

BEHAVIORAL NEUROSCIENCE

Lateralized reward-related visual discrimination in the avian entopallium

Josine Verhaal,^{1,2} Janina A. Kirsch,³ Ioannis Vlachos,³ Martina Manns^{1,2} and Onur Güntürkün^{1,2}¹Biopsychology, Institute for Cognitive Neuroscience, Faculty of Psychology, Ruhr-University Bochum, Universitätsstr. 150, D-44780 Bochum, Germany²International Graduate School of Neuroscience, Ruhr-University Bochum, Bochum, Germany³Bernstein Center Freiburg, Albert-Ludwigs University Freiburg, Freiburg, Germany*Keywords:* asymmetries, conditioning, forebrain, hemisphere, pigeon

Abstract

In humans and many other animals, the two cerebral hemispheres are partly specialized for different functions. However, knowledge about the neuronal basis of lateralization is mostly lacking. The visual system of birds is an excellent model in which to investigate hemispheric asymmetries as birds show a pronounced left hemispheric advantage in the discrimination of various visual objects. In addition, visual input crosses at the optic chiasm and thus testing of each hemisphere is easily accomplished. We aimed to find a neuronal correlate for three hallmarks of visual lateralization in pigeons: first, the animals learn faster with the right eye–left hemisphere; second, they reach higher performance levels under this condition; third, visually guided behavior is mostly under left hemisphere control. To this end, we recorded from the left and right forebrain entopallium while the animals performed a colour discrimination task. We found that, even before learning, left entopallial neurons were more responsive to visual stimulation. Subsequent discrimination acquisition recruited more neuronal responses in the left entopallium and these cells showed a higher degree of differentiation between the rewarded and the unrewarded stimulus. Thus, differential left–right responses are already present, albeit to a modest degree, before learning. As soon as some cues are associated with reward, however, this asymmetry increases substantially and the higher discrimination ratio of the left hemispheric tectofugal pathway would not only contribute to a higher performance of this hemisphere but could thereby also result in a left hemispheric dominance over downstream motor structures via reward-associated feedback systems.

Introduction

For a long time cerebral asymmetries were believed to be specific to humans, characterizing a crucial evolutionary innovation that made *Homo sapiens* unique. During recent decades, however, it has become clear that lateralization is widespread within the animal kingdom (reviewed in Rogers & Andrew, 2002). This delayed recognition of asymmetrical systems in animals has contributed to limiting the knowledge of the neurobiology of lateralized systems (Halpern *et al.*, 2005). Presently, the possibly most thoroughly studied animal model for cerebral asymmetries at the neural and the behavioral levels is the visual system of birds.

In birds, the optic fibres almost completely cross at the optic chiasm. Therefore, the performance of a single hemisphere can be investigated by simply restricting vision to one eye. Using this technique, it has been found that the right hemisphere (RH) is involved in spatial orientation according to geometrical cues and instinct-based behaviors (Vallortigara & Andrew, 1994; Andrew *et al.*, 2004). In contrast, the left hemisphere (LH) is superior for

visual feature discrimination and memorization (Rogers *et al.*, 2007), as well as for categorization of visual patterns (Yamazaki *et al.*, 2007).

In birds, visual information is processed by the thalamofugal and tectofugal pathways, which are equivalent to, respectively, the geniculocortical and extrageniculocortical pathways of mammals (Güntürkün, 2000). The thalamofugal pathway consists of fibres projecting from the retina to the lateral geniculate nucleus and then to the visual Wulst in the telencephalon. The tectofugal pathway consists of fibres projecting from the retina to the tectum. From there, neurons ascend bilaterally to the thalamic nucleus rotundus, which in turn projects ipsilaterally onto the telencephalic entopallium (Güntürkün, 2000). Pigeons demonstrate a lifelong asymmetry of their tectofugal system. This system is characterized by morphological asymmetries at tectal (Güntürkün, 1997; Manns & Güntürkün, 2003, 2005; Manns *et al.*, 2005) and rotundal (Manns & Güntürkün, 1999) levels as well as by asymmetries of ascending fibres that cross from the tectum to the contralateral rotundus (Güntürkün *et al.*, 1998).

When analyzing behavioral visual asymmetries in pigeons, three aspects are especially conspicuous. First, they learn visual discriminations of colours (Prior & Güntürkün, 2001) and patterns (Manns & Güntürkün, 2009) faster with the right eye–left hemisphere. Second, the final discrimination ratio in these tasks is on average higher with

Correspondence: Onur Güntürkün, ¹Biopsychology, as above.
E-mail: onur.guentuerkuen@ruhr-uni-bochum.de

Received 21 November 2011, revised 13 January 2012, accepted 23 January 2012

the right eye (Güntürkün, 1985; Diekamp *et al.*, 1999; Skiba *et al.*, 2002), although no left–right differences in acuity are discernable (Güntürkün & Hahmann, 1994). Third, choices during binocularly performed visual discriminations are mostly under left hemisphere control (Güntürkün, 1985; Nottelmann *et al.*, 2002; Xiao & Güntürkün, 2009; but see Adam & Güntürkün, 2009).

To uncover possible neuronal correlates underlying these behavioural asymmetries, we recorded from neurons in the entopallium of pigeons before and after learning a colour discrimination task.

