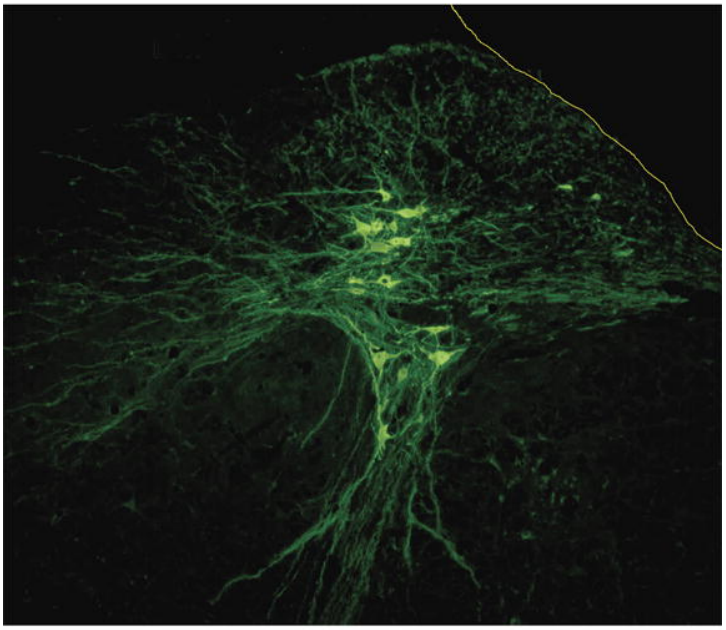
Brain Research



FEBRUARY 9, 2007 | VOLUME 1132 | NUMBER 1
ISSN: 0006-8993

available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Timing of ascending and descending visual signals predicts the response mode of single cells in the thalamic nucleus rotundus of the pigeon (*Columba livia*)

Kristian Folta[a,b,*], *Nikolaus F. Troje*[c], *Onur Güntürkün*[a]

[a]*Institute for Cognitive Neuroscience, Department of Biopsychology, Faculty of Psychology, Ruhr-University Bochum, D-44780 Bochum, Germany*
[b]*Laboratory of Cognitive Neuroscience, German Primate Center, D-37077 Göttingen, Germany*
[c]*Department of Psychology, Queen's University, Kingston, Ontario, Canada K7M 3N6*

ARTICLE INFO

Article history:
Accepted 14 November 2006
Available online 20 December 2006

Keywords:
Bird
Visual system
Rotundus
Tectofugal system
Thalamofugal system
Electrophysiology

ABSTRACT

Neurons of the pigeon's diencephalic n. rotundus were demonstrated to show visual responses of short and long latency representing ascending signals of the retino–tecto–rotundal system and descending signals from telencephalo–tecto–rotundal fibers. Pigeons thus provide an ideal model to investigate the convergence of ascending and descending visual processing streams at single cell level. Although it is known that rotundal responses of long latency show distinct response characteristics, dependent on the stimulus being presented monocularly or binocularly, the mechanisms underlying these response differences are still unclear. While it is possible that the simultaneity of eye stimulation produces a change of processing, it is also possible that the relative timing and order between ipsilateral and contralateral signals are the decisive variable. To test between both possibilities, we recorded from cells in the pigeon's n. rotundus while providing monocular or binocular visual stimulation and varying the delay and order of eye presentations. We revealed that the precise temporal interaction and order of ascending and descending inputs to the tectum decide about late responses with burst or tonic characteristics. When descending signals reached the tectum before the ascending signals, rotundal cells showed late responses that were characterized by burst activity patterns. When ascending input reached the tectum first, responses with tonic characteristic were observed. These effects might become mediated by intratectal mechanisms, the nucleus ventrolateralis thalami, or the bed nuclei of the tectothalamic tract and might constitute the neural basis of a bihemispheric gating function.

1. Introduction

The avian visual system provides an ideal model to investigate the convergence of ascending and descending processing streams at single cell level (Folta et al., 2004; Güntürkün, 2006; Schulte et al., 2006). It also offers an excellent opportunity to analyze the differential cellular integration mode dependent on the relative timing of incoming information from both

* *Corresponding author*. Present address: Laboratory of Cognitive Neuroscience, German Primate Center, D-37077 Göttingen, Germany. Fax: +49 551 3851 452.
E-mail address: kfolta@gwdg.de (K. Folta).

0006-8993/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2006.11.034

streams. In birds, visual information is processed in parallel by the thalamofugal and the tectofugal pathway, the homologues of the mammalian geniculo–cortical and extrageniculo–cortical visual systems, respectively (Fig. 1A; Shimizu and Karten, 1993). The thalamofugal pathway transfers retinal input to the contralateral thalamic nucleus geniculatus lateralis, pars dorsalis (GLd), which projects bilaterally to the visual Wulst of the forebrain (Deng and Rogers, 1998). The tectofugal pathway consists of retinal projections to the contralateral optic tectum (OT), from which fibers lead bilaterally to the thalamic nucleus rotundus (Rt), which then exclusively projects to the ipsilateral entopallium in the forebrain (Engelage and Bischof, 1993; Hellmann and Güntürkün, 1999; terminology according to Reiner et al., 2004). Due to the almost complete decussation of the bird's optic nerves and the limited number of recrossings in ascending pathways, each hemisphere receives information almost exclusively from the contralateral eye.

Visually activated descending telencephalic pathways also reach the tectum (Fig. 1B) and can initiate a second wave of tectal activation (Britto, 1978; Leresche et al., 1983; Dubbeldam et al., 1997; Folta et al., 2004; Schulte et al., 2006). By recording from Rt while using a standardized visual stimulation paradigm of the ipsilateral and contralateral eye, Folta et al. (2004) were able to reveal single visually responsive neurons with responses of short and long response latency. Responses of short latency were attributed to input from the ascending retino–tecto–rotundal system. Those with long latencies probably resulted from input of the telencephalo–tecto–rotundal system (that becomes triggered by the ascending thalamofugal system) since Folta et al. (2004) demonstrated that reversible inactivations of the visual Wulst diminished most of these late responses. Additionally, the authors revealed that rotundal units, which integrate both ascending and descending input, show distinct response characteristics, depending on the visual stimulus being presented monocularly or binocularly. Monocular presentation of a visual stimulus to the contralateral eye produced a short latency response that was probably relayed via the ascending retinotectal projection, started at 30–40 ms, and tapered off at about 80 ms. Ipsilateral visual stimulation, however, produced a brief burst of activity that started after 110–120 ms and was probably relayed via the descending telencephalo–tectal pathway. Binocular stimulation did not produce a mere addition of these response profiles. Instead, a medium latency (about 80 ms), long enduring tonic activity pattern was observed (Folta et al., 2004; see also Fig. 3A). Thus, a simultaneous stimulation of both eyes produced a qualitatively different processing mode at rotundal level. During integration of bilateral visual input within Rt, signals from the ipsilateral eye were shown to become selectively inhibited by GABAergic fibers from a cluster of nuclei, collectively called 'bed nuclei of the tectothalamic tract' (BTT; Mpodozis et al., 1996; Theiss et al., 2003): n. subpretectalis (SP), n. interstitio–pretecto–subpretectalis (IPS), n. subpretectalis–caudalis (SPcd), and n. posteroventralis thalami (PV; see Fig. 4). Detailed electrophysiological analyses of rotundal units revealed that these GABAergic systems not only regulate bilateral integration, but also enable the occurrence of complex computational properties required by a system that processes movement

