



## THE INVOLVEMENT OF DOPAMINE IN THE STRIATUM IN PASSIVE AVOIDANCE TRAINING IN THE CHICK

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**Abstract**—Quantitative receptor autoradiography was used to investigate the distribution of binding of [<sup>3</sup>H]SCH 23390 to dopamine (D<sub>1</sub>) and [<sup>3</sup>H]spiroperone to D<sub>2</sub> receptors in regions of the forebrain of the one-day-old domestic chick (*Gallus domesticus*). High levels of specific binding of the D<sub>1</sub> and D<sub>2</sub> ligands were found in the striatal regions (paleostriatum augmentatum and lobus parolfactorius) of the one-day-old chick, as reported previously in the pigeon, turtle and rat, whilst binding levels were considerably lower in the pallidum (paleostriatum primitivum), hippocampus and hyperstriatum ventrale. The proportions of D<sub>1</sub> and D<sub>2</sub> receptor binding in the chick were relatively similar in the striatum and pallidum, apart from the paleostriatum augmentatum, where D<sub>2</sub> receptors outnumber those of D<sub>1</sub> by a factor of two. Binding of the D<sub>1</sub> and D<sub>2</sub> ligands to forebrain regions was also investigated 30 min after one-trial passive avoidance training of one-day-old chicks in which the aversive stimulus was a bead coated with a bitter tasting substance, methyl anthranilate. These experiments demonstrated a large and highly significant bilateral increase (compared to control birds) in binding to D<sub>1</sub> (but not D<sub>2</sub>) receptors in the lobus parolfactorius. In this striatal region, equivalent to the caudate-putamen of mammals, previous studies have shown that synaptic and dendritic alterations occur following avoidance training.

It is concluded that alterations in dopamine binding may be involved in processes that result in modification of the pecking response in chicks after avoidance training.

*Key words:* dopamine receptors, learning, chick, forebrain.

One-trial passive avoidance training in the domestic chick (*Gallus domesticus*), which has been employed extensively in our laboratory over the past 15 years, makes use of the ability of newly hatched chicks to quickly learn to distinguish between pleasant and unpleasant tasting substances. If the bead is coated with methyl anthranilate, a bitter tasting substance, the chicks peck once, exhibit a disgust response, and will subsequently avoid a similar but dry bead for several days.<sup>9,31</sup> Biochemical, physiological, pharmacological and morphological changes<sup>11,15,33,38-42,43-53</sup> following avoidance training have been found in two forebrain regions, a medial part of the hyperstriatum ventrale (IMHV; also implicated in filial imprinting)<sup>19</sup> and the lobus parolfactorius (LPO). Short- and intermediate-term neurotransmitter changes (from 30 min to 6 h) elucidated to date include altered binding to *N*-methyl-D-aspartate (NMDA) receptors in the IMHV and LPO.<sup>44-46,53</sup> However, it is only in the LPO

that long-term increases in both synaptic<sup>20,47-52,55</sup> and dendritic spine density<sup>24</sup> are found and, moreover, it is only in the LPO that bilateral post-training lesions cause amnesia for the avoidance response.<sup>18,34</sup> However, it is unclear whether these changes represent a memory store *per se*, or are related to modification of neural circuitry connected with the avoidance task.

Although definitive information is lacking on the role of the LPO, it is known to be a major component of the paleostriatal complex of birds. This is comprised of four regions homologous to the basal ganglia of mammals, the paleostriatum augmentatum (PA), paleostriatum primitivum (PP), nucleus accumbens and the LPO.<sup>36</sup> The PP is equivalent to the globus pallidus (pallidum), whilst the PA and LPO are equivalent to the mammalian striatum.<sup>36</sup> The LPO, with its small cells, has been recognized as the equivalent of the medial striatum (caudate-putamen).<sup>2,16</sup> It is reciprocally connected to two midbrain regions that comprise the avian substantia nigra, the tegmenti pedunculopontinus pars compacta<sup>16</sup> and the area ventralis of Tsai (which is part of the ventral tegmentum<sup>5,6,21,22,57</sup>). As in mammals, preliminary experiments in the pigeon have demonstrated that the highest levels of dopamine receptors occur in the striatum.<sup>37</sup>