Materials and methods

Animals

Five pigeons (*Columba livia*) of unknown sex obtained from local breeders were used for this study. The animals were kept on a 12-h dark–light cycle. The animals had *ad libitum* access to water and food prior to training. After each session they had 15 min free access to water and were then deprived for 16–24 h before starting the next experiment. All experiments were performed according to the principles regarding the care and use of animals adopted by the German Animal Welfare Law for the prevention of cruelty to animals. The study was approved by the office of nature, environment, and consumer protection of North Rhine-Westfalia.

Surgery

The animals were anaesthetized using Equithesin (0.3 ml/100 g body weight) or ketamine and Rompun *i.m.* (respectively, 50 mg/kg; Sigma Deisenhofen, Germany; and 10 mg/kg; Bayer, Leverkusen, Germany). The feathers on the head were trimmed and the auditory cavities were locally anaesthetized with lidocaine (AstraZeneca, Wedel, Germany). The anaesthetized pigeons were placed on a foam couch with regulated temperature, and fixed in a stereotactic head holder with the head positioned in the standard 45° position. The skull was exposed. Entry points of the electrodes were 9.5 mm anterior–posterior and 5.5 mm lateral relative to zero according to the Karten & Hodos (1967) stereotactic atlas of the pigeon brain. At these spots the skull was opened, the dura mater carefully removed and a recording chamber which allowed access to the brain during recordings was mounted on each hemisphere. Silver nitrate (0.1%; Sigma-Aldrich, Steinheim, Germany) was used to prevent scar tissue development. The recording chambers were closed using permalastic light body (Kerr, Salerno, Italy). In addition, a fixation block was mounted to the skull allowing fixation of the head during recordings. After surgery the pigeons were given 1 week for recovery.

Classical conditioning paradigm

The experiment was subdivided into three phases – in the preconditioning phase the pigeons did not receive any reward and only the red and green stimuli were presented. During the conditioning and postconditioning phases, one of the colour presentations (red for two pigeons and green for two pigeons; one pigeon was perfused before the postconditioning phase due to sickness) was always followed by delivery of a small water reward irrespective of the behavioural response of the animal.

We recorded the neuronal activity from each pigeon during a preconditioning and a postconditioning phase, each of which consisted of 8–10 recording days. During the conditioning phase neuronal activity was not recorded. Mandibulations or drinking movements were recorded by the use of a laser beam which was interrupted as

soon as the pigeon opened the beak. Once the pigeons made 2/3 of their mandibulations during presentations of the rewarded stimulus (CS+) in the conditioning phase, learning was regarded as completed because they associated CS+ with the following water delivery. From this day onwards the postconditioning phase started.

Recordings

During recordings the head was fixed using the fixation block and the body was restrained loosely by a cloth bag. On each day, recordings were made from the left or the right hemisphere in an alternating order, starting with the left hemisphere in half of the pigeons and with the right hemisphere in the others on the first recording day. Electrodes were advanced to a depth of approximately 3.5–4.5 mm, which corresponds to the core of the entopallium. Multiunit activity was recorded with the Eckhorn multielectrode system (Thomas Recording, Giessen, Germany) using quartz-platinum–tungsten electrodes with an outer diameter of 80 µm and an impedance of 1–6 MΩ at 1000 Hz. Seven electrodes separated by 305 µm were arranged in a circle with one electrode in the middle.

Signals were amplified by a factor of 500–1000 depending on the amplitude of the signal, online bandpass-filtered between 500 and 5000 Hz and monitored on an oscilloscope and an audiomonitor. The recorded signals were sampled at 20 kHz using SPIKE2 software (Cambridge Electronic Design, Cambridge, UK) and stored for offline analysis. After a session, the pigeon was removed from the electrophysiological setup. Silver nitrate (0.1%; Sigma-Aldrich) was applied and the recording chamber was closed again using permalastic light body.

Visual stimulation

On each recording day a pigeon was subjected to four sessions of 40 trials for each stimulus, resulting in total of 320 trials in each recording. The first and last sessions were under binocular seeing conditions, and in between there was a contralateral and an ipsilateral session relative to the recorded hemisphere, in balanced order. Contralateral and ipsilateral seeing conditions were established by occlusion of one of the two eyes by the means of a flexible black board which fitted exactly to the pigeon's head and prevented sight of the monitor. This method allowed switching to the other eye without major manipulations and without loss of neuronal responses.

Two isoluminant colours, red and green, were successively presented on a 15-inch TFT monitor (151M, NFREN, Paris, France) in front of the pigeon. The order of presentation was pseudo-random with the restriction that the same stimulus was presented no more than three times consecutively. Trials were separated by a 7- to 13-s intertrial interval.

During the preconditioning phase of the experiment both stimuli were presented for 3 s to the pigeon. During the postconditioning phase, CS+ was presented for 4.5 s, during the last 1.5 s of which the water delivery occurred. The unrewarded stimulus (CS–) was presented for a total duration of 3 s. The 3 s before stimulus onset was used as a measure of spontaneous activity.

Data analysis

Analysis was done in MATLAB (Mathworks). The analog signal was imported in steps of 100 s and filtered offline (300–3000 Hz). All the signals that passed the threshold of the median amplitude of the raw data trace plus 6 standard deviations were taken into the analysis. This

threshold was taken so that it was above the noise level in all cases but did not discard the smaller spikes. Due to the highly bursty firing pattern of entopallial neurons, spikes of many different amplitudes were found and they were often indistinguishable from each other. Therefore, analyses were performed on the unsorted spikes.