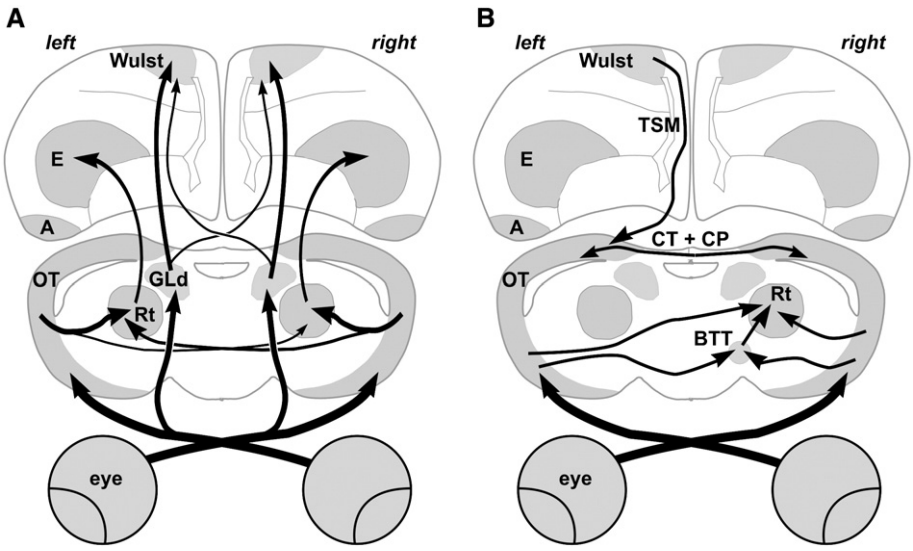


Fig. 1 – Schematic of the ascending tectofugal and thalamofugal visual pathways (A) as well as the descending telencephalotectal and commissural systems (B) which are able to mediate late activations of right-sided rotundal cells. The frontal sections do not represent real anatomical cross-sections, but show structures that are normally not visible within a single plain. Based on evidences outlined in the Discussion section, late rotundal responses become likely mediated by the left tractus septomesencephalicus (TMS) processing from the left Wulst to the left optic tectum (OT). From here projections reach the right OT via the commissura tectalis (CT) and commissura posterior (CP) and, most importantly, via a projection from the left OT to the right nucleus rotundus (Rt). However, rotundal responses become modulated by GABAergic projections of the bed nuclei of the tectothalamic tract (BTT), which integrate input from both tecta and are thus able to regulate the balance between input from both eyes or visual half-fields. Further abbreviations: A: arcopallium; E: entopallium; GLd: n. geniculatus lateralis, pars dorsalis.

analyses and performs detailed feature perceptions based on coarse-coding principles (Gao et al., 1995; Schmidt and Bischof, 2001). While it is possible that the simultaneity of the eye stimulation produces a qualitative change of rotundal processing, it is also possible that it is the relative temporal timing and order between ipsilateral and contralateral signals that are the decisive variables. To test this possibility, we recorded from rotundal cells while varying the temporal delay (0–150 ms) and order between left and right eye stimulation. We show that tonic activity of rotundal cells is not critically dependent on simultaneous visual input from both eyes, but on the precise timing and order of arrival of signals from the ipsilateral and the contralateral eye.

2. Results

We successfully isolated 15 neurons within the right Rt, which showed significant visual responses under monocular and binocular stimulation conditions as compared to spontaneous cell activity (t-tests; $p<0.05$). The location of 'Prussian Blue' marks and the careful reconstruction of the electrode tracks confirmed that all recording sites were located within Rt (Fig. 2). Recorded cell responses were used for calculations of the averaged spike activity and mean values of response latency, response duration, and peak activity strength (Table 1).

In the first experimental session, using brief light flashes, we either stimulated the pigeon's eyes simultaneously or presented the same excitatory visual stimulus to only the ipsilateral or the contralateral eye. Fig. 3A shows the averaged spike activity that results from this stimulation paradigm. Most importantly, we observed early responses with a mean latency of 36 ms after stimulation of the contralateral eye, and a late burst of activity with a mean response latency of 116 ms after visual input to the ipsilateral eye. Simultaneous binocular stimulations generated early and late cell responses (latency of 38 ms vs. 86 ms). However, compared to late monocular responses with burst characteristic, the latter showed reduced latencies (116 ms vs. 86 ms) and were characterized by a tonic activity pattern. For the statistical comparisons of response latency, response duration, and peak activity strength, we calculated two × two ANOVAs with the repeated-measures factors stimulation condition (monocular vs. binocular) and time of response (early vs. late). The analysis of response latencies revealed shorter latencies to binocularly presented stimuli compared to monocular visual stimulations ($F_{(1,14)}=29.192$; $p<0.001$), and shorter latencies of early compared to late responses ($F_{(1,14)}=303.407$; $p<0.001$). Furthermore, we obtained a significant interaction of both factors ($F_{(1,14)}=37.463$; $p<0.001$) that was due to latency differences of late responses after monocular and binocular stimulation conditions. The analysis of response durations revealed no significant difference between monocular and binocular stimulation conditions ($F_{(1,14)}=1.23$; $p>0.05$), but shorter durations of early compared to late responses ($F_{(1,14)}=12.989$; $p<0.01$). The latter effect was attributable to late responses with tonic activity patterns after binocular stimulation compared to patterns of burst activity obtained after monocular stimulation. This was also reflected in a significant interaction of stimulation condition and time of response ($F_{(1,14)}=4.936$; $p<0.05$) that showed comparable durations of early responses after monocular and binocular stimulation, but longer durations of late responses after binocular stimulation of the eyes. The analysis of peak activity strength revealed slightly higher cell activity after monocular compared to binocular stimulation ($F_{(1,14)}=5.0$; $p<0.05$), but no significant difference between early and late responses ($F_{(1,14)}=1.393$; $p>0.05$). A significant interaction of stimulation condition and time of response ($F_{(1,14)}=13.492$; $p<0.01$) was due to comparable peak activity strengths of early and late responses after monocular stimulation, but reduced peak activity strengths of late responses after binocular stimulation of the eyes.

