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Research report

Bilateral striatal lesions disrupt performance in an operant delayed reinforcement task in rats

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ABSTRACT

In order to provide an animal model of the impulsivity observed in Huntington's disease, the effects of bilateral neostriatal lesions in rats were evaluated in an operant delayed reinforcement task. When given a choice between responding to one lever for a small but immediate reward and a second lever for a larger delayed reward, normal rats exhibit a marked preference for responding to the high reward lever when the imposed delay is short, but progressively choose the lever associated with immediate small reward as the delays increase. Following striatal lesions, the animals continue to express similar preferences, but the lesions initially impose a distinct flattening of the delay–preference function, suggesting a relative insensitivity to the increasing delay parameter in making their response choices. However, this deficit declines with extend retraining on the task, such that 1–2 months after lesion the delay-dependent shift of preference from the delayed to the immediate lever as the delays lengthened was comparable in lesion and sham animals. Amphetamine further disinhibited all animals, apparent as a further increase in the number and reduction of the latencies of responses made to the lever associated with immediate reward. Striatal lesions had little influence on the effects of amphetamine on task performance, other than the increase in the numbers of omissions of lever and panel responses induced by the drug was more marked in the lesion than sham animals, and the lesioned animals exhibited less delay-dependency than the controls in their preference for responding to the lever associated with the larger delayed reinforcement at the highest (1.5 mg/kg) dose tested. The present results indicate small but clear effects of dorsal striatal lesions in an operant delayed reinforcement task, suggestive of an initial impairment in response selection and a reduction in their sensitivity to the delay interval itself. This deficit recovered with further training, which may be dependent upon relearning choice response procedures disrupted by the lesion, but might be reinstated by treatment with stimulant drugs.

This article is part of a special issue entitled 'Behavioural, Anatomical, and Genetic Characterisation of Mouse and Rat Models of Huntington's Disease.'

1. Introduction

Although the cognitive and motor symptoms of Huntington's disease (HD) have been well replicated in animal models of striatal degeneration, the third key dimension of the HD syndrome – psychiatric or 'behavioural' symptoms – have been poorly represented. A common feature of the psychiatric impairment is that HD patients exhibit marked impulsivity and disinhibitory symptoms [15]. Over the last decade, an operant delayed reinforcement task has become well established to evaluate impulsivity in rats, and their ability to withhold pre-potent responses to achieve higher rewards [8,12]. In particular, performance on the delayed reinforcement task has been shown to be sensitive to a variety of pharmacological challenges affecting disinhibitory and reward-related processes [8,12,13,18,24] and to lesions in ventral fronto-striatal circuits involved in reward-related motivational processes [5–7,25]. However, the effects of lesions in the dorsal striatum ('neostriatum') – involving the striatal circuits primarily affected in human HD – have not, to our knowledge, been previously explored. In accordance with the hypothesis that the neostriatum may be part of the larger circuitry modulating impulsive behaviours, a recent study using primates has revealed a distinct role for neurons of the dorsal striatum in differentiating the temporally influenced values of reward in a delayed discounting task, while the ventral striatum was more strongly associated with encoding the broad values of the combined delay and reward [4].

The present manuscript reports that bilateral dorsal striatal lesions impair the normal distribution of responses when rats are required to make a choice between an immediate but small reward vs. a larger but delayed reward, assessed in the operant delayed reinforcement task. Bilateral striatal lesions were made by stereotaxic injection of the excitotoxin quinolinic acid, since

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this lesion reproduces well the selective degeneration of medium spiny population of striatal projection neurons seen in the human disease [1,22], and is more reliable and reproducible than alternative metabolic toxins for initial studies of striatal dysfunction. Once stable delayed response performance was re-established following lesion, the effects on performance of low-to-medium doses of amphetamine were assessed in order to probe the extent to which lesion deficits and recovery were dependent upon activation and reward-related processes mediated by dopamine pathways afferent to the striatum [8,12,21,24].