We created raster diagrams and peristimulus time histograms (PSTHs) which showed the activity under the two stimulus conditions. A neuronal recording was called 'responsive' when in both binocular sessions the spike rate in the PSTH during the stimulus presentation was increased or decreased significantly by > 10% compared to spontaneous activity, or when a change was detected using a change point analysis for one of the two colours. Changes in activity were tested using Wilcoxon tests by comparing 60 × 50-ms bins (0 to +3 s) during stimulation with 60 bins (−3 to 0 s) before stimulation. As water supply caused artifacts in several cases, we analysed only responses during the stimulus presentation phase and discarded the water phase completely. Next, responses under the two binocular sessions were averaged. The reward-associated modulation (RAM) was calculated as the response to CS+ relative to CS− and reflects the effect of associative learning.

We calculated the correlation coefficient between the strength of the neuronal response and the number of mandibulations made during the first 3 s of CS+ and CS− presentation under binocular, contralateral and ipsilateral seeing conditions. The responses from the two binocular sessions were analysed separately.

Histology

After the animal finished the experiment we made a lesion in each hemisphere by passing a small (2-mA) DC current of 9 s duration through the electrode to mark the final recording position. The pigeons were deeply anaesthetized (0.45 ml Equithesin per 100 g body weight) and perfused transcardially with 0.9% saline followed by 4% paraformaldehyde solution in 0.1 M phosphate buffer. The brains were embedded in gelatine, cut at 40 μm in the frontal plane, stained with Cresyl violet and microscopically analysed to reconstruct recording positions from the lesions.

Statistics

All statistical procedures were done using SPSS 18. Results with $P < 0.05$ were considered to be significant. The number of visually responsive multi-unit clusters before and after conditioning for the left and the right hemispheres was compared using the Fisher exact test. The neuronal activity (spikes/s) during stimulus presentation, normalized to the average spontaneous activity 3 s before stimulus onset (spikes/s), was analysed with a repeated-measures ANOVA using hemisphere (left and right) as between-subjects factor and seeing condition (binocular, contralateral and ipsilateral) and colour (red/−green prior to conditioning and CS+/CS− after conditioning) as within-subject factors. Pairwise comparisons with a Bonferroni correction were used to test for specific differences. Correlation between the number of mandibulations during a session and increase in activity upon stimulation was calculated with a Pearson correlation coefficient.

Results

Electrophysiological recordings were made from the left and the right entopallium (see Fig. 1 for recording positions) of five pigeons (*Columba livia*). As also noted by previous authors (Engelage &

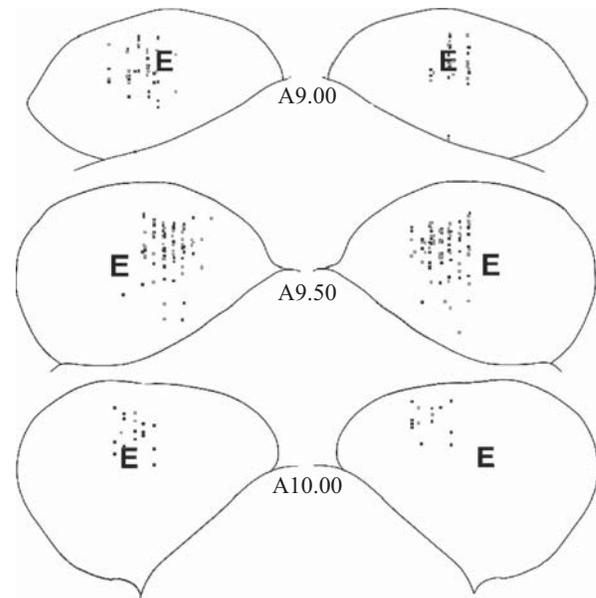


FIG. 1. Reconstruction of the recording position of the electrodes for the preconditioning (gray) and postconditioning (black) phase. The pictures emphasize the entopallium (E). All recording positions were found to be within this brain area. Recording positions were determined relative to the reconstructed recording position of the last recording day, taking into account the horizontal distance of each electrode from the electrode used to make the lesion as well as the depth of each measurement.

Bischof, 1996; Colombo *et al.*, 2001), no differences in firing patterns between subareas of the entopallium were observed. As input from one eye projects to the contralateral hemisphere and only reaches the ipsilateral hemisphere through the commissural recrossings, we investigated responses of the contralateral and ipsilateral hemispheres separately. During the initial recording sessions, a colour stimulus (red or green) was presented to the contralateral, the ipsilateral and both eyes (preconditioning phase).

In order to investigate the effect of learning, the animals were subsequently trained in the discrimination of these two colours by a classical conditioning paradigm. One of the colours (CS+, in balanced manner) was followed by a water delivery while the other colour (CS−) was not. For all pigeons, conditioning was achieved in a single session of 320 trials, as they started to mandibulate during CS+ in expectation of the water reward but not during CS−. After this conditioning session, a second series of recording sessions was performed (postconditioning phase) to compare electrophysiological entopallial responses before and after learning (Figs 2–4).

Responses to colour stimuli prior to conditioning

In the preconditioning phase, we obtained 210 stable multiunit responses (105 from each hemisphere), of which 119 responded to the colour stimuli (54 from the left and 65 from the right hemisphere in a total of five animals). The percentage of visually responsive neuronal multi-unit clusters did not differ significantly between hemispheres (Fisher exact test, $P = 0.164$; Fig. 4).