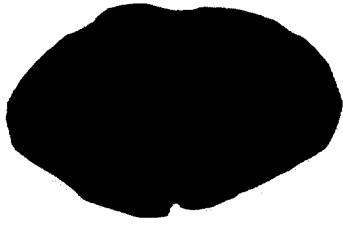
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*Abbreviations:* IMHV, intermediate and medial hyperstriatum ventrale; LPO, lobus parolfactorius (mammalian caudate-putamen); NMDA, *N*-methyl-D-aspartate; PA, paleostriatum augmentatum; PP, paleostriatum primitivum; SP, substance P.

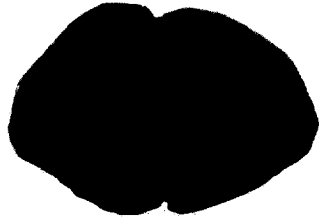
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**D<sub>2</sub>**

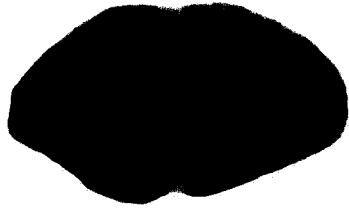
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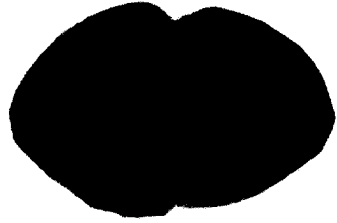
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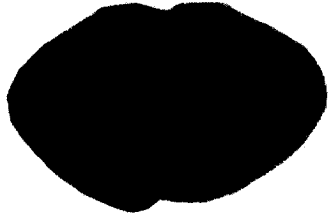
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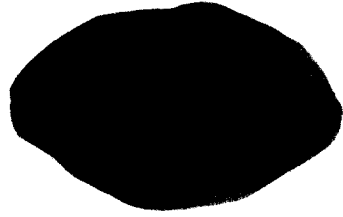
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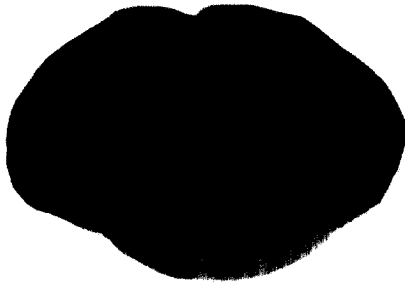
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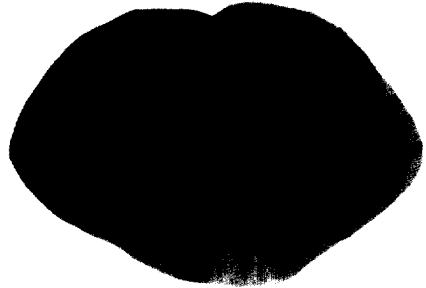
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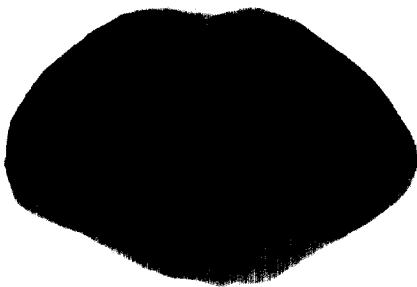
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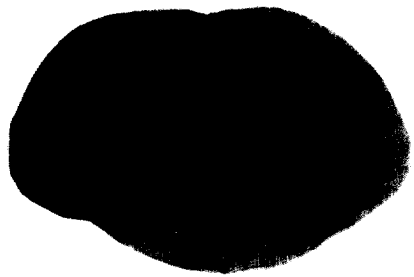
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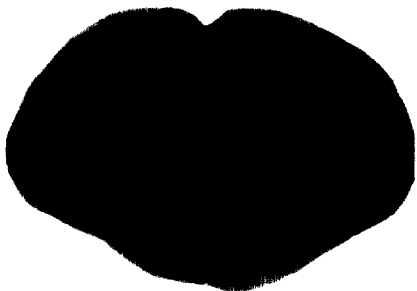
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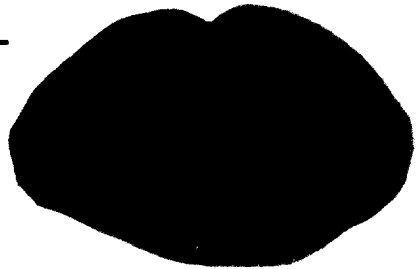
**K**



