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Research report

# Development of the diencephalic relay structures of the visual thalamofugal system in pigeons

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**Abstract**

To compare the developmental pattern of the visual tecto- and thalamofugal pathways in the altricial pigeon, we examined the posthatch differentiation of the retinothalamic system. Choleratoxin was injected into the left and right eye to visualize the retinal innervation pattern of the lateral geniculate nucleus of the thalamus (GLd). The calcium-binding proteins parvalbumin and calbindin and $GABA_{A\beta}$ receptors were used as indicators for the functional development of the GLd. Although all retinorecipient thalamic target structures were invaded by retinal fibers directly after hatching, density of the projection increased during the first week. While the adult GLd was characterized by a substantial number of cells displaying calbindin-immunoreactivity and by a sparse innervation by parvalbumin-immunoreactive fibers, after hatching no labelling for calcium-binding proteins could be detected. Calbindin-immunoreactivity appeared not before posthatching day 7, while parvalbumin-immunoreactive fibers were detected only after the third week. In contrast, a dense but diffuse $GABA_{A\beta}$ receptor-labelling was present from hatching onwards that decreased during development. The delayed expression of calbindin as well as changes in the density of $GABA_{A\beta}$ receptors indicate that maturation of GLd neurons extends long into the posthatch period. It is likely that the GABAergic interneurons mainly develop within this posthatch timeframe. Combined with the delayed development of the parvalbumin-positive innervation, the developmental pattern of GLd neurons suggests that the thalamofugal networks are immature after hatching and therefore still sensitive to modulations of posthatch visual experience.


*Keywords:* $GABA_A$ receptors; Parvalbumin; Calbindin; Retinofugal; Plasticity

## 1. Introduction

Avian brains possess two main visual pathways ascending to the forebrain: the tecto- and the thalamofugal systems. The tectofugal pathway projects via the contralateral mesencephalic optic tectum and the diencephalic nucleus rotundus to the entopallium in the telencephalon. Within the thalamofugal system, retinal ganglion cells terminate in the contralateral thalamic nucleus geniculatus lateralis, pars dorsalis (GLd), which in turn, projects bilaterally to the visual Wulst [3]. Both systems presumably mature with different developmental speeds [14]. In the chicken, the tectofugal system is already differentiated during embryonic development, while the thalamofugal pathway becomes functional only after hatching. These differential developmental patterns are assumed to be the reason for a differential sensitivity of both systems to modulations of the visual experience [2]. In contrast to the precocious chicken, the altricial pigeon hatches with a highly immature tectofugal pathway whose differentiation is only finished 3 weeks after hatching [8,10] and that is still sensitive to modulations of the visual experience [4,9,13]. Since nothing is known about the development of the pigeon's thalamofugal pathway, we examined the posthatch differentiation of the retinothalamic system by anterograde labelling of retinal fibers combined with immunohistochemical detection of the calcium-binding proteins parvalbumin (PV) and calbindin (CB) as well as of $GABA_{A\beta}$ receptors as indicators for the functional maturation of GLd neurons and compared their developmental pattern with that of tectorecipient cells.

## 2. Materials and methods

The original research reported herein was performed in compliance with the guidelines of the National Institutes of Health

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for the care and use of laboratory animals and were approved by a national committee (North Rhine-Westphalia, Germany). To investigate the developmental pattern of retinal projections onto the GLd, we injected 5–10 μl of choleratoxin subunit B (CtB, 0.1% in 2% DMSO) into both eyes of pigeons at different time points after hatching (posthatching day PH1, PH6, PH21, adult) under ketamine (0.2 mg/10 g bodyweight) and xylazine (0.01 mg/10 g bodyweight) anesthesia. After 24 h survival time, the animals were anesthesized with an overdose of equitesin (0.45 ml/100 g body weight) and were transcardially perfused with icecold 4% paraformaldehyde. The brains were postfixed for 2 h, cryoprotected, embedded into 15% gelatine (in case of PH2, PH7 brains), and cryosectioned in the frontal plane at 40 μm. Sections were collected in six parallel series. Subsequently, an immunohistochemical detection of CtB was performed [8]. Complete labelling of the retinotectal projection served as a control for entire ocular injections. Parallel series were immunolabeled with antibodies against parvalbumin (PV: anti-mouse IgG (Sigma) 1/1000), calbindin (CB: anti-mouse IgG (Swant) 1/1000) or $GABA_{A\beta}$ (anti-mouse IgG (Böhringer) 1/50) according to the ABC-method [10,11].

