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Research Report

Increased electrical and metabolic activity in the dorsal raphe nucleus of Parkinsonian rats

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ABSTRACT

Serotonin (5-HT) containing neurons in the dorsal raphe nucleus (DRN) may play important roles in Parkinson's disease (PD). This study investigated neural and metabolic activity of the DRN in animal PD models based on dopamine depletion. The data show both increased firing rate of DRN 5-HT neurons and increased cytochrome oxidase activity in dopamine-depleted rats, as compared to controls. These data support the hypothesis that the DRN 5-HT system is hyperactive in the dopamine-depleted brain.

1. Introduction

Parkinson's disease (PD) is characterised histopathologically by profound and progressive degeneration of nigral dopaminergic neurons, but changes in non-dopaminergic systems are likely to influence the expression of both PD symptoms and treatment outcome (Braak et al., 2003; Del Tredici et al., 2002). One non-dopaminergic system that may be important in this respect is the 5-HT system. For example, recent neurochemical and behavioural data from animal studies suggest that drugs which decrease 5-HT function may be useful to treat dopa-induced dyskinesias (Carta et al., 2007). On the other hand, electrophysiological studies in animals suggest that the psychiatric side-effects of deep brain stimulation in PD, and the emotional and cognitive symptoms of PD more generally, may be prevented by drugs which increase 5-HT function (Temel et al., 2007). The findings of the latter study may be underpinned between a strong functional relationship between the 5-HT system and the subthalamic nucleus (STN), which is a key basal ganglia output station.

The functional status of the DRN 5-HT system in the dopamine (DA)-depleted brain is, however, unclear. For example, some studies in animal models of PD have found hyperinnervation of forebrain 5-HT fibres and elevated brain tissue levels of 5-HT (Commins et al., 1989; Reader and Dewar, 1999; Smits et al., 2008; Zhou et al., 1991). Other similar studies, however, found no or little change in 5-HT levels (Breese et al., 1984; Erinoff and Snodgrass, 1986; Reader and Dewar, 1999). The present study investigated the effect of DA depletion on both the electrical activity of DRN 5-HT neurons and DRN metabolic

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activity as indicated by cytochrome C oxidase (COX) activity. The latter measurements also examined DRN-STN links.

2. Results

Extracellular single-unit recordings were taken from putative 5-HT neurons in rats that were treatment-naïve (10 neurons), sham-lesioned (8 neurons), 6-OHDA-lesioned (17 neurons), or acutely DA-depleted (6 neurons). The electrophysiological properties of neurons in the treatment-naive and sham-lesioned groups were pooled since the values were not statistically different (t's<0.85, $P>0.05$). The mean firing rates of putative 5-HT neurons in both acutely DA-depleted and chronically DA-lesioned rats were significantly higher as compared to the mean firing rate of 5-HT neurons in the control group (F's>3.72, $P<0.032$, Fig. 1). In comparison, putative 5-HT neurons in chronically and acutely DA-depleted rats had similar regularity (coefficient of variation [CV], 0.34±0.02 and 0.31±0.05, respectively) and spike duration (2.26±0.09 and 2.22±0.07 ms, respectively) as 5-HT neurons recorded in the control group.

In line with the electrophysiological results, COX activity in the DRN of chronically DA-lesioned animals was significantly higher (+43.3%; $t=2.62$, $P>0.05$) compared to sham controls. COX activity in the STN was also significantly higher in the DA-lesioned animals compared to sham controls (+44.3%; $t=7.81$, $P>0.001$, Fig. 2). In contrast, COX activity in the cerebral peduncle and periaqueductal gray was not statistically different from controls ($t=-0.56$, $P>0.05$ and $t=1.12$, $P>0.05$, respectively).

Interestingly, there was a significant positive correlation between the levels of COX activity in the DRN and STN (Pearson correlation 0.669, $P>0.05$).

