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Electrophysiological and anatomical evidence for a direct projection from the nucleus of the basal optic root to the nucleus rotundus in pigeons

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Abstract

A direct projection of the nucleus of the basal optic root (nBOR) onto the nucleus rotundus (Rt) in the pigeon would link the accessory optic system to the ascending tectofugal pathway and could thus combine self- and object-motion processes. In this study, injections of retrograde tracers into the Rt revealed some cells in central nBOR to project onto the ipsilateral Rt. Contrary, injections into the diencephalic component of the ascending thalamofugal pathway resulted in massive labeling of neurons in dorsal nBOR. Single unit recordings showed that visual nBOR units could be activated by antidromic stimulation through the Rt. Successful collision tests applied to nBOR cells revealed that the connection between nBOR and Rt is direct. These data provide strong evidence for a direct and differential projection of nBOR subcomponents onto the thalamic relays of the two ascending visual pathways.

Keywords: Birds; Accessory optic system; Tectofugal system; Movement analysis

Our visual world is constantly changing. One of the most important challenges created by these alterations of the visual scenery is to distinguish between self- and object-generated visual motion. Self-generated visual motion stimuli arise while moving through the environment resulting in various patterns of optic flow across the entire retina. Contrary, objects which move through our visual field generate local motion patterns which contrast with a large, stationary or homogeneously moving background [2].

The accessory optic system (AOS) constitutes a group of nuclei which are able to analyze various visual signals generated by self-motion or optokinetic stimuli. In birds, the AOS consists of the nucleus of the basal optic root (nBOR) and the nucleus lentiformis mesencephali (nLM). Neurons within the AOS exhibit directional and/or velocity-selectivity in response to very large visual stimuli and seem to encode self-translation and self-rotation [3,14,16,17]. Feature extraction properties which can be found within the AOS seem to be specialized for detecting optokinetic stimuli rich in luminance contrasts, but not for realizing pattern recognition [3,12]. Cells within the thalamofugal and the tectofugal pathways of birds, which ascend to the forebrain, have strikingly different properties. They respond to moving or stationary stimuli which are smaller and they are mostly inhibited by whole-field motion [2,5,10].

The disambiguation of self- and object-motion possibly requires information processing in the AOS and the ascending visual pathways to be combined. Indeed, several recent studies could show that nBOR and nLM project onto the nucleus geniculatus lateralis thalami, pars dorsalis (GLd), the thalamic relay of the thalamofugal system [16]. Contrary, the nucleus rotundus (Rt), the thalamic relay of the tectofugal system, seems to be innervated, if at all, only by a few terminals stemming from the nBOR [15]. These anatomical data would suggest the existence of only a minute projection. However, electrophysiological data draw a completely different picture. Wang et al. [13] showed that lidocaine injections into nBOR could excite or inhibit about half of the Rt-units, while electrical stimulation of nBOR produced a suppression or enhancement of

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rotundal activity. Thus, anatomical and electrophysiological results seem to be discrepant.

It is possible that Wang et al. [13] had observed their strong nBOR effects on rotundal units due to an indirect projection of the nBOR via an unknown further structure. This then would suggest that AOS projections onto the thalamo- and the tectofugal system are differently arranged to an important extent. The present study aims to clarify this question. We used single-unit recordings and anatomical retrograde tracing techniques to identify and analyze a projection from the nBOR onto the Rt in pigeons.

For neuroanatomical experiments, 11 adult pigeons (*Columba livia*) received injections of the tracer Cholera-toxin subunit B (CtB; Sigma) into the Rt. Prior to surgery, the pigeons were anesthetized with equithesin (0.31 ml / 100 g) and a glass micropipette (outer tip diameter 20 μm) mounted onto a mechanical pressure device (Nanoliterinjector, WPI) was stereotaxically inserted into the left Rt (A 5.5–6.9, D 5.0–7.0, L 1.8–3.1, coordinates of the pigeon brain atlas from Karten and Hodos [9]). 30–40 nl CtB (1% (w/v) in distilled water) was slowly injected over 20 min. After 2 days survival time animals received an injection of 200 units sodium heparin, were deeply anesthetized with equithesin (0.55 ml / 100 g body weight) and perfused intracardially with 100 ml 0.9% (w/v) sodium chloride and 800 ml ice-cold 4% paraformaldehyde in 0.12 M phosphate buffer (PB), pH 7.4. Brains were cut in frontal plane at 35 μm on a freezing microtome and the slices were collected in PB containing 0.1% sodium azide (w/v). Brain slices were reacted free-floating with the immuno-ABC-technique as outlined in detail by Hellmann and Güntürkün [8]. The rotundal tracer injection sites and the resulting retrograde CtB-labeling within nBOR were reconstructed using digitized microscopic images and graphics software.