**F**



**L**



5mm

In mammals,<sup>4</sup> dopamine has been implicated as a modulator of motivational behaviour, and chemical lesions and pharmacological interventions in the dopaminergic system have resulted in various behavioural deficits, including aphagia and akinesia. Dopamine receptors have also been shown to contribute to the maintenance of avoidance responding in mammals.<sup>4</sup> In birds, the striatum (PA and LPO) plays an important role in stereotyped behaviour, and as dopaminergic mechanisms are involved in the induction of stereotypy,<sup>13</sup> and in the control of posture and motor activity,<sup>32</sup> it might be suggested that at least part of its involvement in passive avoidance training is related to modification of the pecking response. Modification of dopamine levels or dopamine binding is therefore likely to be important in this process.

Dopamine receptors are divided into two major subtypes, D<sub>1</sub> and D<sub>2</sub> (D<sub>3</sub>, D<sub>4</sub> and D<sub>5</sub> receptors have been cloned but no selective ligands are available to study their pharmacology in detail). D<sub>1</sub> receptors have a higher affinity for dopamine than D<sub>2</sub> receptors<sup>4</sup> and also have a number of functional differences; D<sub>1</sub> receptor activation enhances neurotransmitter release from striatonigral terminals, whereas D<sub>2</sub> receptor activation inhibits neurotransmitter release from striatopallidal terminals.<sup>56</sup>

The purpose of the present study was to use quantitative receptor autoradiography to investigate changes in the binding to dopamine receptors (D<sub>1</sub> and D<sub>2</sub>) in chick forebrain following passive avoidance training, and to determine whether such changes involved a striatal component, in particular the LPO.

## EXPERIMENTAL PROCEDURES

### Rearing procedure

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).

### Training procedure

The passive avoidance training procedure of Cherkin<sup>9</sup> (as modified by Gibbs and co-workers<sup>31</sup>) has been used in our laboratory for a number of years<sup>7,39,41,47,53</sup> and only a brief description will be given here. Chicks, at 24–30 h post-hatching, were placed in pairs in aluminium pens and pre-trained by three presentations at 5 min intervals of a

small white bead, and then a training trial on a small chrome bead coated with either the aversive substance, methyl anthranilate (trained, M-chicks), or a similar bead coated only with water (control, W-chicks). Chicks were re-tested 30 min later, and only those giving the appropriate response, that is by avoiding if M-trained or pecking if W-control (approximately 85% of chicks), were used for further experimentation.