Colour stimulation resulted in an increase of approximately 20% in spike rate (see Fig. 2) and this increase was higher in the left than in the right hemisphere (interaction between seeing conditions and hemisphere, $F_{2,234} = 18.932$, $P < 0.001$; *post hoc* $P < 0.05$ for all seeing conditions). The onset of the visual stimulus resulted in an

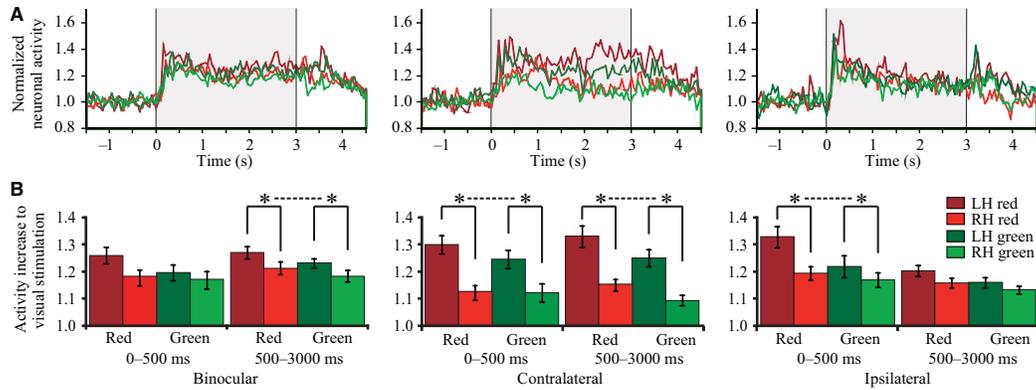


FIG. 2. Preconditioning phase. The graphs show (A) the average neuronal activity normalized for spontaneous activity and (B) the response upon visual stimulation under binocular, contralateral and ipsilateral seeing conditions for the first 500 ms (burst phase) and the period between 500 and 3000 ms (plateau phase). Data were collected from five animals. Multi-unit clusters which were visually responsive: left, $n = 54$; right, $n = 65$. LH, left hemisphere (green); RH, right hemisphere (blue). Bars show SEM; $*P = 0.05$ (colour-independent differences between left and right hemisphere are connected with broken lines).

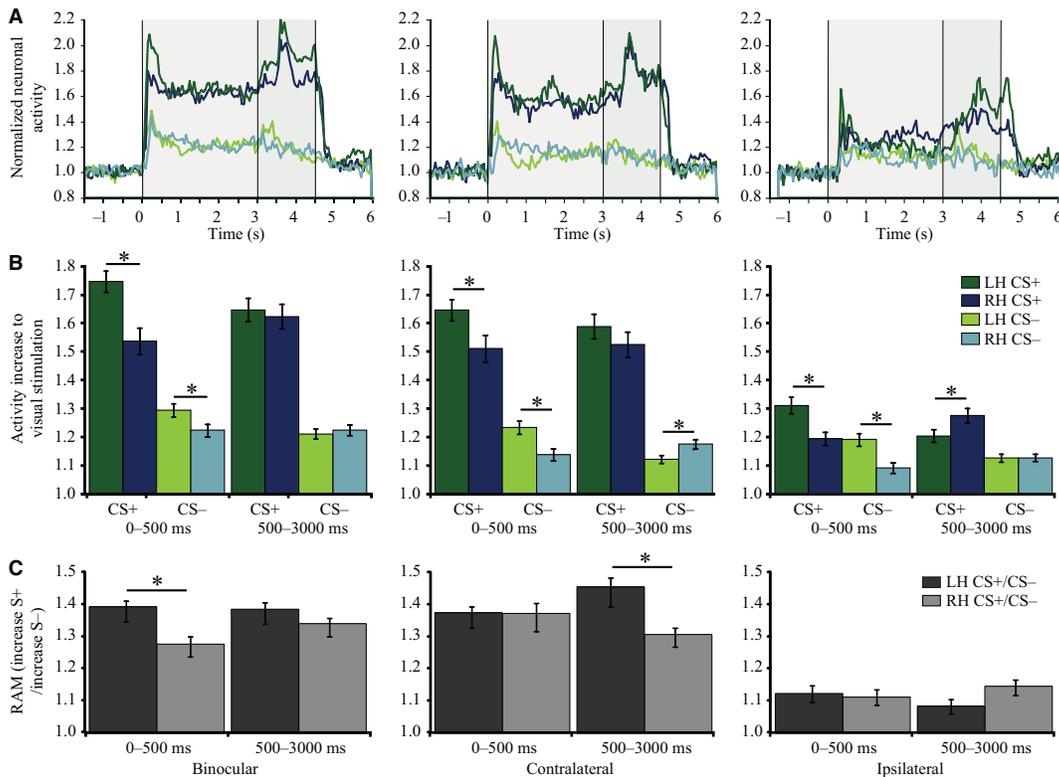


FIG. 3. Postconditioning phase. The graphs show the average neuronal activity normalized for (A) spontaneous activity and (B) increase in activity upon visual stimulation under binocular, contralateral and ipsilateral seeing conditions for the first 500 ms (burst phase) and the period between 500 and 3000 ms (plateau phase). (C) Reward-associated modulation (RAM), which is defined as the increase in activity upon CS+ presentation (spikes during stimulus/spontaneous activity) compared to increase in activity upon CS- presentation. The eyes were stimulated with red or green stimuli, of which one preceded the delivery of water (CS+, shown in dark green for the left hemisphere and in dark blue for the right hemisphere) and the other did not (CS-, shown in light green for the left hemisphere and in light blue for the right hemisphere). Responses during the water phase (3000–4500 ms) were not analysed due to possible artifacts. Data were collected from four animals. Multi-unit clusters which were visually responsive: left, $n = 89$; right, $n = 78$. L, left hemisphere; R, right hemisphere. Bars show SEM; $*P = 0.05$.

