

Brief Report

# Inhibiting effect of D1, but not D2 antagonist administered to the striatum on retention of passive avoidance in the chick

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## Abstract

The avian lobus parolfactorius, equivalent to the medial striatum (caudate-putamen) of mammals, has been shown to be of crucial importance in passive avoidance training in day-old domestic chicks, where the aversive stimulus is the bitter tasting substance methylanthranilate. Here we report that the specific D1 antagonist SCH23390, injected into the lobus parolfactorius of day-old chicks (*Gallus domesticus*) prior to training, impaired performance on testing 30 min post-training at low doses (0.5 and 25 nmol). Sulpiride, a D2 antagonist, had no significant effect on performance in comparable doses. The early D1 activation may signify an essential mechanism leading to storage itself or to the canalisation of the relevant association to a permanent store. © 2003 Elsevier Inc. All rights reserved.

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## 1. Introduction

The dopaminergic system has been implicated in learning new tasks. Striatal dopamine is thought to be involved in inducing selective attention in mammals to novel, or task specific stimuli (Apicella, Legallet, Nieoullon, & Trouche, 1991; Williams, Rolls, Leonard, & Stern, 1993), in reward evaluation (Apicella et al., 1991), reward prediction (Aosaki et al., 1994; Garris et al., 1999), as well as reward dependent synapse potentiation (Reynolds, Hyland, & Wickens, 2001). The dopamine system has also been implicated in the evaluation of aversive stimuli in the basal ganglia of mammals (e.g., Wilkinson et al., 1998).

Memory, as tested by lasting modification of behaviour on experience comprises both the evaluation of stimulus input and the selection of relevant behavioural output (Gurney, Prescott, & Redgrave, 2001). As the dopaminergic system of basal ganglia is considered to affect both functions, it is difficult to tell whether it acts

only as an input and output filter for a separate memory system, or it is specific for the modulation of the coding of learned responses.

Less is known of the role of dopamine in aversive learning in the avian basal ganglia. Since a homology with the corresponding mammalian structures has been established (Reiner, 2002), the avian system might provide a useful comparison in the quest for a functional model. In the present study, we applied one trial passive avoidance training of young chicks (Cherkin, 1969), which exploits an adaptive stereotypic behaviour that involves pecking at conspicuous small objects. In laboratory conditions pecking can be elicited repeatedly by the presentation of a small shiny bead and this stimulus can be made aversive by coating the bead with a bitter tasting substance, methyl anthranilate (MeA). A single peck at the aversive stimulus normally elicits a characteristic disgust response, 80–90% of the trained chicks avoiding a similar but dry bead 1 h after training.

This learning process comprises an initial non-learned pecking response, and the suppression of pecking to specific stimuli, following an aversive experience. Non-learned pecking behaviour is controlled

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by subtelencephalic regions (Kabai, Liker, & Csillag, 1999), whereas learned suppression requires telencephalic centres: the medial striatum known as lobus parolfactorius (LPO) and the intermediate medial part of hyperstriatum ventrale (IMHV) (Rose, 2000).

In our earlier study, learned suppression of pecking was associated with an elevated dopaminergic activity in the telencephalon (Stewart et al., 1996). We found a highly significant increase in binding to D1 (but not D2) receptors in the LPO of day-old chicks 30 min after passive avoidance training.

In view of these findings, the aim of the present study was to examine whether specific dopaminergic agents, D1 or D2 antagonists, SCH23390 or sulpiride, respectively, injected into the LPO of day-old chicks (*Gallus domesticus*) prior to training, would influence performance in a one trial passive avoidance training task.

For obtaining the experimental animals fertile eggs of a commercial strain (Ross Chunky) were incubated in a communal brooder at 38–40 °C under a 12 h light/12 h dark cycle for 21 days until they hatched (day 0). We followed the passive avoidance training procedure described previously (Stewart et al., 1996). In a series of experiments, groups of 50 chicks were placed in pairs in pens under red light. Chicks were pre-trained by presenting a dry white bead, three times to each pair, to encourage pecking. Individuals not responding more than once to the pre-training stimulus were not used in the training trial. In the training trial, chicks were presented with a 3 mm diameter chrome bead dipped in the bitter-tasting substance MeA. Control chicks were offered a similar bead coated with distilled water. The chicks normally peck at the bead spontaneously and indicate that they have tasted the aversive MeA by exhibiting a disgust response, i.e., head shaking, beak wiping, retreat, and eye closure. Those chicks which failed to peck at the bead within 30 s were excluded from the study.

Pharmacological intervention started with intracerebral (i.c.) injections 5–10 min pre-training, using Hamilton micro-syringes fitted with plastic sleeves as stops, and a perspex head holder. The head holder was adjusted to direct injections into the LPO. Prior to the experiments, the location of the injection site was determined using pontamine sky blue. Chicks were given 1 µl i.c. injections (in 0.9% sterile saline) in both right and left hemispheres with a range of dose of the D1 antagonist SCH 23390 (0.05–25 nmol) or the D2 antagonist sulpiride (0.05–200 nmol).

The experiments with different concentrations of SCH23390 and sulpiride were run over a period of several days under similar conditions and randomised to compensate for possible day to day variations. The chicks were tested 30 min after training by presenting a 3 mm diameter chrome dry bead by an experimenter not knowing the chicks treatment history. Those chicks

failing to peck at the test bead within 20 s were considered to have acquired the task. The data were analysed using chi square tests, with a zero hypothesis that the drugs had no effect on memory formation or retention.

All experiments were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals as well as the equivalent regulations currently in force in UK.