Immediately after this re-test, chicks were killed by decapitation and the brains removed from the skull, placed in a brain mould<sup>41</sup> and cut into fore- and hindbrain using guide lines in the mould. Forebrains were then frozen in an isopentane/CO<sub>2</sub> mixture, coded and wrapped in foil before being stored at –70°C until required. All subsequent procedures were performed "blind" with the experimenters not knowing the chick's experimental history. Ten-micrometre coronal sections were cut on a Reichert cryostat at –18°C, from two locations of the chick forebrain. These correspond to levels (a) and (b), respectively, in Fig. 1 of Rose and Csillag,<sup>41</sup> and are also shown in Fig. 1a and b of our previous papers.<sup>45,53</sup> These locations were carefully chosen because the anterior section contains the LPO, and the intermediate level section contains the IMHV, both of which have been identified previously as playing important roles in memory acquisition and storage.<sup>40</sup> In addition, the intermediate section also contains another striatal region, the PA, and the avian equivalent of the globus pallidus, the PP. Sections were collected onto poly-L-lysine-coated coverslips, rapidly air-dried and stored air-tight at –20°C. The sections were brought to room temperature, mounted in coverslip racks and preincubated in 2 × 250 ml of 50 mmol/l Tris-HCl buffer (pH 7.7) at room temperature (23°C) for 4 min to remove endogenous dopamine.

The selective radioligand chosen for D<sub>1</sub> receptors was [<sup>3</sup>H]SCH 23390 (specific activity 79 Ci/mmol), used at 1 nmol/l. Specific binding was determined by the difference in binding in the absence and the presence of (i) 40 nmol/l of the D<sub>2</sub> antagonist spiperone, and (ii) 1 μm butaclamol, a dopamine/serotonin-2 antagonist. Thus, we were able to determine the extent of overlap (if any) of [<sup>3</sup>H]SCH 23390 with D<sub>2</sub> receptors. For D<sub>2</sub> receptors, [<sup>3</sup>H]spiperone (specific activity, 123 Ci/mmol) was used at 0.4 nmol/l. Specific binding was determined by the difference in binding in the absence and the presence of (i) 1 μmol/l of the D<sub>2</sub> antagonist sulpiride, and (ii) 1 μm butaclamol, a dopamine/serotonin-2 antagonist. Sections were incubated *in vitro* at room temperature (23°C) in either 300 ml of 50 mmol/l Tris-HCl containing the appropriate ligand, or ligand plus displacers. For D<sub>1</sub> receptors, incubation time was 1 h, and for D<sub>2</sub> incubation time was 30 min. Incubations were terminated by washing the sections three times in 300 ml Tris-HCl, followed by a brief rinse (5 s) in ice-cold distilled water to remove buffer salts, and then the sections were rapidly air-dried. The processed sections on coverslips were glued onto card, apposed to LKB <sup>3</sup>H-Ultrofilm secured between aluminium sheets and exposed in the dark for up

Fig. 1. Tritium film autoradiograms of [<sup>3</sup>H]SCH 23390 binding to D<sub>1</sub> receptors and [<sup>3</sup>H]spiperone binding to D<sub>2</sub> receptors in coronal sections at two levels of the forebrain of day old chicks. A–C and G–I are from the anterior forebrain and D–F and J–L are from the intermediate part of the forebrain (see Stewart *et al.*<sup>53</sup> for detailed description of the location of these sections). For D<sub>1</sub> receptors, specific [<sup>3</sup>H]SCH 23390 binding was determined by the difference in binding in the absence and the presence of (i) 40 nmol/l of the D<sub>2</sub> antagonist spiperone, and (ii) 1 μm butaclamol, a dopamine/serotonin-2 antagonist. A and D show total binding and B and E show binding in the presence of spiperone. C and F show binding in the presence of butaclamol. [<sup>3</sup>H]SCH 23390 binding is affected little by the presence of the D<sub>2</sub> antagonist spiperone, but is displaced almost entirely by butaclamol. G and J show total [<sup>3</sup>H]spiperone binding to D<sub>2</sub> receptors and H and K binding in the presence of the D<sub>2</sub> antagonist sulpiride; I and L show binding in the presence of butaclamol. [<sup>3</sup>H]spiperone at 0.4 nmol/l. Specific binding was determined by the difference in binding in the absence and presence of (i) 1 μmol/l of the D<sub>2</sub> antagonist sulpiride, and (ii) 1 μm butaclamol, a dopamine/serotonin-2 antagonist. Maximal binding levels are found in the striatal regions. For quantitation of specific binding levels refer to Fig. 2. Hp, hippocampus; see main list for other abbreviations. Scale bar = 5 mm.