3. Discussion

The findings of the present study suggest that the DRN, which is the major source of 5-HT innervation to the forebrain, has increased electrical and metabolic activities in two animal models of PD. The electrophysiological properties of the DRN neurons studied were characteristic of 5-HT neurons (Allers and Sharp, 2003). Since approximately 70% of neurones in the DRN contain 5-HT, it is reasonable to assume that these neurones contribute to the increase in COX activity observed in the DRN of DA-lesioned animals. As such, these data are consistent with reports of increased 5-HT levels in forebrain regions of PD models (Commins et al., 1989; Reader and Dewar, 1999; Sivam, 1995; Smits et al., 2008; Zhou et al., 1991).

The mechanisms underlying the increase in 5-HT cell firing in the DRN observed in the PD models is not clear. Since this effect was observed 24 h after acute DA depletions, there appears to be no requirement for long-term adaptive changes associated with chronic DA lesions, but short-term adaptive changes are possible. There is anatomical and functional evidence of an interaction between DRN 5-HT neurons and midbrain DA neurones (Azmitia and Segal, 1978; Hokfelt et al., 1984; Stern et al., 1979). Although the nature of this interaction is complex, previous observations show

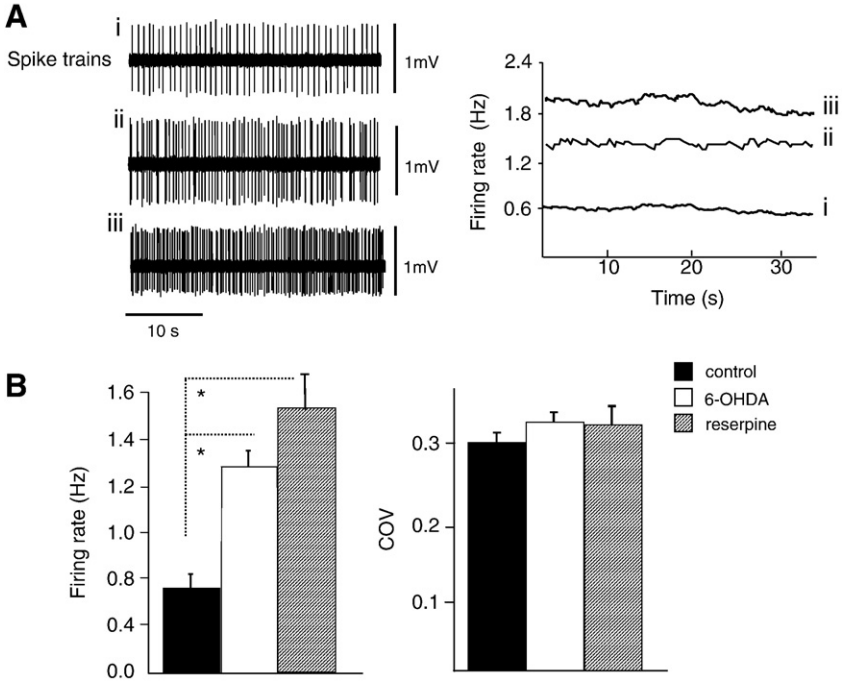

Fig. 1 – Electrophysiological activity of DRN 5-HT neurons in rat PD models. (A) Spike trains and instantaneous firing rates of individual 5-HT neurons following (i) a sham-lesion (ii) chronic DA lesion, and (iii) acute DA depletion. (B) Grouped data for recorded 5-HT neurons. CV, coefficient of variation. Data are presented as means±s.e.m.s. *$P<0.05$ between parkinsonian and control animals.

