

Changes in binding to muscarinic and nicotinic cholinergic receptors in the chick telencephalon, following passive avoidance learning

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Abstract

Changes in nicotinic and muscarinic cholinergic receptors 30 min after one-trial passive avoidance training were studied in day-old chicks (*Gallus domesticus*), by quantitative receptor autoradiography. [³H]- α -bungarotoxin (α -BgT) and [³H]-quinuclidinyl benzilate (QNB) were used to monitor changes in 15 forebrain regions for nicotinic and muscarinic receptors, respectively. A significant increase occurred bilaterally in the quantity of bound α -BgT in the lobus parolfactorius, while the amount of bound QNB decreased significantly, and bilaterally, in the hippocampus, hyperstriatum ventrale, lobus parolfactorius and posterolateral telencephalon, pars dorsalis. The data support an involvement of cholinergic receptor types in the neural mechanisms underlying passive avoidance learning. © 1999 Elsevier Science Ireland Ltd. All rights reserved.

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Day-old chicks peck spontaneously at small objects within their field of view. If the object is coated with an unpleasant-tasting substance, the chicks peck once, exhibit a disgust response and subsequently refuse to peck at similar objects. This early learning ability of young chicks, one-trial passive avoidance learning (PAL), has been used as a model to study the cellular correlates of learning and memory formation. As indicated by biochemical, physiological, pharmacological and morphological changes [3,8,11,13, 15], three telencephalic regions are primarily involved in memory acquisition and storage for such task: the medial hyperstriatum ventrale (MHV), lobus parolfactorius (LPO) and paleostriatum augmentatum (PA). Concerning the functional significance of the brain regions affected, the MHV associates the visual stimulus of the bead with the positive, negative or neutral character of its taste. The LPO is critically involved in long-term memory formation during PAL and in the inhibition of natural behavioural responses, such as pecking. The PA has a role in both the facilitation and inhibition of motor responses and along with the archistriatum it regulates the appearance of the fear and escape

response [3]. In the experiment reported here, binding activity was measured in the lateral telencephalon as well, since the involvement of this region in learning would support the recent hypothesis that the avian lateral telencephalon is an equivalent of the mammalian prefrontal cortex [9,17].

The role of acetylcholine (ACh) in memory formation has long been reported in mammals [6]. Some effects of other neurotransmitters (e.g. dopamine) are also mediated by cholinergic mechanisms [5].

Both nicotinic and muscarinic cholinergic receptor types have been mapped in the avian brain [4,12,18,19]. In addition to identification of two muscarinic receptor subtypes in the pigeon [7], three nicotinic receptor subtypes have been described in the chick brain [20], of which the present study deals with the α -bungarotoxin (α -BgT) sensitive subtype only.

The timing of the present study was based on previous observations. Thirty minutes after PAL the binding to D₁ dopamine receptors was found to increase in the LPO [15], whereas binding to delta opioid receptors increased in the LPO and PA, and decreased in the hyperstriatum dorsale and lateral hyperstriatum [3]. *N*-methyl-D-aspartate-sensitive glutamate receptor binding was enhanced in the left LPO and left intermediate and medial hyperstriatum

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ventrale [13]. Thus, it was reasonable to look for changes in acetylcholine receptor binding 30 min after training.

Thirty-one 1-day-old chicks (Hunnia hybrid, both sexes) were used for the experiment. Sixteen animals were trained to peck at water coated chrome beads (control, W-group) and 15 birds were trained to avoid pecking at beads coated with an aversive substance, methyl anthranilate (MeA) (M-group) as described previously [14]. Chicks were tested 30 min later and since all of them gave the appropriate response, i.e. avoiding the bead if M-trained or pecking if W-trained, no chicks were omitted from the receptor binding study.