initial burst of firing that vanished after approximately 500 ms to be replaced by a sustained plateau (Fig. 2A). Visual responses were stronger in the left hemisphere for the initial 500 ms burst phase

(interaction between seeing condition and hemisphere, $F_{2,234} = 7.321$, $P = 0.001$; both contra- and ipsilateral condition $P < 0.05$) as well as for the subsequent plateau phase (interaction between seeing condition

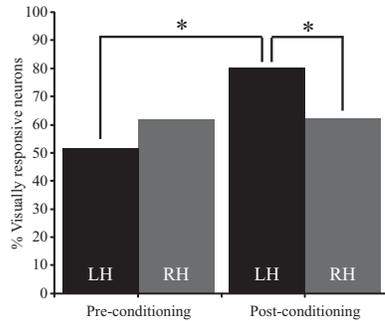


FIG. 4. The percentage of multi-unit clusters which were visually responsive in the pre- and postconditioning phases for the left and the right hemispheres. LH, left hemisphere; RH, right hemisphere; * $P = 0.001$.

and hemisphere, $F_{2,234} = 18.429$, $P < 0.001$; both binocular and contralateral condition $P < 0.05$).

Responses to colour stimuli after conditioning

The conditioning procedure altered the number of responsive multi-unit clusters. In the postconditioning phase, we obtained 236 stable multi-unit clusters (111 left, 125 right), of which 167 were visually responsive (89 from the left and 78 from the right hemisphere in a total of four animals).

Most importantly, we observed a significantly lateralized increase in recruited cellular responses when comparing the activity difference before and after conditioning between left and right. Upon associating the CS+ with reward, there was a significant increase for the left (from 51.4 to 80.2%; Fisher exact test, $P < 0.001$), but not for the right hemisphere (from 61.9 to 62.4%; Fisher exact test, $P > 0.1$; see Fig. 4). This resulted in a significant postconditioning asymmetry in the percentage of visually responsive multi-unit clusters (LH, 80.2%, 89/111; RH, 62.4%, 78/125; Fisher exact test, $P = 0.004$). Possibly, this difference represents the lateralized percentage of the population that is activated within the left and the right entopallium.

Also during the postconditioning phase we found that the onset of the visual stimulus resulted in a 500-ms initial burst of firing that was later replaced by a sustained plateau (see Fig. 3). In the burst phase, responses were, overall, larger for the left than the right hemisphere (interaction seeing condition, reward association and hemisphere, $F_{2,330} = 3.479$ $P = 0.032$; all pairwise comparisons $P < 0.05$). For the plateau phase (500–3000 ms) we found higher right hemispheric responses (interaction seeing condition, reward association and hemisphere, $F_{2,330} = 7.440$, $P = 0.001$) to CS– under contralateral ($P = 0.015$) and to CS+ under ipsilateral conditions ($P = 0.041$).

Reward-associated modulation

In order to evaluate the cellular effect of associative learning, we calculated the RAM, which is defined as the increase in activity upon CS+ (spikes during stimulus/spontaneous activity) compared to CS– presentation. Thus, RAM provides an estimate of the cellular differentiation between CS+ and CS–. RAM was significantly higher in the left entopallium during burst (interaction between seeing condition and hemisphere, $F_{2,330} = 3.589$ $P = 0.029$; binocular condition, LH vs. RH, $P = 0.016$) and plateau phase (interaction between seeing condition and hemisphere, $F_{2,330} = 8.526$ $P < 0.001$; contralateral condition, LH vs. RH $P = 0.011$).

Correlations between neuronal responses and conditioned behavior

The pigeons repeatedly made drinking movements by rapidly opening their beaks during anticipation of water and/or actual drinking. As the entopallium directly projects to the striatum (Krutzfeldt & Wild, 2005) and, via nidopallium caudolaterale, also to the somatomotor arcopallium (Kroner & Güntürkün, 1999), we sought to determine whether strengths of entopallial responses correlate with the number of motor responses after CS+ or CS– delivery. The number of mandibulations did not differ for recordings made from days on which the left or the right hemisphere was recorded (t -test, $P = 0.167$), indicating that there was no motivational confound during recordings. We separately calculated the correlations between cellular responses and the total number of mandibulations during CS+ and CS– trials under the different seeing conditions. For the left hemisphere, these correlations were significant for CS+ delivery for all seeing conditions (binocular, $r = 0.529$, $n = 162$, $P < 0.001$; contralateral, $r = 0.457$, $n = 81$, $P < 0.001$; ipsilateral, $r = 0.297$, $n = 81$, $P = 0.007$). In contrast, for CS– only a weak, although significant, correlation was found for binocular seeing ($r = 0.168$, $n = 162$, $P = 0.033$). For the right hemisphere, however, correlations were significant for both CS+ and CS– (binocular, CS+ $r = 0.418$, CS– $r = 0.268$, $n = 152$, both $P < 0.001$; contralateral, CS+ $r = 0.320$, CS– $r = 0.359$, $n = 76$, both $P < 0.01$).