In a second experimental session of this study, stimulus presentation to the contralateral eye was followed by a temporal delay of 50, 100, and 150 ms, before the ipsilateral eye was visually stimulated. Fig. 3B shows the averaged responses of all cells for the different delay conditions of this stimulation paradigm. While the latency of the contralaterally triggered early response stayed constant, the latency of the ipsilaterally triggered late response increased when the temporal delay between contralateral and ipsilateral stimulation of the eye was increased. Since bilateral cell responses of

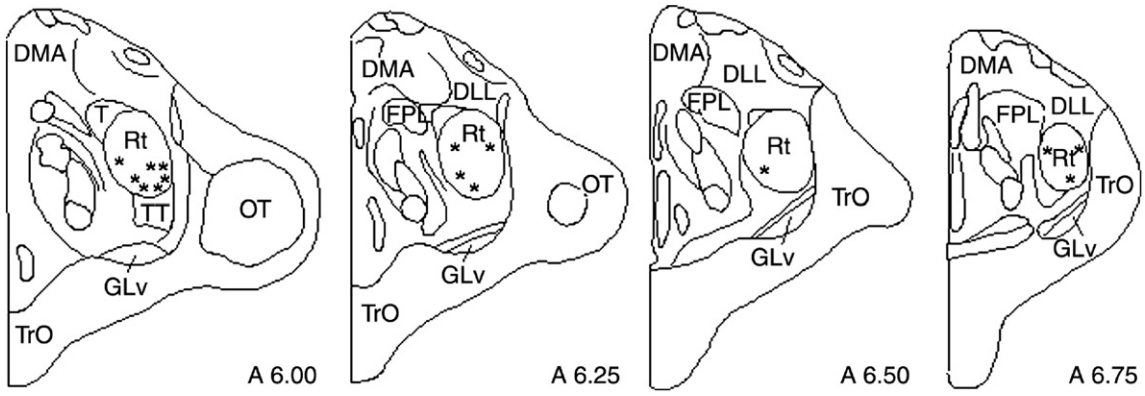


Fig. 2 – Histologically verified recording sites in the nucleus rotundus (Rt). Anterior–posterior levels (A) are according to the atlas by Karten and Hodos (1967). Asterisks indicate the reconstructed recording sites. Abbreviations: DLL: n. dorsolateralis anterior thalami, pars lateralis; DMA: n. dorsomedialis anterior thalami; FPL: fasciculus prosencephali lateralis; GLv: n. geniculatus lateralis, pars ventralis; OT: optic tectum; Rt: n. rotundus; T: n. triangularis; TrO: tractus opticus; TT: tractus tectothalamicus.

Table 1 – Response characteristics of recorded neurons in n. rotundus (Rt)

Response characteristics	Experimental session						
	Session 1: mono- and binocular stimulation			Session 2: contra-before ipsilateral stimulation of the eye		Session 3: ipsi- before contralateral stimulation of the eye	
Spontaneous activity rate (spikes/s)	3.8 (±2.7)			3.3 (±3.0)		3.9 (±3.4)	
Only contralateral visual stimulation							
Latency (ms)	36.0 (±12.0)			–		–	
Duration (ms)	36.0 (±15.3)			–		–	
Peak activity strength (spikes/s)	87.6 (±45.5)			–		–	
Only ipsilateral visual stimulation							
Latency (ms)	116.0 (±7.6)			–		–	
Duration (ms)	38.7 (±27.7)			–		–	
Peak activity strength (spikes/s)	89.0 (±51.4)			–		–	
Contra- and ipsilateral visual stimulation	Delay 0 ms	Delay 50 ms	Delay 100 ms	Delay 150 ms	Delay 50 ms	Delay 100 ms	Delay 150 ms
First response peak							
Latency (ms)	38.0 (±12.4)	37.3 (±11.5)	38.3 (±12.1)	38.0 (±11.6)	78.3 (±3.1)	116.0 (±7.4)	116.0 (±5.7)
Duration (ms)	30.0 (±13.9)	35.3 (±14.2)	33.0 (±14.2)	33.3 (±13.1)	68.7 (±17.5)	61.3 (±20.4)	30.7 (±18.0)
Peak activity strength (spikes/s)	98.5 (±61.5)	112.2 (±88.2)	102.1 (±87.2)	96.4 (±88.8)	105.6 (±58.2)	88.0 (±48.8)	78.3 (±40.5)
Second response peak							
Latency (ms)	86.0 (±18.2)	137.3 (±5.3)	194.3 (±6.8)	242.0 (±5.6)	–	–	189.7 (±14.8)
Duration (ms)	58.7 (±27.2)	57.0 (±17.6)	48.7 (±13.2)	48.0 (±14.9)	–	–	35.7 (±19.1)
Peak activity strength (spikes/s)	56.7 (±31.9)	60.8 (±33.6)	56.9 (±35.3)	58.6 (±33.3)	–	–	93.4 (±60.0)

Values indicate mean (±standard deviation).

our first experimental session represented responses to a delay condition of 0 s, we considered this condition for our analysis and calculated four × two ANOVAs with the repeated-measures factors delay condition (0, 50, 100, 150 ms) and time of response (early vs. late). For reasons of comparison, we subtracted the time differences that were attributable to the temporal delay. The analysis of response latencies revealed no significant main effect of delay condition ($F_{(3,42)}=2.626$; $p>0.05$), but a significant main effect of the factor time of response ($F_{(1,14)}=283.124$; $p<0.001$), and a significant interaction of both factors ($F_{(3,42)}=3.014$; $p<0.05$). Early responses had significantly shorter response latencies than late responses. The significant interaction of the main factors was attributable to very small differences in early responses and can therefore be disregarded. The analysis of response durations revealed no significant main effect of delay condition ($F_{(3,42)}=1.891$; $p>0.05$), but a significant main effect of the factor time of response ($F_{(1,14)}=37.394$; $p<0.001$), and a significant interaction of both factors ($F_{(3,42)}=4.918$; $p<0.01$). Compared to late responses, early responses showed significantly shorter response durations. The significant interaction of the main factors was again attributable to small differences in early responses and can therefore be disregarded. The analysis of peak activity strength revealed no significant main effect of the factors delay condition ($F_{(3,42)}=0.761$; $p>0.05$) and time of response ($F_{(1,14)}=3.857$; $p>0.05$), and no significant interaction of both factors ($F_{(3,42)}=0.337$; $p>0.05$). To summarize, the results of this experimental session indicate that early and late responses differed from each other in their response latency and response duration, but these differences remained constant throughout the different delay conditions. Most important, the tonic response characteristics of late responses were highly comparable in all delay conditions. This clearly shows that binocularly evoked late responses with tonic characteristic are not critically dependent on simultaneous input from both eyes.