to 70 days. The films were developed in Agfa G150 developer for 4 min at 20°C, fixed in Ilford Hypam fixer for 3 min and washed in running water for at least 1 h. Densitometry of the film autoradiograms was performed<sup>54</sup> using the MCID system from Imaging Research (Canada). A series of brain paste standards, prepared according to the method of Unnerstall *et al.*<sup>38</sup> and containing [<sup>3</sup>H]leucine, was exposed to the film with the sections. Five brain regions containing areas of likely interest in memory for the passive avoidance response were measured (both left and right hemispheres separately), in each of the W-control and M-trained birds. These were the LPO, PA, PP, IMHV and hippocampus. As in our previous study, variation in the densities of the autoradiograms of the different brains (both within the trained and the control groups) was standardized in order to permit a comparison of labelling in the different brains. This involved expressing the mean measured value for each region as a percentage of the mean for the whole section in which it was located, multiplied by the mean value for all regions in the brains for each group. In subsequent statistical analysis, these mean standardized values for binding to D<sub>1</sub> and D<sub>2</sub> receptors were used, and values given in figures are expressed as mean ± S.E. For W-chicks there were up to 16 values from the control and 16 from trained chicks. A two-way analysis of variance (ANOVA) was used to analyse all the results. The ANOVA design considered control vs training as one factor, and hemisphere (right vs left) as another. Differences were considered to be significant at values of  $P < 0.05$ .

## RESULTS

### *Specificity of binding of [<sup>3</sup>H]SCH 23390 and [<sup>3</sup>H]spiperone to D<sub>1</sub> and D<sub>2</sub> receptors*

As a description of dopamine receptor binding has not been given before in the domestic chick, and only a brief description was given for pigeon, we present here photographs of the autoradiograms for both D<sub>1</sub> and D<sub>2</sub> binding at the two levels examined in the two-day-old chick (from control birds).

**[<sup>3</sup>H]SCH 23390 binding to D<sub>1</sub> receptors.** Total binding for an anterior section is shown in Fig. 1A, and binding in the presence of spiperone (the D<sub>2</sub> antagonist) in Fig. 1B, whilst binding in the presence of butaclamol (the dopamine/S<sub>2</sub> antagonist) is shown in Fig. 1C. For intermediate level sections, total [<sup>3</sup>H]SCH 23390 binding is shown in Fig. 1D, whilst binding in the presence of spiperone is shown in Fig. 1E, and in the presence of butaclamol in Fig. 1F. [<sup>3</sup>H]SCH 23390 binding is affected little by the presence of the D<sub>2</sub> antagonist spiperone, but is displaced almost entirely by butaclamol.

**[<sup>3</sup>H]Spiperone binding to D<sub>2</sub> receptors.** Total binding to D<sub>2</sub> receptors is shown in Fig. 1G and J for anterior and intermediate level sections, respectively. In the presence of the D<sub>2</sub> antagonist sulpiride (Fig. 1H, K) and butaclamol (Fig. 1I, L), binding is displaced almost entirely.

### *Quantitative analysis of binding of [<sup>3</sup>H]SCH 23390 and [<sup>3</sup>H]spiperone to D<sub>1</sub> and D<sub>2</sub> receptors in forebrain regions*

The pattern of binding to D<sub>1</sub> and D<sub>2</sub> receptors in the forebrain showed a number of similarities. Quantitatively, binding to D<sub>1</sub> and D<sub>2</sub> receptors is markedly

highest in striatal regions (Fig. 2), with that to D<sub>2</sub> receptors in the PA (9.1 fmol/mg protein) higher by a factor of over 2 that for D<sub>1</sub> binding (4.2 fmol/mg protein). Levels of D<sub>1</sub> and D<sub>2</sub> binding (3.8 fmol/mg protein) are similar in the LPO, but binding of each is considerably lower in the PP (1.1 fmol/mg protein) and IMHV (1.9 fmol/mg protein for D<sub>1</sub> and 0.5 fmol/mg protein for D<sub>2</sub>). D<sub>1</sub> binding is very low in the hippocampus (0.4 fmol/mg protein) in comparison with the moderate binding levels to D<sub>2</sub> receptors (2.7 fmol/mg protein).