Electrophysiological experiments were conducted on 22 adult pigeons which were anesthetized with 20% urethane (1 ml / 100 g body weight) during surgery and throughout the recordings. The eyelid and nictitating membrane of the right eye was retracted and kept open. Extracellular single unit recordings were obtained from left nBOR using glass electrodes (4–5 μm tip) filled with a solution of 2% Pontamine sky blue in 2.0 M sodium acetate. For electrical stimulation, the ipsilateral Rt was approached stereotaxically via the rostral optic tectum (10° upward; A 5.75–6.75; Fig. 2A). Bipolar tungsten electrodes (20–40 μm tip, 300–500 μm tip separation), insulated with Epoxy resin (exposed tip $<$ 100 μm) were inserted at a depth of 2.0–2.5 mm from the tectal surface.

Neuronal signals were amplified and filtered using conventional techniques. At depths between 9.5–11.0 mm single-unit spikes with a high signal/noise ratio ($>$ 3:1) were isolated and tested with a visual stimulus (black disc; 5 cm diameter) which was presented manually at various velocities and directions. Only if neurons responded to visual stimulation, they were further tested and their spikes were stored on a digital oscilloscope (WaveStar Lite, Tektronix Inc.) or for off-line processing on a computer (EWB, DataWave Technologies), which allowed an off-line isolation of single units by means of spike sorting and cluster cutting (Fig. 2E). Responses to electrical stimulation in Rt using rectangular current pulses of 100–200 μs duration and 100–450 μA intensity were studied. Antidromic responses were identified based on constant spike latency and high frequency (40 Hz) following in response to electrostimulation as well as a collision test. In the collision test, a spontaneous spike recorded from nBOR triggered the electrical stimulus in Rt which elicited the antidromic spike. At a critical delay between spontaneous spike and electrical stimulus, the orthodromic traveling spike collides with and cancels the antidromic spike [4].

For histological verification the recording site of at least one recording site per experiment was marked by current injection of Pontamine sky blue (negative current pulses of 20 μA, 0.5 s duration, 1 Hz frequency, for 15–20 min). The stimulation site was marked by an electrolytic lesion, passing a positive current (40 μA, 0.5 s, 1 Hz, for 10 min). At the end of each experiment, pigeons were anesthetized (equithesin: 0.6 ml / 100 g body weight) and perfused as described. Brains were cut at 40 μm, mounted and counterstained with cresyl violet. Location of marking sites were histologically determined.

The reconstruction of thalamic CtB injection sites revealed that in three animals tracer spread was almost completely restricted to the Rt. Due to the dorsal approach, all other cases exhibited additional CtB spread within the nucleus superficialis parvocellularis and the GLd, which dorsally adjoins Rt. Within cases of sole rotundal tracer spread, retrogradely labeled somata were frequently labeled ipsilaterally within the central and ventral component of rostral nBORp (nBOR proper, A 4.5–5.0), which adjoins the nucleus of the tecto-thalamic tract medially. Most of these cells were round to ovoid shaped with diameters between 9 and 12 μm. In two cases, few additional neurons were filled within nBORd (dorsal nBOR; nomenclature according to Brecha et al. [1]) over its entire rostro-caudal extent (Fig. 1A). Pigeons that received CtB injections into the GLd exhibited a different retrograde labeling pattern with high numbers of CtB filled somata located within nBORd and the dorsally adjoining area ventralis of Tsai (AVT). Only few cells were labeled within nBORp (Fig. 1B).

In the electrophysiological experiments 85 visually responsive cells could be isolated. The present results exclusively focus on 30 of these cells which were located within nBOR and which responded to electrical stimulation of Rt. Histological verification of Pontamine blue spots confirmed recording sites to be within nBORp or the surrounding nBORd (Fig. 2B). Electrolytic lesions showed both tips of the bipolar stimulation electrode to be located in central and rostral Rt (Fig. 2A).