Finally, we reversed the order of eye stimulation in a last experimental session. The stimulus presentation to the ipsilateral eye was followed by a temporal delay of 50, 100 and 150 ms before the contralateral eye was stimulated. Fig. 3C shows the averaged responses of cells for the different delay conditions of this stimulation paradigm. Most important, only in the 150 ms delay condition, we observed responses of short and long latency. We subtracted the delay time of 150 ms from the latency of the contralateral response and compared early and late responses of this condition with early and late responses of the first experimental session after monocular stimulation of the ipsilateral or contralateral eye. Two × two ANOVAs with the repeated-measures factors session (first session vs. 150 ms delay condition of the third session) and time of response (early vs. late) revealed no significant main effect of the factor session for the statistical comparisons of response latency ($F_{(1,14)}=0.813$; $p>0.05$), response duration ($F_{(1,14)}=2.76$; $p>0.05$), and peak activity strength ($F_{(1,14)}=0.244$; $p>0.05$). The main effect of the factor time of response became significant for the statistical comparison of response latencies ($F_{(1,14)}=447.335$; $p<0.001$), but not for the comparison of response durations ($F_{(1,14)}=0.025$; $p>0.05$) and peak activity strengths ($F_{(1,14)}=0.146$; $p>0.05$). Early responses to contralaterally presented stimuli were significantly shorter than responses of long latency after ipsilateral eye stimulations. There were no significant interactions of both factors for the comparison of response latencies ($F_{(1,14)}=29.881$; $p>0.05$), response durations ($F_{(1,14)}=1.267$; $p>0.05$) and peak activity

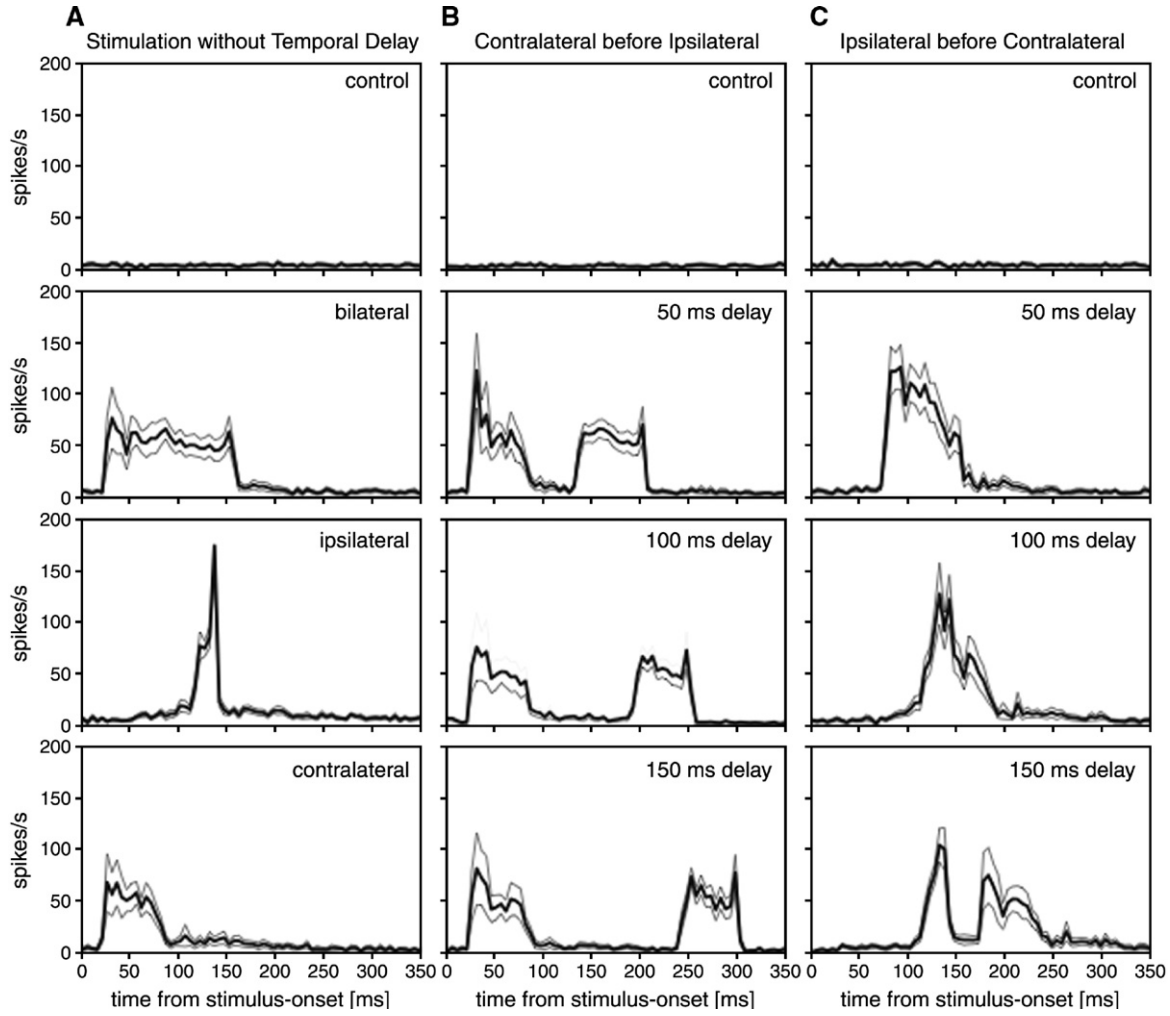


Fig. 3 – Averaged spike activity of rotundal cells with early and late responses under different stimulation conditions: (A) without temporal delay (control without stimulation, binocular stimulation, stimulation of the ipsilateral right eye, stimulation of the contralateral left eye); (B) contralateral left eye becomes stimulated before the ipsilateral right eye; (C) ipsilateral right eye becomes stimulated before the contralateral left eye. In (B) and (C), delays of 50, 100, and 150 ms between the eye stimulations were conducted. Solid thick lines represent the mean spike activity and thin lines the standard error for all bins. Bin width is 5 ms.

strengths ($F_{(1,14)}=2.436$; $p>0.05$). These results clearly indicate that responses observed in the 150 ms delay condition were comparable to late ipsilateral and early contralateral responses of the first experimental session. However, we observed only one response in the 50 ms and 100 ms delay condition. It is highly probable that in these conditions early and late responses merged to a common response. The reduced response latency of about 80 ms for late responses in the 50 ms delay condition indicates that the early contralateral response was combined with a late response with tonic characteristic that we observed after simultaneous bilateral eye stimulation in the first experimental session (without temporal delay). On the other hand, responses with latencies of more than 100 ms, as they were observed in the 100 ms delay condition, indicate a summation of early contralateral and late responses with burst characteristic of the type observed after monocular stimulation of the ipsilateral eye in the first experimental session. To summarize, the response characteristics of rotundal cells were shown to be critically dependent on the temporal delay between each monocular stimulation and the order of eye stimulation. Late tonic activity patterns were independent from simultaneous stimulation of the eyes. All that was required to obtain this kind of response was that contralateral input reached rotundal neurons before the long latency signal arrived. On the other hand, in all conditions in which long latency input reached rotundal cells before an activation by the contralateral visual input, long latency responses were characterized by burst activity patterns.

3. Discussion

The present study shows that single cells in the pigeon's right-sided Rt compute the timing and order of visual stimulation from the ipsilateral or the contralateral eye by altering their response mode from a late, high-frequency response (phasic characteristic) to an earlier tonic firing level, respectively.