## 3. Results

At the second day after hatching, all retinorecipient thalamic target structures including the nucleus geniculatus lateralis, pars ventralis (GLv), the nucleus marginalis tractus optici (nMOT), the nucleus lateralis anterior (LA), as well as the retinorecipient substructures of the GLd (nucleus dorsolateralis anterior thalami, pars magnocellularis (DLAmc), nucleus lateralis dorsalis nuclei optici principalis thalami (LdOPT), nucleus dorsolateralis anterior thalami, pars lateralis (DLL), nucleus supraopticus (SpRt)) were invaded by retinal fibers (Fig. 1). Occasionally, fibers running through the nucleus rotundus could be observed (Fig. 1D). These fibers disappeared within the first week after hatching while, in parallel, the density of the retinothalamic innervation, especially within nMOT and SpRt, increased up to adult levels.

Immunolabelling for the calcium-binding proteins calbindin (CB) and parvalbumin (PV) demonstrated a differential labelling pattern within the GLd that differed substantially from that of the tectorecipient nucleus rotundus. Especially DLL was characterized by cells displaying CB-immunoreactivity whereby their density increased from lateral to medial aspects of the DLL (Fig. 2A). Within DLAmc and SpRT, only sparsely distributed CB-immunoreactive cells could be detected, while LdOPT was devoid of any CB-immunoreactivity. The rostrodorsal aspect of the tectorecipient nucleus rotundus, including the nucleus triangularis, also displayed an intensive labelling of CB-immunoreactive fibers and cells (Fig. 2A), and the complete nucleus rotundus was characterized by a dense network of PV-immunoreactive cells and fibers (Fig. 2B). In contrast, GLd subnuclei did not include any PV-immunoreactive cells, but DLL and SpRt were sparsely innervated by PV-immunoreactive

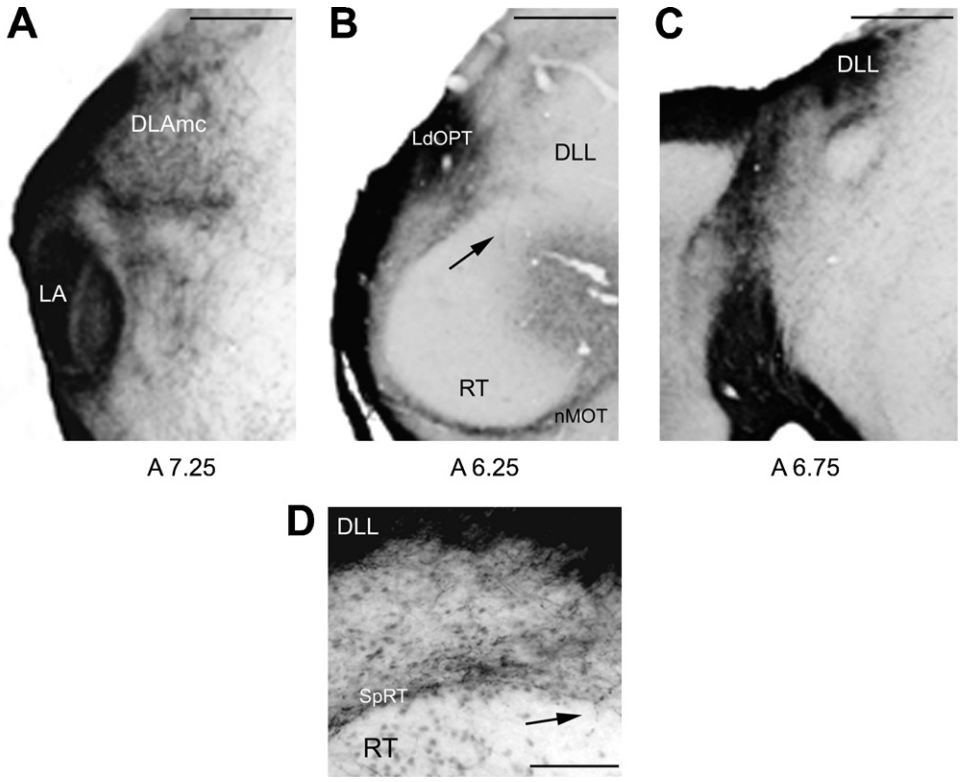


Fig. 1. Retinal innervation pattern of the GLd at PH2; occasionally aberrant fibers could be detected within the rotundus (arrows in B and D). Bars represent 200 μm in A–C, 100 μm in D. DLAmc = nucleus dorsolateralis anterior thalami, pars magnocellularis; DLL = nucleus dorsolateralis thalami, pars lateralis; LA = nucleus lateralis anterior; LdOPT = nucleus lateralis dorsalis nuclei optici principalis thalami; nMOT = nucleus marginalis tractus optici; RT = nucleus rotundus; SpRT = nucleus suprarotundus.