Discussion

We conducted electrophysiological recordings during a colour discrimination task to uncover neuronal correlates of three properties of visual lateralization in pigeons: first, pigeons are known to learn visual discriminations faster with the right eye–left hemisphere; second, with the left hemisphere they reach higher levels of discriminatory performance; third, visual discriminations under binocular conditions are mostly under left hemispheric control. Subsequent to associating certain colours with reward, three prominent electrophysiological left–right differences were observed. First, only in the left hemisphere could an increase in cellular responses to the conditioned stimulus be observed. Second, most biphasic responses, i.e. those starting with a strong burst, were found in the left entopallium. Third, the response difference between CS+ and CS– was larger for the left hemisphere. Together, these effects could constitute important aspects of the cellular basis for the left hemispheric advantage in learning visual discriminations. We will discuss these findings one by one.

Lateralized and learning-dependent recruitment of entopallial cells

Prior to conditioning, the number of visually responsive multi-unit clusters did not differ between the hemispheres. However, subsequent to the bird learning the association between the colour stimulus and reward, we found that a significantly larger number of multiunit responses on the left than on the right side were activated by the CS+. Thus, learning about the appetitive value of a visual stimulus resulted in an asymmetrical recruitment of entopallial cell populations responding to the CS+. This conclusion has to be drawn carefully as we obviously could not observe the recruitment itself but only reconstructed this result from pre- and postconditioning multiunit responses. It is possible that this effect is mediated by a higher number of neurons responding to visual stimulation. However, it is also conceivable that a low number of cells starts to respond vigorously to

the CS+. Although our experiment is unable to differentiate between these two explanations, both would point to a learning-dependent change in response properties that is specific for the left hemisphere.

A left tectofugal increase in cellular responses to conditioned visual cues could gain a higher level of control over downstream associative and motor structures. Thus, visually guided behavior in the context of learned object discriminations would then be prone to being primarily left-hemisphere based. Indeed, several reports in birds have observed that choices during binocularly performed visual discriminations were mostly under left hemisphere control (Güntürkün, 1985; Von Fersen & Güntürkün, 1990; Nottelmann *et al.*, 2002; Xiao & Güntürkün, 2009; but see Adam & Güntürkün, 2009). In chicks it has additionally been shown that they actively look with their right eye at colour cues that are associated with food reward (Vallortigara *et al.*, 1996). Taken together, the larger left-sided neuronal population that responds to learned visual stimuli could contribute to a left-hemispheric dominance during visual discrimination tasks.

Similar to Colombo *et al.* (2001), we observed an initial phasic burst of left entopallial activity after perceiving the conditioned visual colour. This was present for the CS+ and, albeit to a lesser extent, also for the CS-. This kind of activity peak was much smaller and even partly absent on the right side. Thus, the neuronal dynamics after stimulus delivery differed substantially between left and right. As the connection between rotundus and entopallium is likely to be glutamatergic (Vischer *et al.*, 1982; Theiss *et al.*, 1998), the initial burst could result from an asymmetric massive release of glutamate at rotundo-entopallial synapses.

Some of the learning-mediated response properties observed in our study resemble the data from the visual extrastriate cortex of primates in which attention towards relevant cues modulates the strength of neuronal responses (Treue & Maunsell, 1996, 1999; McAdams & Maunsell, 1999; Wannig *et al.*, 2007). The strength of the attentional modulation seems to be related to the behavioral performance (Busse *et al.*, 2008). Indeed, the avian entopallium corresponds to the mammalian extrastriate cortex (Shimizu *et al.*, 2010). This similarity at the cellular level could imply that learning of the association between stimulus and reward coincides with an increase in attentional resources towards the CS+ (Tsushima & Watanabe, 2009). The possible dissociation between lateralized mechanisms of visual learning and asymmetries in shifts of attention has to be analysed in future studies.

Lateralized discriminatory responses

Most relevant for our study was the observation that the response rate difference between CS+ and CS- was significantly larger in the left hemisphere. Thus, the signal delivered by entopallial neurons to downstream structures could be more reliable with respect to reporting the presence of rewarding or non-rewarding cues. The higher discrimination score between CS+ and CS- for the left hemisphere was also evident in the correlations between cellular and behavioral responses: under all CS+ conditions left entopallial responses correlated strongly and significantly with the number of mandibulations, while for CS- only binocular stimulation resulted in a significant, albeit very weak, effect. In contrast, right entopallial responses correlated significantly, but at lower levels, with mandibulations for both CS+ and CS- delivery under binocular and contralateral conditions. Thus, left but not right hemisphere responses were mostly related to a differential behavioral response towards the CS+. This strongly resembles the higher level of performance of pigeons when working in visual discrimination tasks with the right eye. This has been shown for discrimination of natural objects (Güntürkün & Kesch, 1987), colours (Güntürkün & Kesch, 1987;

Prior & Güntürkün, 2001), simple figures (Güntürkün, 1985), memorization of hundreds of complex patterns (Von Fersen & Güntürkün, 1990) and visual categorization of pictorial representation of humans (Yamazaki *et al.*, 2007). As discussed below, a higher level of discrimination at the cellular level also has implications for reward-associated feedback at downstream structures.