### *Effect of passive avoidance training on binding to D<sub>1</sub> and D<sub>2</sub> receptors*

An ANOVA of the data for the effects of passive avoidance training on D<sub>1</sub> and D<sub>2</sub> binding showed no significant differences between hemispheres. The data were therefore combined and analysed for differences in regions between trained and control birds. There is a significant increase of over 100% in binding of the D<sub>1</sub> ligand [<sup>3</sup>H]SCH 23390 in the LPO 30 min post-training (Table 1) in trained compared to control birds ( $F_{1,30} = 4.2$ ,  $P < 0.05$ ), but no significant changes in binding of [<sup>3</sup>H]spiperone to D<sub>2</sub> sites. There were no other significant differences in binding to D<sub>1</sub> and D<sub>2</sub> receptors between trained and control birds in the other four forebrain regions examined (despite the apparent increases in binding to D<sub>1</sub> receptors in the PA and PP).

## DISCUSSION

The data presented here show that [<sup>3</sup>H]SCH 23390 and [<sup>3</sup>H]spiperone bind respectively to D<sub>1</sub> and D<sub>2</sub> receptors at high levels to the striatum and pallidum of the two-day-old chick, as reported in the pigeon, turtle and rat.<sup>37</sup> In addition, as in these species, the proportions of D<sub>1</sub> and D<sub>2</sub> receptors in the chick are

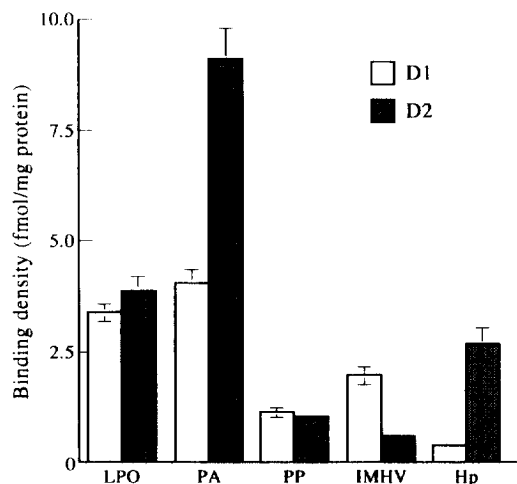


Fig. 2. Specific binding of [<sup>3</sup>H]SCH 23390 to D<sub>1</sub> and [<sup>3</sup>H]spiperone to D<sub>2</sub> receptors in forebrain regions of the chick. Vertical bars represent S.E.M. ( $n = 16$ ). Hp, hippocampus; see main list for other abbreviations.

Table 1. Specific binding (fmol/mg protein) of [<sup>3</sup>H]SCH 23390 to D<sub>1</sub> and [<sup>3</sup>H]spiroperone to D<sub>2</sub> receptors in five forebrain regions of the one-day-old chick 30 min after training on a one-trial passive avoidance task, and in control birds

	D <sub>1</sub> binding		D <sub>2</sub> binding	
	Trained	Control	Trained	Control
LPO*	9.2 ± 1.0*	3.3 ± 0.4*	3.9 ± 0.4	5.1 ± 0.5
PA	8.1 ± 1.3	4.2 ± 0.5	8.2 ± 0.8	9.0 ± 0.8
PP	1.8 ± 0.2	1.1 ± 0.2	1.1 ± 0.2	1.0 ± 0.1
IMHV	2.0 ± 0.2	2.0 ± 0.1	0.6 ± 0.1	0.6 ± 0.1
Hippocampus	0.4 ± 0.1	0.4 ± 0.0	2.6 ± 0.2	2.5 ± 0.2

