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Evidence that the effect of 5-HT_2 receptor stimulation on temporal differentiation is not mediated by receptors in the dorsal striatum

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Abstract

5-HT_2 receptor stimulation alters temporal differentiation in free-operant timing schedules. The anatomical location of the receptor population responsible for this effect is unknown. We examined the effect of a 5-HT_2 receptor agonist and antagonists, injected systemically and into the dorsal striatum, a region that is believed to play a major role in interval timing. Rats were trained under the free-operant psychophysical procedure to press levers A and B in 50 s trials in which reinforcement was provided intermittently for responding on A in the first half, and B in the second half of the trial. Percent responding on B (%B) was recorded in successive 5 s epochs of the trials; logistic functions were fitted to the data from each rat to derive timing indices (T_{50}: time corresponding to %B = 50; Weber fraction: $[T_{75} - T_{25}]/2T_{50}$, where T_{75} and T_{25} are the times corresponding to %B = 75 and %B = 25). Systemic treatment with the 5-$HT_{2A/2C}$ receptor agonist 2,5,-dimethoxy-4-iodo-amphetamine (DOI) (0.25 mg/kg, s.c.) reduced T_{50}; the 5-HT_{2A} receptor antagonist MDL-100907 (0.5 mg/kg, i.p.) did not affect performance, but completely blocked the effect of DOI. DOI (1 and 3 μg) injected bilaterally into the dorsal striatum did not alter T_{50}. The effect of systemic treatment with DOI (0.25 mg/kg, s.c.) was not altered by intra-striatal injection of MDL-100907 (0.3 μg) or the 5-HT_{2C} receptor antagonist RS-102221 (0.15 μg). The ability of systemically administered MDL-100907 to reverse DOI's effect on T_{50} confirms the sensitivity of temporal differentiation to 5-HT_{2A} receptor stimulation. The failure of intra-striatal MDL-100907 to antagonize the effects of DOI suggests that 5-HT_{2A} receptors in the dorsal striatum are unlikely to be primarily responsible for DOI's effects on timing. Furthermore, the results provide no evidence for a role of striatal 5-HT_{2C} receptors in DOI's effect on timing.

Keywords: Free-operant psychophysical procedure; Temporal differentiation; 5-HT_2 receptors; Corpus striatum; DOI; MDL-100907; RS-102221

1. Introduction

Two major divisions of interval timing behaviour are temporal discrimination, in which the organism emits discriminative responses depending on the duration of an exteroceptive stimulus, and temporal differentiation, in which the organism's behaviour comes under the control of time during an ongoing interval (Platt, 1979; Richelle and Lejeune, 1980). These two forms of interval timing can be revealed by retrospective and immediate timing schedules, respectively (Killeen and Fetterman, 1988; Killeen et al., 1997). Although performance on most, if not all, known timing schedules probably entails elements of both temporal discrimination and temporal differentiation, the finding that pharmacological challenges can have qualitatively different effects on performance in retrospective and immediate timing schedules lends weight to the notion that there may be important differences between the neural substrates of temporal discrimination and temporal differentiation (see Al-Ruwaitea et al., 1997; Ho et al., 2002).

The experiments described in this paper were concerned with temporal differentiation performance in the free-operant psychophysical procedure (Stubbs, 1976, 1980; Chiang et al., 1998). In this schedule reinforcement is provided intermittently for responding on two levers, A and B; reinforcement is provided for responding on lever A in the first half, and on lever B in the second half of each trial. Temporal differentiation is assessed

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quantitatively from the sigmoid psychometric function relating proportional responding on lever B (%B) to time measured from the onset of the trial. This function is approximately logistic in form, and is characterized by the indifference point, T_{50} (the time as which %B = 50), and a slope parameter, ε; these parameters may be used to derive the Weber fraction, which expresses the precision of temporal differentiation (Killeen et al., 1997).

Performance on the free-operant psychophysical procedure is sensitive to manipulation of central 5-hydroxytryptaminergic (5-HTergic) mechanisms. Chronic central 5-HT depletion has relatively little effect on the principal indices of timing, T_{50} and the Weber fraction (Chiang et al., 1999). However, acute systemic treatment with drugs acting at specific 5-HT receptors can have marked effects on these measures (Body et al., 2001, 2002a,b, 2003, 2004). Thus, stimulation of postsynaptic 5-HT_{1A} receptors induces a leftward shift of the psychometric curve, and hence a reduction of T_{50} (Body et al., 2002b). A similar effect is produced by the 5-HT_2 receptor agonist 2,5-dimethoxy-4-iodoamphetamine (DOI) (Body et al., 2003) and the mixed 5-$HT_{2/3}$ receptor agonist quipazine (Body et al., 2005); the effects of both these agonists can be reversed by the 5-HT_2 receptor antagonist ketanserin (Body et al., 2003, 2005). Since ketanserin is relatively selective for the 5-HT_{2A} receptor (see Barnes and Sharp, 1999; Hoyer et al., 2002), it is likely that the effects of DOI and quipazine are mediated by this receptor subtype. Interestingly, the 5-HT-releasing agent fenfluramine also reduces T_{50}, and this effect can be reversed by ketanserin but not by the selective 5-HT_{1A} receptor antagonist *N*-[2-(4-[2-methoxyphenyl]-1-piperazinyl)ethyl]-*N*-2-pyridinylcyclohexanecarboxamide (WAY-100635), suggesting that the effect of endogenous 5-HT on temporal differentiation may be mediated principally by 5-HT_{2A} receptors (Body et al., 2004).