Thus, the avian tectofugal system differentiates at single cell level the timing and order of sequences with respect to input from the two eyes.

We confirmed previous observations in the pigeon's diencephalon (Folta et al., 2004) that Rt units are visually responsive with either early and/or late responses. Early responses become activated with latencies of about 30–40 ms, which are compatible to activations via the ascending retino–tecto–rotundal system (Fig. 1A; Letelier et al., 2000; Schmidt and Bischof, 2001; Folta et al., 2004; Schulte et al., 2006). Late responses with latencies of more than 80 ms possibly reflect activity patterns of descending pathways (Fig. 1B; Folta et al., 2004). The two prominent telencephalo–tectal pathways of the pigeon brain are the tractus septomesencephalicus (TSM), originating mainly in the visual Wulst (Miceli et al., 1987), and the tractus occipitomesencephalicus (TOM), originating in the arcopallium (Zeier and Karten, 1971; Dubbeldam et al., 1997). The visual Wulst is the primary telencephalic representation of the thalamofugal system (Güntürkün et al., 1993; Shimizu and Karten, 1993), whereas the arcopallium receives secondary visual input from the tectofugal system (Husband and Shimizu, 1999) and a small projection from the Wulst (Shimizu et al., 1995). Reversible inactivations of the visual Wulst resulted in a decrease of late rotundal responses, whereas early responses were not significantly affected (Folta et al., 2004). This decrease in spiking activity was observed for both late burst and tonic responses, which supports the view that descending signals become mediated by the TSM, and not the TOM. Additionally, a recent quantitative tract tracing study confirmed that the number of arcopallial neurons projecting via TOM to the tectum is minor, compared to the number of cells that project from the visual Wulst via TSM (Manns et al., 2005). We therefore concentrate our discussion on descending projections from the visual Wulst, although we cannot exclude that arcopallial projections to the tectum might have an additional effect.

Descending fibers of the TSM project to cells in deep tectal layers of the OT (Miceli et al., 1987), which are in part the source of diverse descending tectomotor output pathways, but also project to Rt (Hellmann et al., 2004). Tectal neurons of lamina 13 integrate retinotectal input as well as telencephalotectal information and thus constitute a central relay station between ascending tectofugal and descending telencephalo–tectal streams of processing (Bagnoli et al., 1977, 1979, 1980; Leresche et al., 1983; Folta et al., 2004; Schulte et al., 2006). In addition, they serve as an important link between the thalamofugal and tectofugal system. Most likely, the observed activity pattern of rotundal cells reflects these tectal processes of signal convergence. Up to now, it was hypothesized that a binocular tectal input modifies rotundal responses since the very same excitatory stimulus was shown to produce two very different descending signals in Rt, dependent on whether the stimulus was presented monocularly or binocularly. Late responses showed a short burst of firing after monocular stimulation and showed a tonic firing pattern with reduced latencies after binocular stimulation of the eyes. The results of this study clearly indicate that responses with tonic response characteristic are not critically dependent on simultaneous tectal input. Instead, we revealed that the precise temporal interaction and order of ascending and descending inputs to OT determine if responses have a burst or tonic characteristic. In all cases, where descending signals reached the OT before the ascending signals, rotundal cells showed late responses that were characterized by burst activity patterns. On the other hand, when ascending input reached the OT first, responses with tonic characteristic were observed.

Several nuclei, pathways, or modulatory subcircuits could mediate the observed effect. The first possibility is an intratectal mechanism that differentiates between input from the contralateral left and the ipsilateral right eye. The latter input could be transferred via the left GLd-right Wulst-right tectum. However, Folta et al. (2004) could show that activations from the right eye become reduced by injections of lidocaine into the left and not the right Wulst. Thus, this first option is not very likely. The second possibility also involves an intratectal mechanism but assumes that left and right eye inputs are transferred via the mainly inhibitory intertectal commissures (Robert and Cuénod, 1969; Hardy et al., 1984; Keysers et al., 2000). This assumption is, however, also not very likely since the tecto–tecal inhibition is mostly mediated via the substantia nigra, and only few fibers directly constitute an intertectal interaction (Bischof and Niemann, 1990; Hellmann et al., 2004). The third possibility could involve the nucleus ventrolateralis thalami (VLT) since the partly GABAergic VLT output to deep tectal layers requires a bilateral activation of the telencephalo–tecto–VLT system and projections of VLT provide input to the contralateral VLT (Domenici et al., 1988; Schulte et al., 2006) and to tectal layers 11–14 (Hunt and Brecha, 1984) on the ipsilateral side. Thus, VLT neurons not only modulate their counterparts in the contralateral half brain, but also tectal cells. Since VLT receives input from the ipsilateral visual Wulst (Schulte et al., 2006), an inactivation of Wulst neurons might also influence information processing in VLT, OT, and Rt. Unfortunately, information on VLT is very limited. More is known about the BTT, which constitute an essential link of the fourth possibility (Fig. 1B). It was shown that SP, SPcd, IPS and PV (Fig. 4) project to different subdivisions of Rt (Mpodozis et al., 1996), indicating the existence of functional differences among these inhibitory subnuclei. Additionally, SP receives only ipsilateral and PV only contralateral tectal input (Tömböl et al., 1999; Schmidt and Bischof, 2001; Theiss et al., 2003). This indicates that these two structures mediate the balance of input between the information streams representing the two eyes and thus the two visual half-fields within each Rt (Schmidt and Bischof, 2001; Theiss et al., 2003; Voss and Bischof, 2003). If our effects are mediated by the BTT (Fig. 1B), it might be understandable why a binocular stimulation induces the same rotundal activity pattern as the stimulation sequence of 'first left, then right eye' but unlike the 'first right, then left eye' sequence: during binocular stimulation, the left eye input reaches the right Rt and the right BTT faster than the right eye input. It thus involves essentially the same sequence of events as in the 'first left, then right eye' stimulation paradigm. Only the 'first right, then left eye' sequence ensures that right Rt and right BTT are first activated by right eye input. We do not claim that our model (Fig. 1B) gives a complete explanation of all processes involved in information integration from both eyes. Probably, further nuclei and projections may have to be included, e.g. the telencephalo–tecto–VLT system (Schulte