Of the forebrain regions in which D<sub>1</sub>/D<sub>2</sub> receptor binding changes were measured, significant differences were found only in a part of the striatum of the chick (the LPO\*, equivalent to the mammalian caudate-putamen),  $P < 0.05$ . Data are given for left and right hemispheres combined, as there were no hemispheric differences between control and trained birds, and are expressed as mean ± S.E.M. ( $n = 16$ ).

relatively similar in the striatum and pallidum, apart from the PA, where D<sub>2</sub> receptors outnumber those of D<sub>1</sub> by a factor of two. Because filtration assay experiments using [<sup>3</sup>H]spiroperidol (which binds to D<sub>2</sub> receptors) have shown that there is a large increase (almost two-fold) in the number of receptors from one to 16 days post-hatch in the chick,<sup>28</sup> we cannot determine whether the pattern we have observed is similar in older birds, although the appearance of binding in our autoradiograms is similar to that of the adult pigeon in the study of Richfield *et al.*<sup>37</sup>

In the passive avoidance experiments, the data show a large bilateral increase in binding to D<sub>1</sub> (but not D<sub>2</sub>) receptors in part of the striatum, the LPO. There are also increases in binding in the PP and PA, but these are below the level of significance. The significance to the passive avoidance process of the changes in the striatum, and their pharmacological basis, may be examined in relation to the connections of the LPO. The LPO of the bird is reciprocally connected to the substantia nigra and ventral tegmentum.<sup>5,6,22,57</sup> The descending striatonigral and striatopeduncular pathway contains a projection arising from two main striatal cell types: substance P (SP)/dynorphin-containing neurons and enkephalin-containing neurons, both also co-localizing GABA as neurotransmitter.<sup>1,35</sup> Ascending nigral and ventral tegmental projections constitute the main source of dopamine, a major afferent input to the striatum. The action of dopamine is mediated by specific receptors, of which the best known are the D<sub>1</sub> and D<sub>2</sub> subtypes. According to data from mammals, D<sub>1</sub> receptors are postsynaptic, associated with SP and acetylcholine neurons, whereas D<sub>2</sub> receptors are presynaptic, acting as autoreceptors or heteroreceptors (see review by Di Chiara *et al.*<sup>14</sup>). Functionally, the effect of D<sub>1</sub> receptors is largely modulatory. By inactivation of the slow K<sup>+</sup> current and removal of the Mg<sup>2+</sup> blockade,<sup>10</sup> these receptors restore NMDA transmission (otherwise inactivated) and thereby an active state of the medium-size spiny striatal neurons. By so doing, D<sub>1</sub>

receptors facilitate the NMDA-related burst firing of striatal neurons.<sup>14</sup> An up-regulation of D<sub>1</sub> receptors, as observed in the present study, is likely to be associated with increased burst activity. An elevation of bursting has indeed been observed in the LPO of chicks post-training,<sup>17</sup> although the time scale of changes was different. In the active state of striatal neurons, however, D<sub>1</sub> receptors inactivate the slow Na<sup>+</sup> current, which has a stabilizing effect on the neuronal membrane.<sup>8,14</sup> As a result, D<sub>1</sub> receptors set the excitability of the striatal output neurons "within a range most efficient for burst firing in response to excitatory phasic input".<sup>14</sup>

D<sub>2</sub> receptors, on the other hand, do not seem to act directly on NMDA transmission. These receptors mainly regulate transmitter release from presynaptic terminals in the striatum, including the release of dopamine itself via autoreceptors. Furthermore, both D<sub>1</sub> and D<sub>2</sub> receptors affect acetylcholine transmission in reciprocal fashion.<sup>3,14</sup>