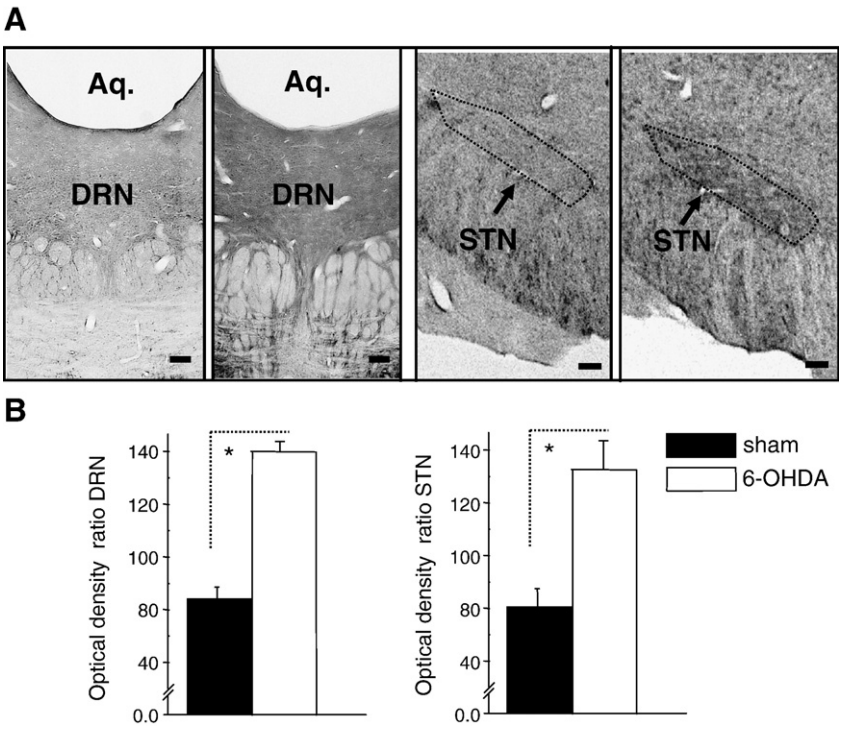

Fig. 2 – Cytochrome C oxidase (COX) activity in the DRN and STN of rats with chronic DA lesions. (A) Representative sections stained for CO at the level of the DRN (bar = 200 μm) and STN (bar = 150 μm), respectively. The left hand section of each pair was taken from sham-lesioned rats, and the right hand section from DA-lesioned rats. Aq. = aquaduct of Sylvius, STN = subthalamic nucleus, and DRN = dorsal raphe nucleus. (B) Grouped data for COX activity in the DRN and STN. Data are presented as means ± s.e.m.s of optical density ratio. *P < 0.05 between DA-depleted and sham-lesioned animals.

that when administered acutely, DA receptor agonists increase whereas antagonists decrease 5-HT cell firing (Martin-Ruiz et al., 2001). The latter data suggest that the increase in 5-HT cell firing in the PD models is not simply due to an immediate fall in DA availability. A more likely scenario is changes in 5-HT neuron control over hours/days following the loss of dopamine.

Data in the present study suggest that one possible mechanism is altered function of basal ganglia output, specifically at the level of the STN. Thus, our COX experiments detected an increase in metabolic activity in the STN following DA lesions as expected from previous studies (Blandini et al., 2007; Breit et al., 2007). Moreover, this increase in metabolic activity in the STN significantly correlated with the increase in metabolic activity in the DRN. The importance of STN–DRN link is further evidenced by our recent finding that chemically-induced inhibition of the STN decreased the firing of DRN 5-HT neurons (Temel et al., 2007). Therefore, it is plausible that increased activity of DRN 5-HT neurons in the PD models is a consequence of increased STN activity.

In summary, the present data support the hypothesis that 5-HT neurons undergo adaptive increases in firing activity in the Parkinsonian condition, possibly due to changes in basal ganglia output. This hypothesis may be relevant to recent links between 5-HT and the clinical effects of L-DOPA treatment (Carta et al., 2007) and non-motor symptoms of PD (Temel et al., 2007).

4. Experimental procedures

Male rats for electrophysiological (270–330 g; Sprague–Dawley Harlan Olac, Bicester, U.K., $n = 10$) and histological (270–330 g; Lewis, Maastricht University, The Netherlands, $n = 10$) studies were housed in groups with food and water freely available. Electrophysiological experiments were carried out in accordance with the U.K. Home Office Animals (Scientific Procedures) Act (1986), and histological experiments were carried out in accordance with the Animal Experiments and Ethics Committee of Maastricht University. All studies were compliant with minimal standards as defined by the European Communities Council Directive of 24 November 1986.