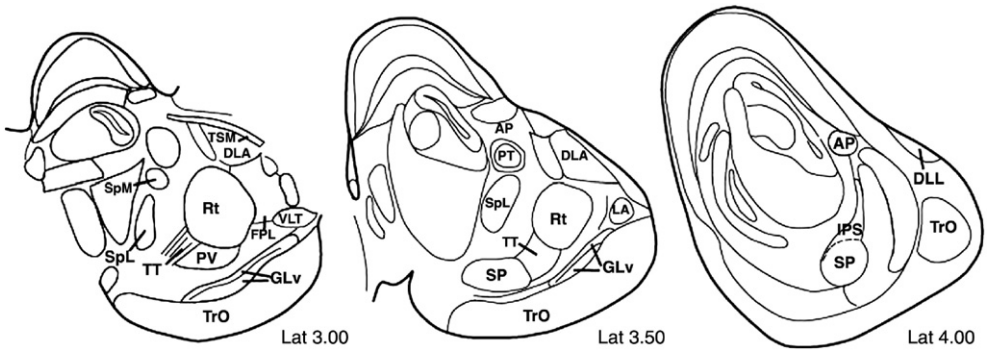


Fig. 4 – Sagittal sections depicting the exact locations of the bed nuclei of the tectothalamic tract (BTT): n. subpretectalis (SP), n. interstitio–pretecto–subpretectalis (IPS), and n. posteroventralis thalami (PV). Not illustrated is the n. subpretectalis–caudalis (SPcd), which lies interposed between the SP and the n. spiriformis lateralis (SpL) and extends along the caudomedial margin of SP proper. In addition to the BTT, n. ventrolateralis thalami (VLT) and n. rotundus (Rt) are depicted. Lateral levels (Lat) are according to the atlas by Karten and Hodos (1967). Further abbreviations: AP: area pretectalis; DLA: n. dorsolateralis anterior thalami; DLL: n. dorsolateralis anterior thalami, pars lateralis; GLv: n. geniculatus lateralis, pars ventralis; FPL: fasciculus prosencephali lateralis; LA: n. lateralis anterior thalami; PT: n. pretectalis; SpM: n. spiriformis medialis; TSM: tractus septomesencephalicus; TrO: tractus opticus; TT: tractus tectothalamicus.

et al., 2006), or GABAergic projections of the reticularis superior thalami (RS) to Rt (Mpodozis et al., 1996).

We can only speculate about the significance of burst and tonic activity patterns. They probably serve different computational functions. Late responses with burst characteristic might act as an effective alarm signal, which in case of sudden appearance of an object in the presently unattended visual hemifield sends an interrupt to the hemisphere which is busy with processing information from the attended side (Voss and Bischof, 2003). This interrupt might allow to direct the attention to the other visual hemifields. Studies on the somatosensory thalamus of awake, behaving rabbits revealed that a burst of spikes is more likely to activate target cells than tonic firing (Swadlow and Gusev, 2001). If late responses with burst characteristic act as an effective signal, they might trigger a head-turning or attention switching to the relevant side. Pigeons that fixate stimuli with their left or their right lateral visual field often subsequently make a head or body movement to this target (Friedman, 1975). Since n. rotundus does not send direct efferent projections to the motor system, rotundal responses with burst characteristic have first to be transmitted to the entopallial system. From there, they might activate cells in defined regions of the arcopallium, which project back to deep layers of the optic tectum (Karten et al., 1993). In the owl, Knudsen et al. (1995) presented evidence that the arcopallium in the forebrain of the barn owl mediates gaze changes independently of the optic tectum and that it projects in parallel to both the optic tectum and to saccade-generating circuitry in the brainstem tegmentum. That means that parts of the arcopallium and the optic tectum have independent access to premotor circuitry for generating head and eye saccades. Although the arcopallium exhibits species-specific anatomical differentiation, the different response modes revealed in our study might be used by such a system. We think that the descending telencephalo-tectal projections via TSM and TOM play a key role in mediating head or even body movements to the left or to the right (Güntürkün and Hoferichter, 1985). In both cases, visuomotor systems in both half brains have to be coordinated. Accordingly, the physiological properties of responses with burst and tonic activity patterns make a bihemispheric gating function possible.

4. Experimental procedures

4.1. *Subjects*

The successful cases of this study totaled seven adult naive homing pigeons (*Columba livia*) of unknown sex, which were obtained from local breeders in Germany. All experiments were performed in accordance with the ECC directive of 24th November 1986 (86/609/EEC) and the specifications of the German Animal Welfare Law for the prevention of cruelty to animals.

4.2. *Surgery and recording*

Prior to surgery and throughout the recordings, each pigeon was anesthetized with 25% urethane (1 ml/100 g, i.m.) and was placed in a stereotaxic headholder. Body temperature was maintained using an electrical heating pad. The brain was exposed at the appropriate stereotaxic coordinates, and an incision was made in the dura mater. The surface of the brain was covered with mineral oil to prevent it from drying. The eyelids were held open with adhesive tape.

Extracellular single cell responses were recorded from the right Rt using single glass coated platinum–iridium electrodes with ~1.0 MΩ resistance. Stereotaxic coordinates for the electrode positions were derived from the atlas of the pigeon brain (Karten and Hodos, 1967). Spikes were amplified ($\times 10^4$) and filtered (0.3–10 kHz). Single-unit spikes with a high signal/noise ratio ($\geq$3:1) were sampled at 9600 Hz and were isolated with the aid of the window discriminator of the acquisition program 'Experimenter's Workbench' (EWB, DataWave Technologies 1993–1995). Off-line spike-sorting- and cluster-

cutting routines allowed the sorting of neuronal responses according to shape and amplitude.

At the end of each experimental session, the position of the last electrode and the inner and outermost borders of all recording sites were marked by inserting a metal electrode and applying a small electrical current for a 'Prussian Blue' reaction (Green, 1958; Fung et al., 1998). Afterwards, the animal was perfused transcardially with 100 ml 0.9% (w/v) sodium chloride followed by 800 ml ice-cold 4% paraformaldehyde plus 15% potassium ferricyanide (for the 'Prussian Blue' reaction) in 0.12 M phosphate buffer, pH 7.4. After the perfusion, the brain was removed from the skull and was postfixed overnight in a 4% solution of paraformaldehyde plus 30% sucrose and 15% potassium ferricyanide. Next, it was cryoprotected for 24 h with 30% sucrose in 0.12 M phosphate buffer. The brains were sagittally cut at 50 μm on a freezing microtome, and the brain sections were processed with standard histological methods. The Nissl stained sections and the lesion marks from the Prussian Blue reaction served for verification of the electrode tracks, which were reconstructed according to the Karten and Hodos (1967) atlas of the pigeon brain. The coordinates of all electrode penetrations and the depths of recording sites relative to the lesion marks allowed a good reconstruction of the location of all recorded neurons.

4.3. *Visual stimulation*

We adapted the stimulation procedure described by Folta et al. (2004) and presented bilateral or monocular light flashes of 500 ms duration to the ipsilateral and/or contralateral eye (with respect to the recording electrode in Rt). These light flashes were produced by a 15 V, 150 W halogen light with a luminance of 40 cd/m^2 (background illumination: 5 lux). The light was gated by two mechanical shutters (rise/fall times 27 ms each) and was transmitted to the bird's eyes by two light-conducting oculars of 15 cm length and with a diameter of 1.5 cm. They were arranged along the optical axes, i.e. in an angle of about 60° to the left and right from midline. This guaranteed that light was presented only to the appropriate eye. Although many rotundal cells respond to moving stimuli (Wang et al., 1993; Sun and Frost, 1998), a substantial proportion is tuned to other aspects, like color and luminance, without responding to movement (Granda and Yazulla, 1971; Wang et al., 1993). However, since virtually all rotundal units are excited by light flashes (Revzin, 1970; Granda and Yazulla, 1971), this stimulus ensured a high probability of obtaining recordings from the majority of rotundal units and ensured to reveal cellular activations due to ascending and descending signals (Folta et al., 2004).