Most striatonigral SP neurons express D<sub>1</sub> receptors, whilst D<sub>2</sub> receptors are typical of striatopallidal enkephalin neurons. Therefore, the observed massive increase in D<sub>1</sub> receptor binding would indicate that the passive avoidance response is associated with the functional state of striatonigral circuitry. It should be noted, however, that a considerable number of striatal neurons were found to contain both types of dopamine receptor.<sup>56</sup>

That the dopamine receptor responses reported here might also be relevant to memory formation associated with avoidance conditioning is supported by earlier reports. Dopamine receptor action was found to retard response suppression learning in young chicks,<sup>29</sup> in apparent contrast with our finding, but receptor binding levels were not measured in the cited study.

Part of the avian LPO corresponds to mammalian nucleus accumbens.<sup>35</sup> Dopaminergic afferents terminating in the nucleus accumbens has been implicated in the reinforcement of behaviour,<sup>4</sup> primarily as positive reinforcers but also as mediators of the avoidance response.<sup>27</sup> The measured differences in receptor binding may have included a component that can be ascribed to the nucleus accumbens-equivalent subdivision, although we did not attempt to refine the regional analysis within the LPO. A further question is whether the dopamine receptor changes reflect increased attention/stress levels in trained birds. Only a time course would tell how long this elevation persists, but increased attention (even stress) might be an integral component of memory formation from aversive experience.

Injections of 6-hydroxydopamine into the ventral tegmental area of rats result in inattention as a consequence of the loss of dopamine fibres in the forebrain.<sup>23,25</sup> The dopamine agonist apomorphine potentially restores attention when injected into the neostriatum, but not other dopamine-recipient regions such as the nucleus accumbens, lateral septum

or frontal cortex.<sup>26</sup> In young, five-day-old chicks, apomorphine has been shown to increase the number of directed pecks and other behaviours relevant to attention rather than fear.<sup>12</sup> However, random pecking was not increased in these experiment, nor was avoidance testing applied. Therefore, the apparent discrepancy between a dopamine agonist enhancing pecking in some experiments and an elevation of D<sub>1</sub> receptors together with the suppression of pecking may be resolved. In fact, it makes it even more likely that the apparent up-regulation of D<sub>1</sub> receptor binding sites in trained chicks is distinct from other dopaminergic behavioural effects, not involving memory formation, since the latter would be expected to result in changes of the opposite direction.

According to the observation of Zolman and McDougall,<sup>60</sup> dopaminergic synaptic transmission as measured by the D<sub>2</sub> antagonist sulpiride is different at one compared with four days of age of chicks. The total number of spiroperidol binding sites in the chick forebrain base (including the striatum) has been

found to rise significantly between one and four days post-hatch,<sup>28</sup> so a developmental element may also be involved in the observed D<sub>1</sub> vs D<sub>2</sub> changes reported in the present study, especially since, in the rat, dopamine D<sub>1</sub> receptors develop anatomically in a quite distinct manner.<sup>30</sup> Therefore, despite our finding that avoidance training involves only alterations in binding to D<sub>1</sub> receptors in the striatum, we cannot rule out the possibility that D<sub>2</sub> receptors may still play a role in aversive conditioning, similar to their involvement in the mediation of response suppression learning observed in rat pups.<sup>29</sup>

*Acknowledgements*—Grateful thanks are expressed for a Royal Society twinning grant to Dr M. G. Stewart, a British Council grant to Dr M. Stewart and Dr M. Kossut, and a grant (OKTI-ETT T-04 369/93) from the Hungarian Ministry of Welfare to Dr Andras Csillag. Thanks also to colleagues in the Open University and Semmelweis University for helpful comments on the manuscript, and Dr Paul Kelly of the University of Edinburgh for advice on dopamine receptor binding.

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(Accepted 8 August 1995)