Both chronic and acute models of PD were utilised. For chronic DA neuron lesions, rats were pretreated with desipramine (25 mg/kg, i.p.) and anaesthetised with halothane prior to infusion (over 2 min) of 6-OHDA (250 μg in 10 μl) or vehicle (1% ascorbic acid/saline) into the lateral ventricle (AP −0.9, ML 1.4, DV −4.0) (Rodriguez Diaz et al., 2001). Experiments were carried out 14–16 days post-surgery. Compared to sham-lesioned, animals treated with 6-OHDA showed a significant ($p < 0.05$)

loss of striatal DA (−82%) and dihydroxyphenylacetic acid (−89%), as measured by HPLC with electrochemical detection. Electrophysiological recordings were also obtained in animals with acute DA depletion. In this case, animals were treated with reserpine (5 mg/kg, i.p., 20 h before recording), and then α-methyl-*p*-tyrosine (250 mg/kg, i.p., 4 h before recording) according to Garcia et al. (Garcia et al., 2003). A control group of treatment-naïve rats was also included. DA-lesioned and depleted rats were akinetic prior to electrophysiological and metabolic experiments.

Extracellular single-unit recordings of DRN 5-HT neurons were made in rats anaesthetised with chloral hydrate (460 mg/kg, i.p.), as described previously (Boothman et al., 2003). Putative 5-HT neurons satisfied at least 3 of the following extracellular electrophysiological and pharmacological criteria (Allers and Sharp, 2003; Hajos et al., 1995): slow (0.5–2 Hz) and regular (CV: 0.3–0.5) firing, a triphasic spike waveform of relatively long duration (1.9–2.5 ms), and an inhibitory response to administration of the 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (10 μg/kg, i.v.). The latter was only performed during the final cell recording. For each cell, recording was obtained over a period of at least 10–15 min. After recordings were completed, the final position of the recording electrode was marked by iontophoretic Pontamine dye injection (20 μA, negative current, 1 Hz pulses of 300 ms duration for 20 min). Rats were then perfused transcardially with phosphate-buffered saline and fixative prior to standard histological analysis.

COX activity was measured in separate groups of DA-lesioned (6-OHDA i.c.v.) and control animals, using a previously published method but with minor modifications (Silverman and Tootell, 1987). In brief, coronal tissue sections (30 μm) including the DRN and STN were immersed in 0.1 M phosphate buffer containing 30% sucrose, and then incubated for 10 min in 0.05 M Tris buffer (pH 7.6) containing 10% sucrose, 0.275 mg/ml cobalt chloride and 5 μl/ml dimethylsulfoxide (Sigma-Aldrich, St. Louis, MO, USA). After rinsing steps, sections were then incubated for 1 h at 37 °C in 0.1 M phosphate buffer (pH 7.4) containing 0.5 mg/ml 3,3′-diaminobenzidine-4HCl, 0.4 mg/ml cytochrome *C* type III (C2506, Sigma-Aldrich), 4% sucrose, 0.18 mg/ml catalase (C9322, Sigma, Aldrich) and 2.5 μl/ml dimethylsulfoxide, followed by fixation in 4% neutral buffered paraformaldehyde for 10 min. Finally, sections were rinsed, dehydrated and cover-slipped.

COX activity was quantified by image analysis (analySIS Imaging System, Münster, Germany) of digital photos taken using an Olympus U-CMAD-2 digital camera connected to an Olympus AX 70 microscope. Densitometric measurements (Image J software version 1.38x; Wayne Rasband, NIH, Bethesda, USA) were obtained from 3 DRN images (anteroposterior; −7.3, −7.6, and −8.0 relative to bregma) and 3 STN images (−3.6, −3.8, −4.16) per rat. Measurements of COX activity were also taken from the cerebral peduncle and periaqueductal gray. COX data are expressed as optical density ratios (optical density/area of interest in mm^2).

Data were analysed statistically by the Independent-Samples *t*-Test and one way ANOVA. *P* values < 0.05 were considered significant.

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