Data acquisition started 100 ms before stimulus onset, defined as the time, when luminance had reached 10% of its maximum. In a first stimulation paradigm, four different stimulus conditions were tested: monocular stimulation of the eye either ipsilateral or contralateral to the recording site, simultaneous stimulation of both eyes, and a control condition without stimulation. For investigations of precise temporal interactions of ascending and descending signals at rotundal level, two further stimulation paradigms were used. Firstly, the ipsilateral eye was stimulated followed by a stimulation of the contralateral eye after a temporal delay of 50, 100, and 150 ms, and a control condition without any visual stimulation. In each of these conditions, both shutters closed 500 ms after presentation of the ipsilateral stimulus, resulting in contralateral light flashes of 450, 400, and 350 ms duration, respectively. Secondly, the same units were stimulated contralaterally followed by a stimulation of the ipsilateral eye after a temporal delay of 50, 100, and 150 ms, and a control condition without any visual stimulation. Again, both shutters closed 500 ms after presentation of the contralateral stimulus, resulting in ipsilateral light flashes of 450, 400, and 350 ms duration, respectively. Thus, our experiment consisted of three experimental sessions and stimulation procedures with four stimulation conditions. Under each of these conditions, spike trains of 1 s duration were acquired. A total of 20 trials per stimulation condition were recorded for each single unit.

4.4. *Data analysis*

Peristimulus-time histograms (PSTHs, 5 ms bin width) were calculated over all trials of each stimulation condition. Spike activity was measured within the first 350 ms after stimulus onset since all isolated neurons responded exclusively to stimulus onset. Dependent *t*-test comparisons confirmed the statistical significance ($p<0.05$) of cell responses to visual stimulation versus spontaneous activity for each isolated single unit.

Normalized PSTHs were calculated for each unit by dividing the number of spikes for each bin by the maximum number of spikes per bin for each stimulation condition, resulting in bin values between 0 and 1. In the case of monocular cell responses, latency was calculated as the lower time limit of the first bin for which the normalized cell response exceeded values of 0.2, if this bin was immediately followed by a second bin above threshold. Similarly, response offset was defined as the upper time limit of the last significant bin that was followed by at least two bins below the threshold of 0.2. Since binocular cell responses showed two response components merging to a common response, the offset of the first (phasic) response component was defined as the upper time limit of the last significant bin that was followed by at least one bin below the threshold of 0.3. Response latency of the second (tonic) response component was calculated as the lower time limit of the first bin after response offset of the first response component for which the normalized cell response exceeded values of 0.3, if this bin was immediately followed by a second bin above threshold. In all cases, response duration was calculated as the time between response onset and offset. Peak activity strength was calculated by averaging the non-normalized discharge rate (spikes/s) during the interval of significant spiking activity, as defined by response latency and duration. Finally, repeated measurement ANOVAs were used to test for differences in response latency, response duration, and peak activity strength.

Acknowledgments

We thank Ariane Schwarz and Bettina Diekamp for their excellent support and Stefan Treue for helpful comments on the manuscript. This work was supported by the Deutsche

Forschungsgemeinschaft (Sonderforschungsbereich 509: Neurovision).

REFERENCES

Bagnoli, P., Francesconi, W., Magni, F., 1977. Visual Wulst influences on the optic tectum of the pigeon. Brain Behav. Evol. 14, 217–237.

Bagnoli, P., Francesconi, W., Magni, F., 1979. Interaction of optic tract and visual Wulst impulses on single units of the pigeon's optic tectum. Brain Behav. Evol. 16, 19–37.

Bagnoli, P., Grassi, S., Magni, F., 1980. A direct connection between visual Wulst and tectum opticum in the pigeon (*Columba livia*) demonstrated by horseradish peroxidase. Arch. Ital. Biol. 118, 72–88.

Bischof, H.J., Niemann, J., 1990. Contralateral projection of the optic tectum in the zebra finch (*Taenopygia guttata castanotis*). Cell Tissue Res. 262, 307–313.

Britto, L.R., 1978. Hyperstriatal projections to primary visual relays in pigeons: electrophysiological studies. Brain Res. 153, 382–386.

Deng, C., Rogers, L.J., 1998. Bilaterally projecting neurons in the two visual pathways of chicks. Brain Res. 794, 281–290.

Domenici, L., Waldvogel, H.J., Matute, C., Streit, P., 1988. Distribution of GABA-like immunoreactivity in the pigeon brain. Neuroscience 25, 931–950.

Dubbeldam, J.L., den Boer-Visser, A.M., Bout, R.G., 1997. Organization and efferent connections of the archistriatum of the mallard, *Anas platyrhynchos* L.: an anterograde and retrograde tracing study. J. Comp. Neurol. 388, 632–657.

Engelage, J., Bischof, H.J., 1993. The organization of the tectofugal pathway in birds: a comparative review. In: Zeigler, H.P., Bischof, H.J. (Eds.), Vision, Brain, and Behavior in Birds. MIT Press, Cambridge, MA, pp. 137–158.

Folta, K., Diekamp, B., Güntürkün, O., 2004. Asymmetrical modes of visual bottom–up and top–down integration in the thalamic nucleus rotundus of pigeons. J. Neurosci. 24, 9475–9485.

Friedman, M.B., 1975. How birds use their eyes. In: Wright, P., Caryl, P.G., Vowles, D.M. (Eds.), Neural and Endocrine Aspects of Behaviour in Birds. Elsevier, Amsterdam, pp. 181–204.

Fung, S.H., Burstein, D., Born, R.T., 1998. In vivo microelectrode track reconstruction using magnetic resonance imaging. J. Neurosci. Methods 80, 215–224.

Gao, H.F., Wu, G.Y., Frost, B.J., Wang, S.R., 1995. Excitatory and inhibitory neurotransmitters in the nucleus rotundus of pigeons. Vis. Neurosci. 12, 819–825.

Granda, A.M., Yazulla, S., 1971. The spectral sensitivity of single units in the nucleus rotundus of pigeon. *Columba livia*. J. Gen. Physiol. 57, 363–384.

Green, J.D., 1958. A simple microelectrode for recording from the central nervous system. Nature 182, 962.

Güntürkün, O., 2006. Avian cerebral asymmetries: the view from the inside. Cortex 42, 104–106.

Güntürkün, O., Hoferichter, H.H., 1985. Neglect after section of a left telencephalotectal tract in pigeons. Behav. Brain Res. 18, 1–9.