Asymmetries of tectofugal neuronal responses and lateralized behavior: a hypothetical scenario

The present study was undertaken to reveal possible cellular correlates of the asymmetrically organized visual system of pigeons. The tectofugal system is the critical pathway for visually guided learning and behavior in pigeons. Lesions of tectofugal components strongly degrade colour, brightness and pattern discriminations of frontally presented cues (Hodos & Karten, 1966; Hodos, 1969; Karten *et al.*, 1973; Hodos *et al.*, 1984; Bessette & Hodos, 1989). In particular, lesions of left-sided tectofugal structures or left-sided forebrain components with descending projections to the midbrain tectum reduce visual performance (Güntürkün & Hoferichter, 1985; Güntürkün & Hahmann, 1999; Valencia-Alfonso *et al.*, 2009). The current study provides hints about the mechanisms by which the left tectofugal systems could gain control over visually guided behavior.

Left entopallial neurons responded more strongly to unconditioned colour cues. Additionally, a higher proportion of left-sided cellular responses differentially coded for rewarded and unrewarded colours. It is conceivable that these two features contribute to the right eye-left hemisphere being faster in learning various visual discrimination tasks as left entopallial neurons would already start from a higher activity level and a large number of left hemisphere neurons could be recruited into an associative network (Skiba *et al.*, 2000; Prior & Güntürkün, 2001). The observed advantage of left entopallial cells in differentiating between CS+ and CS- is a probable correlate of the finding that pigeons reach higher levels of discriminatory performance with the right eye in various visual discrimination tasks (Güntürkün, 1985; Güntürkün & Bohringer, 1987; Diekamp *et al.*, 1999; Skiba *et al.*, 2002). At the same time, the left hemispheric tectofugal pathway would thereby gain a higher predictive value for reward during choice situations in discrimination tasks. In this case, reward-associated feedback systems could differently adjust left and right visuomotor circuits, thereby increasing the synaptic weights of left tectofugal output pathways (Durstewitz *et al.*, 1999; Montague *et al.*, 2004). As a consequence, the left tectofugal system could slowly start to dominate visually guided behavior of the animal during object discriminations tasks.

Obviously, this scenario is speculative at the moment. However, beyond serving as a working hypothesis for future experiments, it also highlights an important and unanswered question. Presently, we do not know why left-sided entopallial cells are able to differentiate to a higher degree between rewarded and unrewarded visual stimuli. Additional work is needed to provide a more complete account on the neuronal mechanisms of visual asymmetry in birds.

Acknowledgements

This research was financed by a Marie Curie Early Stage Research Training Fellowship of the European Community's Sixth Framework Programme under contract number MESTCT-2005-020385 (J.V.), BMBF Cognition 01GW0542 (I.V. and J.A.K.), Neurex Interreg-IV (I.V.) 01GQ0420 to BCCN Freiburg (J.A.K.), 01GQ0830 to BFNT Freiburg-Tübingen (J.A.K.), and the Deutsche Forschungsgemeinschaft through its SFB 874 (O.G.). We would like to thank the Bernstein Center Freiburg (BCF) for supporting a stay in Freiburg (J.V.). We also thank Ariane Schwarz for assistance with histology and Tobias Otto for technical support.

Abbreviations

CS–, un rewarded stimulus; CS+, rewarded stimulus; LH, left hemisphere; PSTH, peristimulus time histogram; RAM, reward-associated modulation; RH, right hemisphere.

