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Context-driven changes in l-DOPA-induced behaviours in the 6-OHDA lesioned rat

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ABSTRACT

Both contralateral rotational behaviour and dyskinetic abnormal involuntary movements (AIMs) are induced by the administration of L-DOPA in the unilateral 6-OHDA lesioned rat model of Parkinson's disease. Since rotational responses can be conditioned to environmental cues we have investigated the extent to which drug-induced AIMS may also be conditioned by exteroceptive cues and experience. In Experiment I, 6-OHDA lesioned rats received repeated daily injections of L-DOPA either in their home cage (control) or in association with a brief (20 mins) exposure to the rotometers (paired). To assess conditioning, all animals then received two tests in the rotometer bowls. Following injection of saline the paired group both rotated more contralaterally and displayed manifest AIMs, neither of which were exhibited by the control rats. Moreover, following injection of L-DOPA, the paired group showed a trend for increased AIMs compared to controls.
Two further studies provided longer exposure to the conditioning environments in counterbalanced designs. Although, using these parameters, re-exposure in the presence of saline did not induce context-dependent AIMs, a strong context-specific component of the sensitised response to L-DOPA was seen; chronic administration of drug produced a significantly stronger behavioural response in animals paired with a particular environment for drug administration than controls.
This data suggests that part of the sensitisation of behavioural responding to L-DOPA administration is not solely a pharmacological phenomenon, but is also conditioned to the environmental context in which the drug is administered. This has clear implications for the clinical observation and experimental measurement of drug-induced dyskinesia in Parkinson's disease patients and animal models.

Introduction

Parkinson's disease is a motor disorder caused largely by the degeneration of the dopaminergic nigrostriatal pathway. Unfortunately, the most efficacious treatment for the disorder, the dopamine precursor 3,4-L-dihydroxyphenylalanine (L-DOPA), is also responsible for the insidious onset of L-DOPA-induced dyskinesia (LID), abnormal involuntary movements (AIMs) affecting the limbs, torso and facial area. Both anti-parkinsonian therapies and anti-dyskinetic agents are commonly assessed preclinically in the unilateral 6-hydroxydopamine (6-OHDA) lesioned rat model, which has a near complete unilateral depletion of the nigrostriatal dopaminergic pathway created by the discrete administration of 6-OHDA into one median forebrain bundle (Ungerstedt, 1976). The resulting motor asymmetry produces a tendency for spontaneous locomotion to be directed towards the lesioned hemisphere (ipsilateral). Conversely, anti-parkinsonian pharmacotherapies such as dopamine agonists and L-DOPA act on the supersensitive dopamine receptors in the lesioned striatum to evoke contralateral circling (Mendez and Finn, 1975). Repeated administration causes this response to sensitise, with a more rapid time to onset, greater magnitude and duration (although this last has been debated), all thought to relate to a change in the post-synaptic dopamine receptors or post-receptor mechanisms following intermittent stimulation (Carey, 1991; Lindgren et al., 2007; Papa et al., 1994; Ravenscroft et al., 2004). In addition to the rotational response, L-DOPA-induced dyskinesias are also represented in this model, as torsional twisting of the body axis, hyperkinesia and dystonia of the forelimb, dystonia of the hindlimb and orofacial movements develop with repeated L-DOPA administration, all of which are focused on the side contralateral to the lesion (Cenci et al., 1998; Dekundy et al., 2007; Lundblad et al., 2002; Winkler et al., 2002).

Context-dependent conditioning and context-specific sensitisation are two behavioural phenomena that have been demonstrated in intact animals typically following administration of psychostimulants, often with dopaminergic activity. In context-dependent conditioning a particular environment (conditioned stimulus) is paired with the administration of a drug (unconditioned stimulus) such that if the animal is re-exposed to the environment in the absence of the drug, the behaviour will be evident (conditioned response). Context-specific sensitisation describes a different drug/context interaction in which the behavioural response to the drug increases in magnitude with repeated administration. This sensitisation is associated with the context, such that in a novel

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environment the behaviour will return to baseline (Keller et al., 2002; Szumlinski et al., 1997; Tirelli and Terry, 1998). L-DOPA-induced contralateral rotations are vulnerable to context-dependent conditioning, repeated pairings of the drug and environment leading to a contralateral rotational response when returned to the paired environment in the absence of L-DOPA (Carey, 1992). Given the potential for context-dependency in the basic rotational response to L-DOPA, this then raises 2 questions. First, do the non-locomotor AIMs develop context-dependency in a similar fashion? Second, are the developments in rotational responses and AIMs with chronic L-DOPA treatment purely due to pharmacological events, or, given that animals are repeatedly tested in the same environment, is there an element of context-specific sensitisation that contributes to the severity of these behaviours?

The clinical relevance of these phenomena is underlined in the significant development of L-DOPA-induced dyskinesia with repeated use of L-DOPA as well as informal clinical reports that patients' L-DOPA-induced dyskinesia can increase or wane in different contexts such as doctors' surgery *vs* home or in response to stress. The present study uses three Pavlovian pairing paradigms to determine the behavioural consequences of associating drug exposure with a particular context on different components of the L-DOPA-induced response in the standard 6-OHDA model. In the first experiment short exposures to the rotometer environment during drug activity replicating protocols used in evaluation of conditioned rotational behaviour. A second experiment tests the same conditioning process during longer exposure times to determine if there was context specific sensitisation, whilst the final experiment characterised this behaviour in a counter-balanced design, assessing paired and unpaired behaviours in the same apparatus but with different contexts. Behavioural outcome is assessed in association with the levels of striatal fosB/ΔfosB immediate early gene activation, a characterised biochemical correlate of AIMs severity (Andersson et al., 1999).

