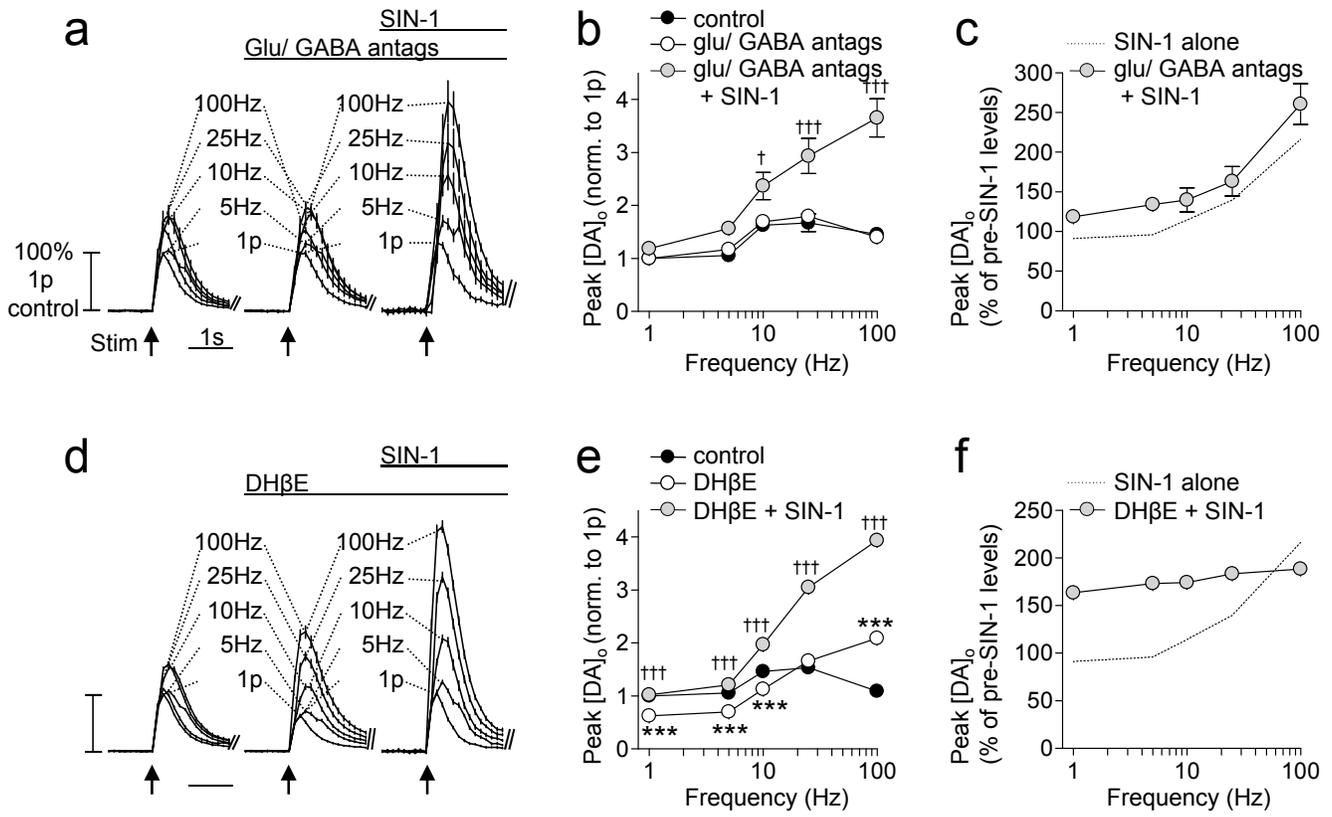


Figure 3

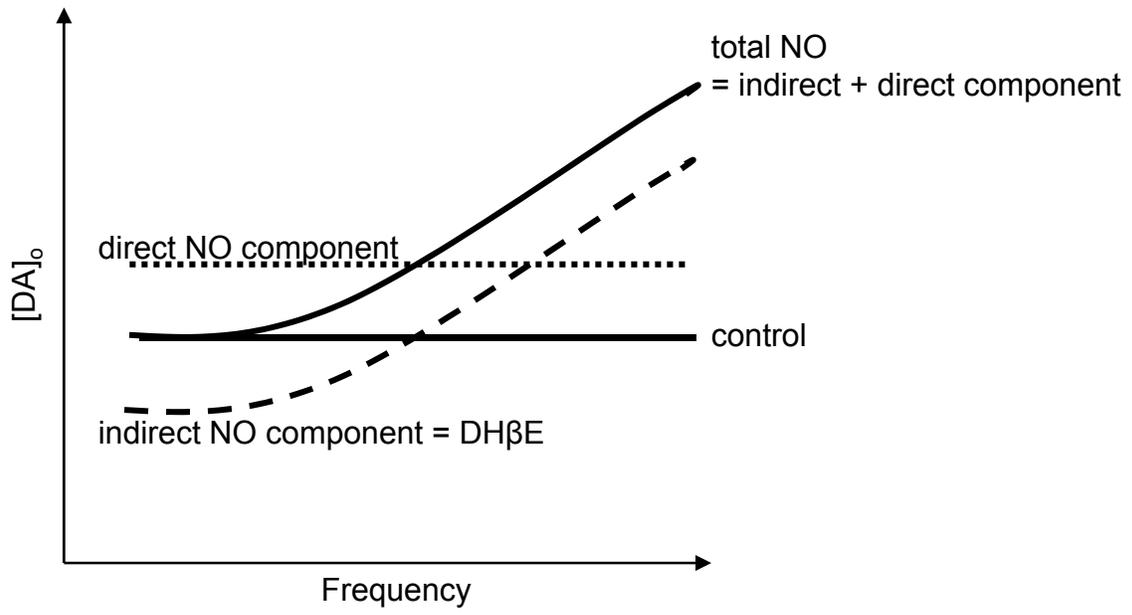


Figure 4