After the test the chicks were decapitated within 5 min and the forebrains were removed within 1 min, marked and frozen in an isopentane/CO₂ mixture and stored at –25°C until required. Coronal sections (10 µm) were cut at –18°C from two levels of the forebrain, one of which contained the LPO and hyperstriatum ventrale (HV) and the other the archistriatum, HV and thalamus. Twelve sections were cut from each level in each brain, three for muscarinic and three for nicotinic receptor binding assays and three each for determining non-specific binding. Sections were collected onto poly-L-lysine coated coverslips, air-dried and stored at –25°C until analysis. The sections were brought to room temperature, mounted in coverslip racks and preincubated in 50 mmol/l Tris–HCl buffer (pH 7.4) + 1 mg/ml BSA at room temperature for 30 min to remove endogenous acetylcholine. Muscarinic receptors were labelled with [³H]-QNB (1 nmol/l, specific activity 49 Ci/mmol), while nicotinic receptors were labelled with [³H]-α-BgT (0.5 nmol/l, specific activity 77 Ci/mmol). Incubations with QNB were performed in subdued light. Non-specific binding was determined in sections incubated with atropine sulphate (1 µmol/l) for muscarinic receptors and with cold α-BgT (1 µmol/l) for nicotinic receptors. All sections were incubated for 1 h at 26°C, then rinsed in ice cold PBS buffer 3 × 1 or 3 × 5 min for muscarinic and nicotinic receptors, respectively. The sections were then dipped in ice cold distilled water to remove buffer salts and rapidly air-dried. The processed sections on coverslips were glued onto card and apposed to [³H]-Ultrafilm Amersham secured between aluminium sheets, and exposed in the dark for up to 30 days for muscarinic receptors and 120 days for nicotinic receptors. 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 1 h. A series of brain paste standards containing [³H]-leucine was prepared according to the method of Unnerstall et al. [16] and exposed to the film together with the sections. Densitometry of the film autoradiograms was performed on a Joyce–Loebl Magiscan MD image analysis system. Fifteen forebrain regions were drawn around individually on the computer screen, with the help of a light pen, left and right hemispheres separately in each section. The optical density of respective brain regions was expressed as the average of the entire area measured. A standard curve of optical density vs. radioac-

tivity was generated. Since the radioactivity of the sections with non-specific binding was negligible, these sections were not included in the calculations. Owing to variations in the level of binding between birds (both within the W- and the M-group), the data were transformed in order to permit a realistic comparison between the different conditions. This involved expressing the mean measured value for each region as a percentage of the mean for the hemisphere in which it was located and multiplied by the mean hemispheric value for all brains, in order to restore the original dimension of data. The mean transformed values for each brain were used in the subsequent statistical analysis. A two-way analysis of variance (ANOVA) was performed, in which the design considered control vs. training as one factor and right vs. left hemisphere as another. Differences were considered to be significant at $P < 0.05$.

There were no significant interactions between side and treatment in any of the groups tested.

No significant lateral differences were observed in the amount of bound [³H]-QNB. However, the concentration of bound [³H]-α-BgT was significantly greater in the right hemisphere in the dorsal archistriatum ($P < 0.008$; $F = 8.347$; d.f. = 1) and the paleostriatum primitivum ($P < 0.033$; $F = 5.043$; d.f. = 1) in both the W- and the M-groups.

Bound [³H]-α-BgT was rather evenly distributed among the telencephalic regions, with some local enhancements in the PA and LPO. (Fig. 1) Training induced changes were not detected at α-BgT, with the exception of the LPO, where the amount of bound [³H]-α-BgT was significantly greater in the M-group ($P < 0.0001$; $F = 16.128$; d.f. = 1).

Bound [³H]-QNB was heterogeneously distributed in both the W-group and in the M-group: binding was low in the posterolateral telencephalon pars dorsalis (PLTd), ectostriatum and hippocampus and intermediate in the archistriatum dorsale, archistriatum ventrale, hyperstriatum accessorium, HV, lateral neostriatum, medial neostriatum, paleostriatum primitivum, substance-P field, thalamus and posterolateral telencephalon pars ventralis (PLTv). The highest values were measured in the LPO and PA. (Fig. 1)

Concerning the effects of training, the amount of bound [³H]-QNB was significantly reduced in the M-group, as compared with the W-group, in the following regions: the hippocampus ($P < 0.001$; $F = 25.246$; d.f. = 1), HV ($P < 0.011$; $F = 7.247$; d.f. = 1), LPO ($P < 0.012$; $F = 7.038$; d.f. = 1) and PLTd ($P < 0.004$; $F = 9.446$; d.f. = 1) (Table 1).