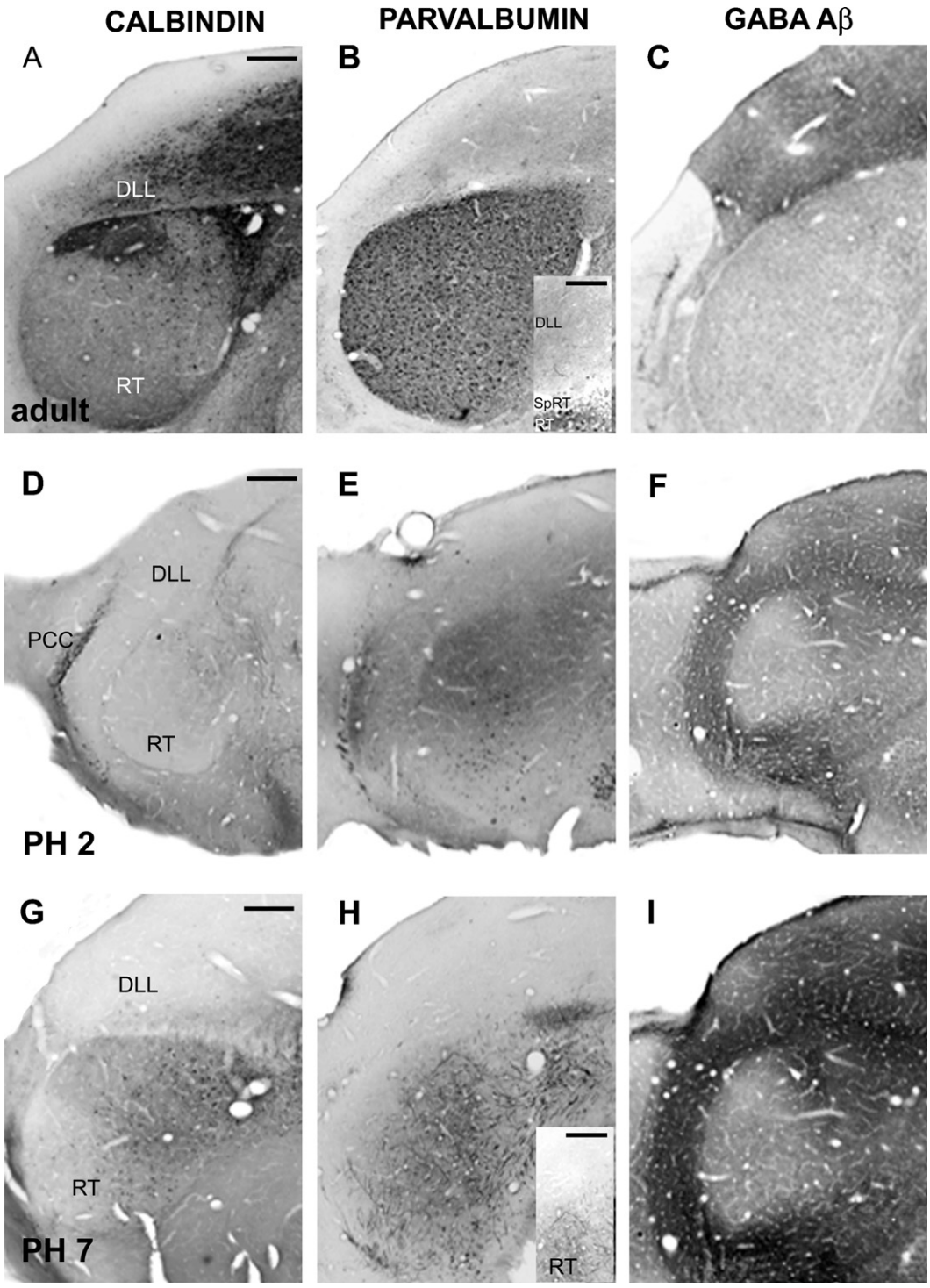


Fig. 2. Distribution of calbindin-, parvalbumin- and GABA $_{A\beta}$-immunoreactivity in adult (A–C), PH2 (D–F), and PH7 (G–I) brains. Bars represent 200 μm in A–I, 100 μm in inserts of B and H. DLL = nucleus dorsolateralis thalami, pars lateralis; PPC = nucleus principalis precommissuralis; RT = nucleus rotundus.