Güntürkün, O., Miceli, D., Watanabe, M., 1993. Anatomy of the avian thalamofugal pathway. In: Zeigler, H.P., Bischof, H.J. (Eds.), Vision, Brain, and Behavior in Birds. MIT Press, Cambridge, MA, pp. 115–135.

Hardy, O., Leresche, N., Jassik-Gerschenfeld, D., 1984. Postsynaptic potentials in neurons of the pigeon's optic tectum in response to afferent stimulation from the retina and other visual structures: an intracellular study. Brain Res. 311, 65–74.

Hellmann, B., Güntürkün, O., 1999. Visual-field-specific heterogeneity within the tecto–rotundal projection of the pigeon. Eur. J. Neurosci. 11, 2635–2650.

Hellmann, B., Güntürkün, O., Manns, M., 2004. The tectal mosaic: organization of the descending tectal projections in comparison to the ascending tectofugal pathway in the pigeon. J. Comp. Neurol. 472, 395–410.

Hunt, S.P., Brecha, N., 1984. The avian optic tectum: a synthesis of anatomy and bio-chemistry. In: Vanagas, H. (Ed.), Comparative Neurobiology of the Optic Tectum. Plenum Press, New York, pp. 619–648.

Husband, S.A., Shimizu, T., 1999. Efferent projections of the ectostriatum in the pigeon (*Columba livia*). J. Comp. Neurol. 406, 329–345.

Karten, H.J., Hodos, W., 1967. A Stereotaxic Atlas of the Brain of the Pigeon. The Johns Hopkins Press, Baltimore.

Karten, H.J., Cox, K., Mpodozis, J., Bischof, H.J., Shimizu, T., 1993. Little cells ending on big cells: an oligosynaptic retino–tecto–pulvinar system in pigeon. Abstr.-Soc. Neurosci. 19, 969.

Keysers, C., Diekamp, B., Güntürkün, O., 2000. Evidence for asymmetries in the phasic intertectal interactions in the pigeon (*Columba livia*) and their potential role in brain lateralisation. Brain Res. 852, 406–413.

Knudsen, E.I., Cohen, Y.E., Masino, T., 1995. Characterization of a forebrain gaze field in the archistriatum of the barn owl: microstimulation and anatomical connections. J. Neurosci. 15, 5139–5151.

Leresche, N., Hardy, O., Jassik-Gerschenfeld, D., 1983. Receptive field properties of single cells in the pigeon's optic tectum during cooling of the 'visual Wulst'. Brain Res. 267, 225–236.

Letelier, J.C., Mpodozis, J., Marin, G., Morales, D., Rozas, C., Madrid, C., Velasco, M., 2000. Spatiotemporal profile of synaptic activation produced by the electrical and visual stimulation of retinal inputs to the optic tectum: a current source density analysis in the pigeon (*Columba livia*). Eur. J. Neurosci. 12, 47–57.

Manns, M., Güntürkün, O., Heumann, R., Blöchl, A., 2005. Photic inhibition of TrkB/Ras activity in the pigeon's tectum during development: impact on brain asymmetry formation. Eur. J. Neurosci. 22, 2180–2186.

Miceli, D., Reperant, J., Villalobos, J., Dionne, L., 1987. Extratelencephalic projections of the avian visual Wulst. A quantitative autoradiographic study in the pigeon (*Columba livia*). J. Hirnforsch. 28, 45–57.

Mpodozis, J., Cox, K., Shimizu, T., Bischof, H.J., Woodson, W., Karten, H.J., 1996. GABAergic inputs to the nucleus rotundus (pulvinar inferior) of the pigeon (*Columba livia*). J. Comp. Neurol. 374, 204–222.

Reiner, A., Perkel, D.J., Bruce, L.L., Butler, A.B., Csillag, A., Kuenzel, W., Medina, L., Paxinos, G., Shimizu, T., Striedter, G., Wild, M., Ball, G.F., Durand, S., Güntürkün, O., Lee, D.W., Mello, C.V., Powers, A., White, S.A., Hough, G., Kubikova, L., Smulders, T.V., Wada, K., Dugas-Ford, J., Husband, S., Yamamoto, K., Yu, J., Siang, C., Jarvis, E.D., 2004. Revised nomenclature for avian telencephalon and some related brainstem nuclei. J. Comp. Neurol. 473, 377–414.

Revzin, A.M., 1970. Some characteristics of wide-field units in the brain of the pigeon. Brain Behav. Evol. 3, 195–204.

Robert, F., Cuénod, M., 1969. Electrophysiology of the intertectal commissures in the pigeon. II. Inhibitory interaction. Exp. Brain Res. 9, 123–136.

Schmidt, A., Bischof, H.J., 2001. Integration of information from both eyes by single neurons of nucleus rotundus, ectostriatum and lateral neostriatum in the zebra finch (*Taeniopygia guttata castanotis* Gould). Brain Res. 923, 20–31.

Schulte, M., Diekamp, B., Manns, M., Schwarz, A., Valencia-Alfonso, C., Kirsch, J.A., Güntürkün, O., Folta, K., 2006. Visual responses and afferent connections of the n. ventrolateralis thalami (VLT) in the pigeon (*Columba livia*). Brain Res. Bull. 68, 285–292.

Shimizu, T., Karten, H.J., 1993. The avian visual system and the evolution of the neocortex. In: Zeigler, H.P., Bischof, H.J. (Eds.),

Vision, Brain, and Behavior in Birds. MIT Press, Cambridge, MA, pp. 104–114.
Shimizu, T., Cox, K., Karten, H.J., 1995. Intratelencephalic projections of the visual Wulst in pigeons (*Columba livia*). J. Comp. Neurol. 359, 551–572.
Sun, H., Frost, B.J., 1998. Computation of different optical variables of looming objects in pigeon nucleus rotundus neurons. Nat. Neurosci. 1, 296–303.
Swadlow, H.A., Gusev, A.G., 2001. The impact of 'bursting' thalamic impulses at a neocortical synapse. Nat. Neurosci. 4, 402–408.
Theiss, M.P., Hellmann, B., Güntürkün, O., 2003. The architecture of an inhibitory sidepath within the avian tectofugal system. NeuroReport 14, 879–882.
Tömböl, T., Nemeth, A., Sebesteny, T., Alpar, A., 1999. Electron microscopic data on the neurons of nuclei subpretectalis and posterior-ventralis thalami. A combined immunohistochemical study. Anat. Embryol. 199, 169–183.
Voss, J., Bischof, H.J., 2003. Regulation of ipsilateral visual information within the tectofugal visual system in zebra finches. J. Comp. Physiol., A. Neuroethol. Sens. Neural. Behav. Physiol. 189, 545–553.
Wang, Y.C., Jiang, S., Frost, B.J., 1993. Visual processing in pigeon nucleus rotundus: luminance, color, motion, and looming subdivisions. Vis. Neurosci. 10, 21–30.
Zeier, H., Karten, H.J., 1971. The archistriatum of the pigeon: organization of afferent and efferent connections. Brain Res. 31, 313–326.