Contrary to our expectations based on the results of an earlier study [10], the binding to muscarinic receptors was decreased, rather than increased, in the M-group. This apparent discrepancy might be due to the different methods used in the two experiments. Rose et al. [10] measured receptor binding capacity in homogenized optic lobe and forebrain preparations, while we used receptor autoradiography, which enables the analysis of single brain regions. In addition, in our study the birds were tested for retention

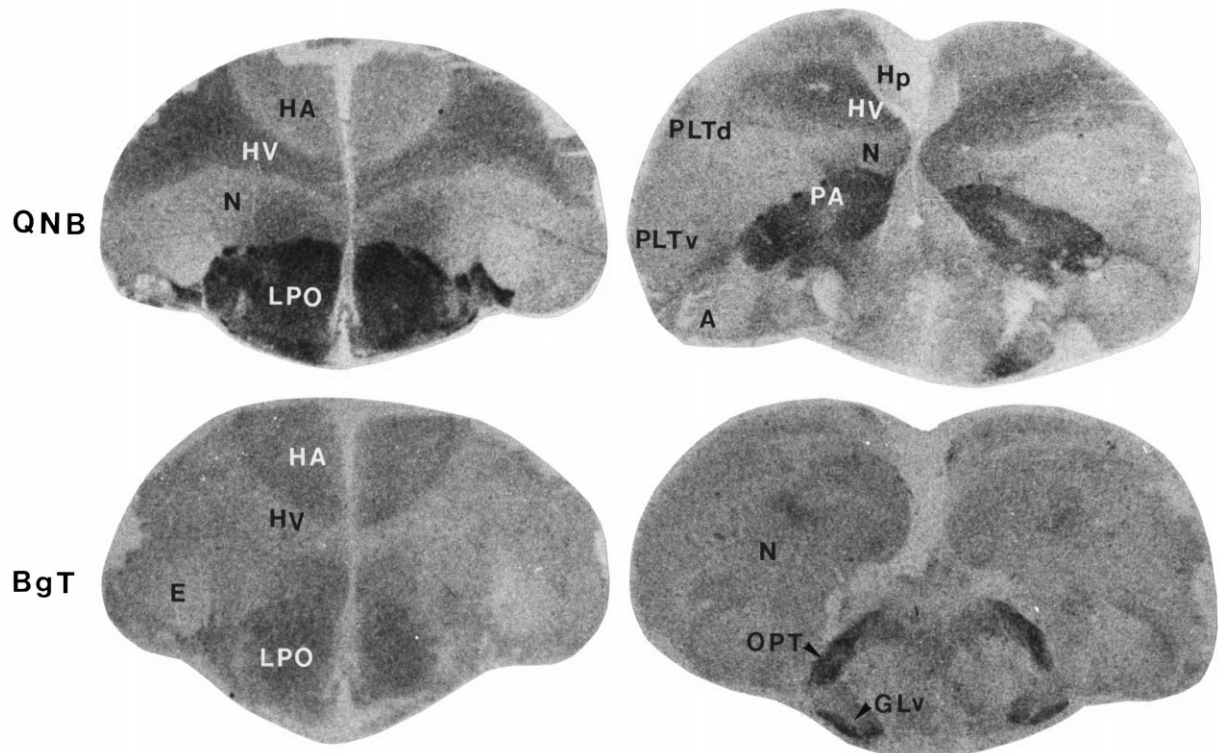


Fig. 1. Original autoradiograms of coronal chick brain sections from the W-group (control), taken at section planes (A) 11.2 – 11.6 (images on the left) and (A) 7.5 – 8.0 (images on the right), following the binding of [3 H]-quinuclidinyl benzilate (QNB) or [3 H]- α -bungarotoxin (BgT). A, archistriatum; E, ectostriatum; GLv, nucl. geniculatus lateralis. pars ventralis; HA, hyperstriatum accessorium; Hp, hippocampus; HV, hyperstriatum ventrale; LPO, lobus parolfactorius; N, neostriatum; OPT, optic thalamic complex; PA, paleostriatum augmentatum; PLTd, posterolateral telencephalon pars dorsalis; PLTv, posterolateral telencephalon pars ventralis.