2. Methods

2.1. Subjects

Twenty-four male rats of the hooded Lister strain were housed in groups of 4 rats per cage in a light and temperature controlled animal house environment. Water was available *ad libitum*. During all periods of behavioural testing, the animals were subject to a food restriction schedule, fed an average of 10 g chow per day following completion of the daily tests so as to maintain approx. 90% of free-feeding body weight. All experiments were conducted subject to local ethical review, licences and inspection under the UK Animals (Scientific Procedures) Act 1986.

2.2. Delayed reinforcement task

Testing was undertaken in a bank of 12 operant chambers under the online control of the BNC Control software (Cambridge Cognition/Campden Instruments, Loughborough, UK). Each operant chamber had two retractable levers, located on either side of a central reinforcement magazine. The magazine was covered by a retractable transparent Perspex panel, and into which could be delivered 45 mg saccharine reward pellets. Panel retractions were monitored to detect rats' nose poke responses into the magazine. The chambers each had a grid floor, a panel light to illuminate the magazine from within, three stimulus lights located above each lever and the magazine, and a central house light. The delayed reinforcement was implemented according to the Cue protocol of Cardinal et al. [8]. Briefly, each trial in the delayed reinforcement task was of 100 s duration, and commenced by illumination of the panel light. Nose poke at the panel resulted in the panel light switched off and presentation of the two levers. As soon as the rat pressed either lever, both levers were retracted. If the rat pressed the left lever, a single pellet was delivered immediately to the magazine and the magazine was illuminated. Alternatively, if the rat pressed the right lever, the stimulus light above the lever was illuminated for the duration of a scheduled delay period (see below); following which the stimulus light was turned off, four reward pellets were delivered to the magazine and the panel light switched on. Collection of the reward was recorded by a panel nose poke; whereupon all lights were then turned off until the end of the trial. Limited holds were applied to the latencies for each response: At trial outset, the animal was required to respond to the illuminated panel presentation within 10 s to trigger lever presentation, failure of which resulted in the panel light being turned off and lever presentation no longer being available on that trial; the house light turned on for 10 s to signal a panel press omission error (type 'E1'). Following lever presentation, the rat was required to make a lever press response within 10 s, failure of which resulted in the levers being retracted and the house light turned on for 10 s to signal a lever press omission error (type 'E2'). If the animal failed to collect the reward within 10 s, a reward collection error (type 'E3') was recorded and the panel light was turned off. On trials in which the animal responded to the right (delayed reinforcement) lever, anticipatory nose pokes at the magazine panel during the cued delay interval were recorded but had no scheduled consequences.

All rats were initially habituated to the food pellets, familiarized with the magazine delivery and panel press operation in the operant chambers, and then trained to lever press for food reward in a simple discrete trial schedule over 3 days, prior to being transferred directly to training on the delayed reinforcement schedule. Each session comprised 60 trials divided into 5 blocks of 12 trials utilizing reinforcement delays of 0, 10, 20, 40 and 60 s, consecutively, in successive blocks. In each block, the first two trials were information trials, in which just the left lever for immediate 1-pellet reward or just the right lever for 4-pellet delayed reinforcement (at the delay scheduled for that block) were presented, in random order. On the subsequent 10 trials of the block both levers were presented, and the rat's choice for the immediate or the delayed reinforcement lever (or a limited hold error of types E1 or E2) was recorded. All rats were trained to a stable high level of performance over 30 test sessions, before receiving experimental surgery.

2.3. Striatal lesions

Animals were returned to free feeding for 1 week prior to receiving bilateral excitotoxic lesions of the neostriatum. The animals were allocated to two equal groups ($n = 12$) matched on choice performance during the last 12 days of the baseline period. Each animal was anaesthetised with 1.5–2% isoflurane in a 2:1 mixture of O_2 and NO and mounted in a Kopf stereotaxic frame with the incisor bar set at −2.3 mm. Lesion animals received stereotaxic infusions of 0.09 M quinolinic acid (Sigma, Poole, Dorset, UK) dissolved in 0.1 M phosphate buffered 0.9% saline (PBS, pH 7.4) delivered via a 30 gauge stainless steel cannula connected via polyethylene tubing attached to a 10 μl glass microsyringe mounted in a microdrive pump. Injections were made of 0.25 μl into each of four sites into each striatum, in two tracks each at two depths at stereotaxic coordinates: $A = 0.4$ and 1.6 mm anterior to bregma, $L = \pm 3.4$ and ± 2.6 mm, respectively, either side of the midline, and $V = 4.0$ and 5.0 mm below dura. Each injection was administered over 1 min with 2 min allowed for diffusion prior to positioning of the next needle track. Control animals received an identical injection of PBS vehicle. At completion of surgery, each animal was injected with 5 ml glucose saline s.c. in the flank and 0.15 ml (5 mg/ml) diazepam i.m. in the hind leg. Paracetemol analgesia was administered via the drinking water (1 g/l) for 48 h after surgery.