All neurons were spontaneously active with average firing rates of 7.32 ± 1.64 (SEM) spikes/s (range: 0.30–33.5/s) (Fig. 2C). Visual stimulation led to a noticeable

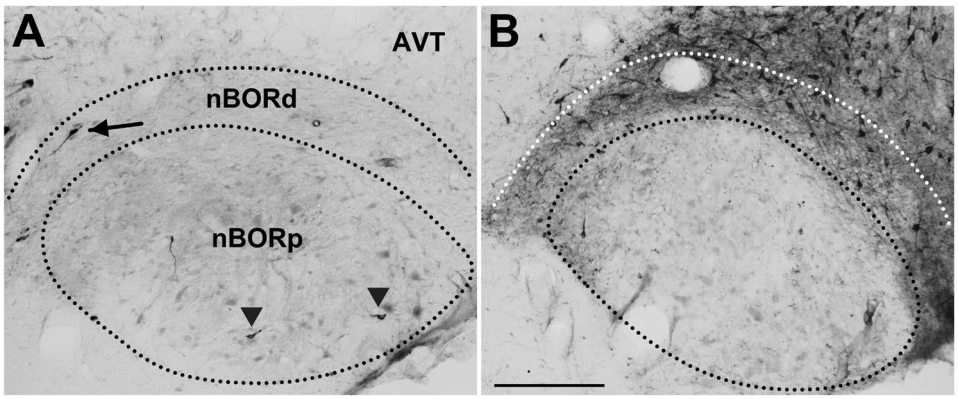

Fig. 1. Retrogradely labeled neurons within the nBOR after CtB injections restricted to the Rt (A) or primary located within GLd (B). (A): Dotted lines indicate the borders of the nBORp and nBORd. Arrowheads point to CtB labeled neurons within the ventral parts of the rostral nBORp (A 4.8). One additional cell is labeled within nBORd (arrow). (B): Retrogradely labeled neurons within nBORd (A 4.9). Somata cluster within its dorsal most component. AVT, area ventralis of Tsai. Medial is to the right and dorsal is upward. Scale bar = 200 μm.

increase in firing rate to an average of 20.79 ± 3.70 spikes/s (range: 7.11–66.40/s) (Fig. 2D). All neurons were activated by Rt-stimulation with short pulses. Action potentials were elicited reliably and with a relatively fixed spike latency of 6.44 ± 0.38 ms (range: 3.18–12.07 ms, except one cell with 23.92 ms) (Fig. 2F). Several cells were also tested for their ability to follow stimulations at a high frequency (40 Hz). On the average, 60.1% of the repetitive Rt-stimulation pulses elicited an antidromic spike at the expected, cell-specific latency.

In eight neurons, we could show a direct link between nBOR and Rt employing a collision test. For these cells, the mean delay between the spontaneous spike and the electrical impulse at which the antidromic spike could be extinguished was between 2.38–9.00 ms (Fig. 2G). When increasing the interval between the spontaneous spike and Rt-stimulation, these neurons responded with an antidromic spike to the stimulation pulse with the same latency that they showed during random stimulation (Fig. 2H).