Methods

Animals

Female Sprague Dawley rats were obtained from Charles River, UK, 200 g on arrival and kept in standard housing, in a 14 h light/10 h dark cycle, 3–5 per cage with *ad libitum* access to food and water. All procedures were carried out in accordance with the UK Animals (Scientific Procedures) Act 1986, project licence number 30/1968. 6-hydroxydopamine HBr (6-OHDA), ascorbic acid, 3,4-L-dihydroxphenylalanine methyl ester (L-DOPA), D-amphetamine sulphate and benserazide were obtained from Sigma Aldrich (UK).

Experimental design

Experiment I, Short duration L-DOPA conditioning, paired vs unpaired controls

Two groups of 6-OHDA lesioned rats allocated on the basis of amphetamine-induced rotations (2.5 mg/kg) were injected with L-DOPA (10 mg/kg s.c. in saline, with 15 mg/kg benserazide) daily for 2 weeks. One group of rats was placed in rotometer bowls for 20 min, 40 min after drug injection ('paired' group, n = 25); the second group was similarly placed in rotometer bowls for 20 mins daily but administered the same dose of L-DOPA separately in their home cages later in the day ('control' group, n = 16). After L-DOPA treatment (14 sessions), all rats were placed in the rotometer bowls following saline injection and rotations and AIMs were assessed for 10 mins. In the subsequent session, all rats were placed in the rotometers following L-DOPA administration and rotations and AIMs scored to confirm the presence of AIMs in response to L-DOPA (see Fig. 1A).

Experiment II, Long duration conditioning, paired vs unpaired controls

6-OHDA lesioned rats were injected with a single dose of L-DOPA and placed in rotometers for 3 h. Rotation and AIMs were recorded and on the basis of the behavioural response rats were divided into two even behavioural groups. The 'paired' group was injected with L-DOPA every other day for 6 weeks in the rotometers and the 'control' group was injected at the same time but in their home cage in an administration regime consistent with the approach taken above (See Fig. 1B). After 6 weeks, rats were all retested 'on' L-DOPA in the rotometers and rotations and AIMs were rescored. 24 h after L-DOPA administration rats were transcardially perfused with paraformaldehyde, cryoprotected and sectioned. FosB immunohistochemistry was performed on sections of the striatum. An additional group of age-matched 6-OHDA lesioned rats not treated with L-DOPA (n = 6) was included in this part of the study to determine baseline FosB expression in the intact and lesioned hemispheres.

Experiment III, Long duration conditioning in two rotometer environments

6-OHDA lesioned rats were allocated to two equal groups on the basis of amphetamine-induced rotations (n = 14–16). In a counter-balanced design, 2 environments (E1 and E2) were generated within the rotometer room; E1, standard rotometers with standard sawdust, quiet ambient noise; and E2, standard rotometers with coloured, cinnamon scented sawdust, dimmed light and white noise. On alternate days Group A received L-DOPA (as above) in E1 and saline in E2 whilst Group B was the reverse, given saline in E1 and L-DOPA in E2. After 6 weeks, there were 2 probe tests; 1) both groups received saline in their 'L-DOPA' environment, and 2) on the following day, they received L-DOPA in the 'saline' environment (see Fig. 1C).

6-hydroxydopamine lesion

To produce unilateral lesions of the ascending nigrostriatal dopamine tract, Sprague Dawley rats were anaesthetised in an induction chamber with 4% isoflurane in 95% O_2/5% CO_2 before being transferred to a stereotaxic frame and maintained at 1.5–3% isoflurane. A sagittal cut exposed bregma and a hole was drilled. A total volume of 3 μl of 3 μg/μl 6-OHDA HBr in 0.02% ascorbic acid was injected at a rate of 1 μl/min via a 30 gauge stainless steel cannula targeted at the median forebrain bundle using the following coordinates AP −4.4 mm, ML −1.0 mm, and DV −7.8 mm, with the tooth bar set at −2.3 mm below the interaural line (Paxinos and Watson, 1998). Following injection the cannula was left in place for 3 min for diffusion prior to retraction. Post-surgical analgesia of soluble paracetamol in the drinking water was administered for 3 days (1 g/l).

Behaviour — rotation

Rotation was assessed in a bank of 16 automated rotometer bowls (Rotorat, Med Associates, Georgia, VT) modelled after the design of Ungerstedt and Arbuthnott (1970) which record the frequency of rotations in the clockwise and anticlockwise directions. Two weeks post-lesion, all animals were injected with 2.5 mg/kg D-amphetamine sulphate and rotation monitored over 90 mins. Only animals scoring a net ipsilateral rotation rate of 6 turns/min were selected for further analysis, as this rate has been established to be associated with striatal depletion >95–97% on the lesioned side (Carey, 1991; Heikkila et al., 1981; Schmidt et al., 1983). This was confirmed by tyrosine hydroxylase immunohistochemistry post-mortem which showed a greater than 95% depletion of tyrosine hydroxylase positive cell bodies in the substantia nigra of the lesioned hemisphere (data not shown). Rotations in response to L-DOPA were performed in the same rotometer apparatus. In Experiment III the sawdust in the bowls was replaced with cinnamon scented green sawdust, dim light was used and white noise played for the duration of the experiment.