Food deprivation was reintroduced 6 weeks after surgery, and after 3 days stabilization, the animals were tested in the delayed reinforcement task for a further 3 weeks (15 days).

2.4. Amphetamine treatment

After the series of drug free trials, the rats were tested under 4 doses of amphetamine (vehicle, 0.5, 1.0 or 1.5 mg/kg d-amphetamine sulphate, Sigma) dissolved in 1 ml/kg 0.9% saline, and injected i.p., 20 min prior to the test session. Each drug test was administered on separate days in a Latin square design, repeated three times, with one drug-free test day to restore baseline responding between each drug dose.

2.5. Statistical analysis

The primary dependent variables were responses to the delayed lever (DLR) or the immediate lever (ILR) at each delay, the latencies of responding to the two choice levers (calculated as geometric means), and the incidence of E1, E2, E3 errors, as defined above.

A preference index was calculated at each delay (d) as the preference for responding to the delayed reinforcement lever over the immediate reinforcement lever, as the percentage:

$$\text{preference}, \quad P(d) = 100 \times \frac{\text{DLR} - \text{ILR}}{\text{DLR} + \text{ILR}}\%, \quad \text{for } d = 0, 10, 20, 40 \text{ or } 60\,\text{s}.$$

The (negative) slope of the delay–preference function was calculated from the preference values at each delay (d) by linear regression as a best-fit straight line for each animal, according to the formula:

$$\text{slope} = \frac{\sum_{d=1}^{5} d \times P(d) - 3 \times \sum_{i=1}^{5} P(d)}{10}$$

All behavioural data were analysed by 2-, and 3-factor split plot analyses of variance with Groups (Lesion vs. Control) as the between subjects factor, and Delays (0, 10, 20, 40 and 60 s), Times (Baseline and Weeks post lesion), Sides (Delayed vs. Immediate reward levers) and/or Doses (Baseline, Saline, 0.5, 1.0 and 1.5 mg/kg Amphetamine) as within-subject factors. Analyses were undertaken using the Genstat software (v.13.1, VSN International, Oxford), with Newman–Keuls' and Sidak's tests for multiple post hoc comparisons, as appropriate [19,23].

2.6. Histology

At the completion of behavioural testing, all animals were perfused under deep barbiturate anaesthesia (1 ml i.p. Euthetal, Merial Animal Health, Harlow, UK) and perfused via the ascending aorta with approx. 100 ml PBS followed by approx. 250 ml 1.5% paraformaldehyde in PBS. The brains were removed, immersed for 2 h in fixative and then in 20% sucrose solution until the brains sank. Sections were cut at 40 μm thickness, and collected in 1:6 series for staining with cresyl violet for cell bodies, acetylcholinesterase by the thiocholine method, and immunohistochemically by the biotin–streptavidin reaction (DAKO kit, Ely, UK) with primary antibodies against NeuN (1:5000; Chemicon) and DARPP-32 (1:30,000; the gift of Prof. H. Hemmings).

3. Results

All rats quickly learned to lever press for food pellet reward. Moreover, they quickly learned to press the right lever for the larger rewards, in particular in the first block when the delay was 0 s and hence the choice was effectively between two immediate rewards to responses on a high-reward lever and a low-reward lever. All rats showed an increasing tendency to make some responses to the lower reward left (immediate) lever as the delay intervals increased, with a corresponding decline in responses to the high-reward delayed reinforcement lever even at the longest delays they continued to exhibit a modest preference for the high reward