The anatomical location of the 5-HT_{2A} receptors that mediate these effects on temporal differentiation remains unknown. 5-HT_{2A} receptors are widely distributed in the brain, the densest populations being found in the basal ganglia and cerebral cortex (Barnes and Sharp, 1999; Hoyer et al., 2002; Leysen, 2004). The present experiments examined the possibility that the 5-HT_{2A} receptor population relevant to temporal differentiation may be located in the dorsal striatum. There is a great deal of evidence that the striatum plays a major role in voluntary timing behaviour (see Gibbon et al., 1997; Hinton and Meck, 1997, 2004; Harrington et al., 1998; Meck and Benson, 2001; Ferrandez et al., 2003; Matell et al., 2003; Nenadic et al., 2003; Pastor et al., 2004). The presence of a dense population of 5-HT_{2A} receptors in this structure suggests that this may be an appropriate starting point for a search for the location of the 5-HT_{2A} receptors that mediate effects on temporal differentiation. One aim of the present experiments was to establish whether the effect of systemically administered DOI on temporal differentiation could be attenuated by the highly selective 5-HT_{2A} receptor antagonist MDL-100907 (see Barnes and Sharp, 1999; Hoyer et al., 2002), administered either systemically or directly into the dorsal striatum. Secondly, we examined whether the effect of systemically administered DOI would be reproduced when the agonist was injected directly into the striatum. Thirdly, we examined whether intra-striatal injection of the highly selective 5-HT_{2C} receptor antagonist RS-102221 (Bonhaus et al., 1997) could block DOI's effect on temporal differentiation.

2. Experiment 1: effects of systemic DOI and MDL-100907 treatment

2.1. Methods

The experiment was carried out in accordance with UK Home Office regulations governing experiments on living animals.

2.1.1. Subjects

Twenty female Wistar rats aged approximately 4 months and weighing 250–290 g at the start of the experiment were housed individually under a constant cycle of 12 h light and 12 h darkness (lights on 07.00–19.00 h), and were maintained at 80% of their initial free-feeding body weights by providing a limited amount of standard rodent diet after each experimental session. Tap water was freely available in the home cages.

2.1.2. Apparatus

The rats were trained in operant conditioning chambers (CeNeS Ltd., Cambridge, UK) of internal dimensions 25 cm × 25 cm × 22 cm. One wall of chamber contained a recess fitted with a hinged Perspex flap, into which a peristaltic pump could dispense the liquid reinforcer (0.6 M sucrose solution). Apertures were situated 2.5 cm above the floor and 2.5 cm on either side of the recess; a motor-driven retractable lever could be inserted into the chamber through each aperture. Each lever could be depressed by a force of approximately 0.2 N. The chamber was housed in a sound-attenuating chest; masking noise was provided by a rotary fan. A microcomputer (CeNeS Ltd., Cambridge, UK) programmed in Arachnid BASIC, and located in an adjoining room, controlled the schedules and recorded the behavioural data.

2.1.3. Behavioural training

At the start of the experiment, the food-deprivation regimen commenced and the rats were gradually reduced to 80% of their free-feeding body weights. They were then trained to press the levers, and were exposed to a discrete-trials continuous reinforcement schedule, in which the two levers were presented in a random sequence, for three sessions. Thereafter, the rats underwent 50 min training sessions under the free-operant psychophysical procedure, 7 days a week, at the same time each day during the light phase of the daily cycle (between 08.00 and 13.00 h). The reinforcer, a 0.6 M solution of sucrose in distilled water, was prepared daily before each session.

The free-operant psychophysical procedure was similar to that used by Bizo and White (1994a,b). Each session consisted of fifty 50 s trials, successive trials being separated by 10 s intertrial intervals. In 40 of the 50 trials reinforcement was provided on a constant-probability variable-interval 30 s schedule (Catania and Reynolds, 1968). The same schedule ran continuously throughout the session, apart from interruptions during intertrial intervals and probe trials (see below). The levers were