fibers (Fig. 2B insert). Within DLAmc and LdOPT, no PV-immunoreactive elements could be detected at all. Moreover, contrary to the rotundus, the complete GLd was characterized by diffuse $GABA_{A\beta}$ receptor-immunoreactivity (Fig. 2C).

Directly after hatching, an intensive $GABA_{A\beta}$ receptor-immunoreactivity was observed within the complete GLd (Fig. 2F). In contrast, no labelling for calcium-binding proteins could be detected, whereas the presence of CB-immunoreactive cells in, e.g., the pretectal nucleus principalis precommissuralis indicated that calcium-binding protein expression was not generally delayed (Fig. 2D and E). CB-immunolabelling appeared 1 week after hatching within all GLd subnuclei (Fig. 2G). In parallel, the first CB-immunoreactive components were detected within the tectum [11] and the nucleus rotundus (Fig. 2G). More-

over, first PV-immunoreactive fibers emerged within the nucleus rotundus but not within the GLd (Fig. 2H). PV-immunoreactive fibers within DLL and SpRT could only be observed 3 weeks after hatching at a time point when PV-positive cells in the optic tectum were already visible [10]. Parallel to the maturation of calcium-binding-immunoreactive elements within the GLd, the intensity of $GABA_{A\beta}$ receptor-immunoreactivity peaked between PH7 and PH21 and decreased to a moderate level in adult brains (Fig. 2I).

## 4. Discussion

The present study shows that although the geniculate complex is completely innervated by retinal fibers after hatching, the retinothalamic system is not fully differentiated and matures with a developmental speed comparable to tectorecipient elements [8,10].

The CB-immunolabelling pattern within the GLd resembles that of GAD-immunoreactivity, which represents GABAergic interneurons [5]. Since CB is often co-localized with GABA [1], it is very likely that CB-immunoreactivity characterizes the inhibitory subpopulation of GLd components. CB-immunoreactivity is not detectable before 1 week after hatching, although the GLd displays immunolabelling for $GABA_{A\beta}$ receptors from hatching onwards. An early expression of GABA receptors is often observed due to the trophic role of GABA in proliferation, migration, differentiation and synapse maturation [12]. However, since the expression levels of calcium-binding proteins control neuronal $Ca^{2+}$ buffer capacities and hence regulate synaptic functioning [7], the late development of CB-immunoreactivity probably indicates an immature status of CB-expressing neurons. Since retinal input is already present after hatching and probably contacts geniculate neurons at excitatory synapses, local inhibitory connections within GLd presumably differentiate later than excitatory ones. In the mammalian visual cortex, a comparable prolonged maturation of GABAergic subsystems is identified as crucial for ontogenetic plasticity [6]. Accordingly, the delayed CB-expression as a likely marker for the maturation of GABAergic GLd neurons might indicate plasticity-related processes of the thalamofugal system. Therefore, it is conceivable that this system is still sensitive to modulations of the posthatch visual experience as already shown for the tectofugal system [9,13]. Here, it is interesting that the first tectal and rotundal CB-immunoreactive neurons appeared parallel to CB-immunoreactive GLd cells, indicating comparable developmental speeds of CB-immunoreactive cells within both visual pathways.

Moreover, GLd is innervated by PV-immunoreactive fibers that develop within the first 3 weeks after hatching and hence after development of CB-immunoreactivity. PV expression is also delayed in the mammalian visual cortex, and its emergence closely corresponds to the onset of a critical period with a high level of plasticity [6]. Since PV-immunoreactivity within the GLd arises parallel to the development of PV-immunoreactive tectal cells [10] and since the GLd is innervated by the tectum [15], it is likely that the PV-immunostaining labels tectal afferents. In this case, maturation of the thalamofugal system is finished only late in development indicating prolonged plasticity of the tecto–thalamic interactions.

In sum, the present data provide evidence for posthatch functional maturation of thalamofugal subsystems that are regarded as critical for ontogenetic plasticity. Therefore, posthatch modulation of visual experience may affect both thalamo- as well as tecto-fugal pathways.

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