The present study clearly shows that neurons of the nBOR

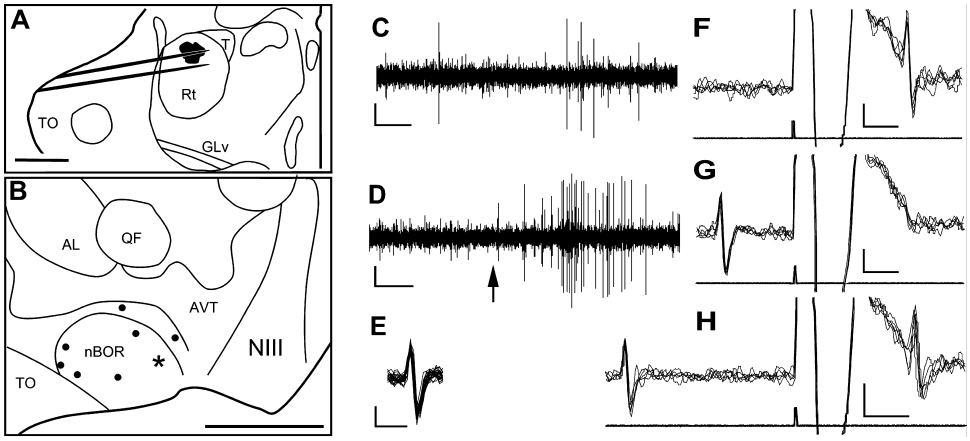

Fig. 2. Schematic diagram of a typical stimulation site within Rt (A) and the location of eight neurons within nBOR (B) which were tested with collision stimuli. Recording sites were marked by Pontamine blue spots. The star indicates the location of the neuron whose responses are shown in (C-H). Spontaneous firing (C) and response during manually applied visual stimulation (D; the arrow indicates the approximate onset of the visual stimulus). Off-line spike sorting was applied to isolate single-unit activity from these recordings (E). Five superimposed responses to antidromic stimulation of Rt show a constant latency of 6.62 ± 0.14 ms (F). The onset of the stimulus in Rt is shown on the lower trace of the recording. Antidromic spikes failed to occur in the collision test, when a stimulus pulse was applied to Rt 3.9 ms after the occurrence of a spontaneous spike (G). Delays of up to 8.0 ms between the spontaneous spike and electrical stimulation within Rt resulted in collision between the spontaneous, orthodromic and elicited, antidromic spike. Electrical stimulation with a delay of 9.1 ms after a spontaneous action potential again elicited an antidromic spike with the expected latency of 6.42 ms (H). AL, ansa lenticularis; AVT, Area ventralis of Tsai; GLv, n. geniculatus lateralis thalami, pars ventralis; NIII, nervus oculomotoris; QF, tractus quintofrontalis; T, n. triangularis; TO, tectum opticum. Scale bars: (A,B) = 1000 μm; (C,D) = 2.0 s × 2.0 mV; (E) = 1.0 ms × 2.0 mV, (F,G,H) = 2.0 ms × 2.0 mV.

directly project onto the Rt of the same hemisphere. In conjunction with studies showing a projection from the nBOR onto the GLd [15,16], our data demonstrate that the AOS is able to modulate directly both visual pathways which project onto the forebrain. It is conceivable that these projections constitute an essential link to distinguish self- and object-motion processed by the AOS and the ascending pathways, respectively.

Our anatomical data demonstrate that the projection of the nBOR onto GLd and Rt are differently organized. While the GLd is reached by a massive projection which mainly arises from nBORd and AVT, the efferents to Rt are weaker and mostly stem from nBORp. The nBORd is known to receive a substantial projection from the contralateral nBORp [1,15]. It is possible that this projection enables binocular interactions necessary to process whole field optical flow patterns. Indeed, Wylie and Frost [14] showed that especially nBORd and AVT cells have spatially separated receptive fields on both sides of the animal, which either code for translational or rotational visual flow. These receptive fields were clearly outside the frontal area of binocular overlap [14]. This is in perfect agreement with the visual field specialization's of the tecto- and the thalamofugal system in pigeons. While the thalamofugal system which ascends via the GLd to the forebrain nearly exclusively represents the lateral visual fields, the tectofugal pathway via the Rt represents both frontal and lateral visual fields [6,7]. Therefore, the differential thalamic projections of the nBOR subcomponents are probably related to diverse modulation properties of lateral and frontal processes. Thus, it is possible that the disambiguation of self- and object-motion is differentially regulated for frontal and lateral field of view.

The direct projection from nBOR onto Rt does not seem to be massive, although more substantial than previously assumed [15]. Since, however, Wang et al. [13] observed that about half of all recorded Rt-units changed their firing properties and their receptive field sizes after stimulation or inhibition of nBOR, this projection seems to have widespread intrarotundal effects. The Rt contains a homogenous network of GABAergic fibers which preferentially synapse on perikarya and proximal dendrites of relay neurons, and thus could exert a massive control of Rt-activity patterns [11]. Some of these GABAergic synapses probably arise from interneurons while others stem from the nucleus spiriformis (SP) and nucleus interstitio-pretecto-subpretectalis (IPS). If the direct projections of the nBOR would contact the intrarotundal GABAergic system, even a small group of terminals could exert a strong effect. Since the nBOR is in addition known to project to the SP/IPS complex, nBOR-activity levels would be able to modulate the tectofugal system by direct as well as by indirect projection.

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