Pre-training intracerebral injection of the D1 antagonist SCH23390 potently attenuated avoidance learning at 0.5 and 25 nmol doses. Treatment with the lowest dose (0.05 nmol) had no significant effect on learning (Fig. 1).

In contrast, the D2 antagonist sulpiride had no significant effect on the percentage avoidance in the amounts used for the D1 antagonist. The performance was significantly affected only at and above 100 nmol. Sulpiride showed an apparent, although not significant tendency to attenuate learning in the lowest dose applied (Fig. 1).

It has to be noted that, in the passive avoidance paradigm, poor learning performance is reflected by active pecking on the aversive stimulus. Therefore, treatments attenuating learning are not likely to interfere with motor control.

Pre-training intracerebral injections of the D1 antagonist SCH23390, but not the D2 antagonist sulpiride significantly impaired performance when the birds were tested 30 min post-training. Such effect of D1 antagonist is likely to have resulted from an attenuation of memory formation or recall. Previously we demonstrated that, 30 min post-training, there was a significant bilateral increase in the binding of the D1 antagonist <sup>3</sup>H-SCH23390, but not of the D2 antagonist <sup>3</sup>H-spiperone, in the LPO of chicks (Stewart et al.,

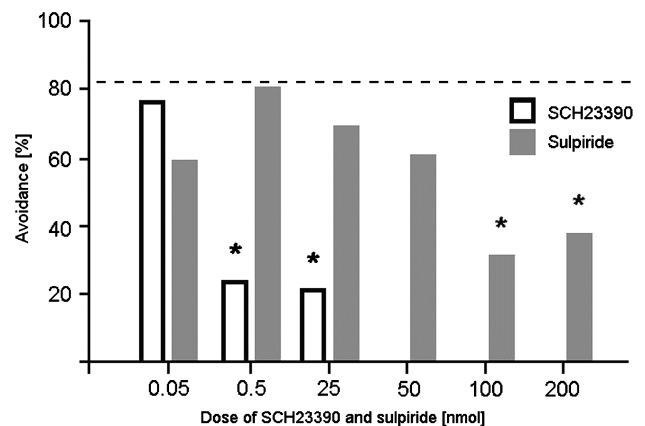


Fig. 1. Effects of SCH23390 (open bars) and sulpiride (shaded bars) on passive avoidance performance. Compared to performance of saline injected birds (dashed line), SCH23390 significantly attenuated inhibition of pecking on the aversive stimulus at low doses, while sulpiride was only effective at extreme doses (asterisks indicate significance at the 0.05 level tested by  $\chi^2$  test,  $n$  = number of animals).

1996). Taken together, these data strongly suggest that there is a D1 dependent process in the early post-training phase of the learning task, with an apparent upregulation of receptors in the LPO.

In the present study, the apparent paradoxical effect of the D2 antagonist at the lowest dose as compared to higher doses might be associated with a preferentially pre-synaptic action of sulpiride at low concentration (Ichihara, Nabeshima, & Kameyama, 1988).

The possibility that the dopamine antagonist interferes with pecking or visual capabilities per se is unlikely. If the antagonist SCH23390 had simply prevented pecking, it would have resulted in apparent 'avoidance' and increased retention, i.e., the opposite to what was observed. In fact, one advantage of the passive avoidance learning paradigm is that it clearly distinguishes any effects on learned pecking from those affecting spontaneous pecking. However, it cannot be ruled out that other aspects of recall or the canalisation of memory to a permanent store were affected.

Concerning the role of striatal dopamine receptors in the passive avoidance learning process of chicks, an important question is whether the level of dopamine alters as a result of memory formation. Given the upregulation of D1 receptor binding, there is no compelling reason to expect such a change. In a previous study, no significant changes in the concentration of dopamine or its metabolites were detected in tissue extracts from the LPO of trained and control chicks (Csillag, 1999) within the 30 min time frame. Consequently, any upregulation of the D1 receptor should be attributed to a post-synaptic effect within the LPO.

The organisation of the avian and mammalian basal ganglia is remarkably similar (Reiner, 2002; Schnabel et al., 1997), and dopamine receptors (both D1 and D2) are present at very high levels in the chick striatum, similarly to mammals. The striato-nigral and striato-tegmental pathways are also similar in birds and mammals (Szekely, Boxer, Stewart, & Csillag, 1994). In mammals, D1, as opposed to D2 receptors are always post-synaptic and associated with SP and ACh neurons (Di Chiara, Morelli, & Consolo, 1994). Functionally, D1 receptors are largely modulatory in the interaction with NMDA receptors. On the one hand, by causing inactivation of the slow K<sup>+</sup> current and removal of the Mg<sup>2+</sup> blockade, they restore NMDA transmission and an active state of the medium-size spiny striatal neurons. Thus, D1 receptors facilitate the NMDA-related burst firing of striatal neurons (Di Chiara et al., 1994) and set the excitability of the striatal output neurons within a range most efficient for burst firing in response to excitatory phasic input. On the other hand, NMDA activation has been demonstrated to selectively recruit D1 receptors to be incorporated from internalised pools to the plasma membrane (Scott et al., 2002). Overall, activation of D1 receptors is likely to be associated with

afferent dopaminergic reward/aversion signals, potentially determining whether memory is formed for the given pairing of experience (e.g., visual image of the bead and adverse taste). Such mechanism has to be reconciled with the report of Hale and Crowe (2002), in which dopaminergic D1, D2, D3, and D4 agonists did not interfere with the early phase of passive avoidance learning in day-old chicks.

The present experiment is relevant to the early phase of the cascade of events leading to lasting memory formation for the avoidance task (Rose, 1995). The early D1 dopaminergic activation may signify an essential mechanism leading to storage itself or to the canalisation of the relevant association to a permanent store.

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