Table 1

The mean transformed values of total binding of [3 H]-QNB (QNB) and [3 H]- α -BgT (α -BgT) in 15 forebrain regions, in the W-group (control, $n = 16$) and in the M-group (trained, $n = 15$), 30 min after passive avoidance learning, expressed as fmol/mg tissue (mean \pm SE)^a

Region	QNB		α BgT	
	W-group	M-group	W-group	M-group
Archistriatum dorsale	61293 \pm 22690	62992 \pm 16355	5.16 \pm 0.07	5.27 \pm 0.09
Archistriatum ventrale	1647 \pm 250	2041 \pm 261	4.35 \pm 0.04	4.36 \pm 0.04
Ectostriatum	139 \pm 30	117 \pm 15	3.29 \pm 0.21	3.42 \pm 0.32
Hyperstriatum accessorium	1540 \pm 62	776 \pm 59	4.58 \pm 0.04	4.56 \pm 0.04
Hippocampus	589 \pm 62*	310 \pm 21*	3.97 \pm 0.05	3.85 \pm 0.10
Hyperstriatum ventrale	22289 \pm 4375*	7713 \pm 704*	4.63 \pm 0.04	4.50 \pm 0.03
Lateral neostriatum	1162 \pm 411	317 \pm 27	4.54 \pm 0.04	4.28 \pm 0.04
Lobus parolfactorius	831052 \pm 171293*	298898 \pm 46460*	5.52 \pm 0.16*	6.57 \pm 0.14*
Medial neostriatum	7305 \pm 3047	2791 \pm 273	4.90 \pm 0.05	4.98 \pm 0.05
Paleostriatum augmentatum	272415 \pm 6839	77271 \pm 8292	4.76 \pm 0.06	4.76 \pm 0.08
Paleostriatum primitivum	32651 \pm 10313	13565 \pm 3126	3.80 \pm 0.08	3.86 \pm 0.13
Posterolateral telencephalon, pars dorsalis	816 \pm 60*	445 \pm 45*	4.56 \pm 0.05	4.54 \pm 0.04
Posterolateral telencephalon, pars ventralis	1030 \pm 89	644 \pm 96	4.51 \pm 0.05	4.64 \pm 0.06
Substance-P field	2235 \pm 438	3380 \pm 1247	5.64 \pm 1.14	4.30 \pm 0.09
Thalamus	80666 \pm 71969	3472 \pm 510	5.99 \pm 0.52	5.45 \pm 0.12

^a *Significant changes between the W- and the M-group ($P < 0.05$).

before they were sacrificed, whereas this was not the case in each series of experiments in the study of Rose et al. [10]. It should be noted, that in a previous study, muscarinic receptor binding during imprinting did not show a significant difference between overtrained and undertrained birds [1].

Since in the present study we only investigated the amount of bound α -BgT, it cannot be excluded that changes of kappa-BgT sensitive or BgT-insensitive nicotinic receptors also occur during PAL.

ACh has differential effects in the brain: it selectively suppresses intrinsic but not extrinsic (afferent) synaptic transmission. During learning this suppression prevents the interference of incoming new information with older memories, while a decreased cholinergic regulation sets the suitable background for recall [6]. The concentration of ACh decreases in the LPO and PA but not in the MHV 2 and 24 h after PAL [2]. The amount of a transmitter and the affinity of its receptors do not necessarily change directly proportionally. However, according to the previously described role of ACh in learning and recall it can be supposed that during PAL the amount of ACh and the binding affinity of its receptors increase in the chick telencephalon, while during recall they both decrease. It should be noted, that since in the present study the birds were invariably tested prior to sampling, the observed alterations of receptor binding may well have been associated, at least in part, with the mechanism of recall.

It is noteworthy, that a pronounced change of opposite direction in muscarinic and nicotinic receptor binding was observed only in the LPO, a region which has been implicated in the cellular correlates of PAL in the domestic chick [3,8,11,13,15]. The mechanisms underlying such differential effects of cholinergic receptor binding are subject to further investigation.

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