References

- Adam, R. & Güntürkün, O. (2009) When one hemisphere takes control: metacontrol in pigeons (*Columba livia*). *PLoS ONE*, **4**, e5307.
- Andrew, R.J., Johnston, A.N., Robins, A. & Rogers, L.J. (2004) Light experience and the development of behavioural lateralisation in chicks. II. Choice of familiar versus unfamiliar model social partner. *Behav. Brain Res.*, **155**, 67–76.
- Bessette, B.B. & Hodos, W. (1989) Intensity, color, and pattern discrimination deficits after lesions of the core and belt regions of the ectostriatum. *Vis. Neurosci.*, **2**, 27–34.
- Busse, L., Katzner, S. & Treue, S. (2008) Temporal dynamics of neuronal modulation during exogenous and endogenous shifts of visual attention in macaque area MT. *Proc. Natl Acad. Sci. USA*, **105**, 16380–16385.
- Colombo, M., Frost, N. & Steedman, W. (2001) Responses of ectostriatal neurons during delayed matching-to-sample behavior in pigeons (*Columba livia*). *Brain Res.*, **917**, 55–66.
- Diekamp, B., Prior, H. & Güntürkün, O. (1999) Lateralization of serial color reversal learning in pigeons (*Columba livia*). *Anim. Cogn.*, **2**, 187–192.
- Durstewitz, D., Kroner, S. & Güntürkün, O. (1999) The dopaminergic innervation of the avian telencephalon. *Prog. Neurobiol.*, **59**, 161–195.
- Engelage, J. & Bischof, H.J. (1996) Single-cell responses in the ectostriatum of the zebra finch. *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.*, **179**, 785–795.
- Güntürkün, O. (1985) Lateralization of visually controlled behavior in pigeons. *Physiol. Behav.*, **34**, 575–577.
- Güntürkün, O. (1997) Morphological asymmetries of the tectum opticum in the pigeon. *Exp. Brain Res.*, **116**, 561–566.
- Güntürkün, O. (2000) Sensory physiology: vision. In Causey Whitton, G (Ed.), *Sturkie's Avian Physiology*. Academic Press, Manoa, pp. 1–19.
- Güntürkün, O. & Bohringer, P.G. (1987) Lateralization reversal after intertectal commissurotomy in the pigeon. *Brain Res.*, **408**, 1–5.
- Güntürkün, O. & Hahmann, U. (1994) Visual acuity and hemispheric asymmetries in pigeons. *Behav. Brain Res.*, **60**, 171–175.
- Güntürkün, O. & Hahmann, U. (1999) Functional subdivisions of the ascending visual pathways in the pigeon. *Behav. Brain Res.*, **98**, 193–201.
- Güntürkün, O. & Hoferichter, H.H. (1985) Neglect after section of a left telencephalotectal tract in pigeons. *Behav. Brain Res.*, **18**, 1–9.
- Güntürkün, O. & Kesch, S. (1987) Visual lateralization during feeding in pigeons. *Behav. Neurosci.*, **101**, 433–435.
- Güntürkün, O., Hellmann, B., Melsbach, G. & Prior, H. (1998) Asymmetries of representation in the visual system of pigeons. *NeuroReport*, **9**, 4127–4130.
- Halpern, M.E., Güntürkün, O., Hopkins, W.D. & Rogers, L.J. (2005) Lateralization of the vertebrate brain: taking the side of model systems. *J. Neurosci.*, **25**, 10351–10357.
- Hodos, W. (1969) Color-discrimination deficits after lesions of the nucleus rotundus in pigeons. *Brain Behav. Evol.*, **2**, 185–200.
- Hodos, W. & Karten, H.J. (1966) Brightness and pattern discrimination deficits in the pigeon after lesions of nucleus rotundus. *Exp. Brain Res.*, **2**, 151–167.
- Hodos, W., Macko, K.A. & Bessette, B.B. (1984) Near-field acuity changes after visual system lesions in pigeons. II. Telencephalon. *Behav. Brain Res.*, **13**, 15–30.
- Karten, H.J. & Hodos, W. (1967) *A Stereotaxic Atlas of the Brain of the Pigeon*. Johns Hopkins UP, Baltimore, MD.
- Karten, H.J., Hodos, W., Nauta, W.J. & Revzin, A.M. (1973) Neural connections of the “visual wulst” of the avian telencephalon. Experimental studies in the pigeon (*Columba livia*) and owl (*Speotyto cunicularia*). *J. Comp. Neurol.*, **150**, 253–278.
- Kroner, S. & Güntürkün, O. (1999) Afferent and efferent connections of the caudolateral neostriatum in the pigeon (*Columba livia*): a retro- and anterograde pathway tracing study. *J. Comp. Neurol.*, **407**, 228–260.
- Krutzfeldt, N.O. & Wild, J.M. (2005) Definition and novel connections of the entopallium in the pigeon (*Columba livia*). *J. Comp. Neurol.*, **490**, 40–56.
- Manns, M. & Güntürkün, O. (1999) ‘Natural’ and artificial monocular deprivation effects on thalamic soma sizes in pigeons. *NeuroReport*, **10**, 3223–3228.
- Manns, M. & Güntürkün, O. (2003) Light experience induces differential asymmetry pattern of GABA- and parvabumin-positive cells in the pigeon’s visual midbrain. *J. Chem. Neuroanat.*, **25**, 249–259.
- Manns, M. & Güntürkün, O. (2005) Differential effects of ocular BDNF-injections onto the development of tectal cells characterized by calcium-binding proteins in pigeons. *Brain Res. Bull.*, **66**, 475–478.
- Manns, M. & Güntürkün, O. (2009) Dual coding of visual asymmetries in the pigeon brain: the interaction of bottom-up and top-down systems. *Exp. Brain Res.*, **199**, 323–332.
- Manns, M., Güntürkün, O., Heumann, R. & Blochl, 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.
- McAdams, C.J. & Maunsell, J.H. (1999) Effects of attention on the reliability of individual neurons in monkey visual cortex. *Neuron*, **23**, 765–773.
- Montague, P.R., McClure, S.M., Baldwin, P.R., Phillips, P.E., Budygin, E.A., Stuber, G.D., Kilpatrick, M.R. & Wightman, R.M. (2004) Dynamic gain control of dopamine delivery in freely moving animals. *J. Neurosci.*, **24**, 1754–1759.
- Nottelmann, F., Wohlschlager, A. & Güntürkün, O. (2002) Unihemispheric memory in pigeons-knowledge, the left hemisphere is reluctant to share. *Behav. Brain Res.*, **133**, 309–315.
- Prior, H. & Güntürkün, O. (2001) Parallel working memory for spatial location and food-related object cues in foraging pigeons: binocular and lateralized monocular performance. *Learn. Mem.*, **8**, 44–51.
- Rogers, L.J. & Andrew, R.J. (2002) *Comparative Vertebrate Lateralization*. Cambridge University Press, Cambridge.
- Rogers, L.J., Andrew, R.J. & Johnston, A.N. (2007) Light experience and the development of behavioural lateralization in chicks III. Learning to distinguish pebbles from grains. *Behav. Brain Res.*, **177**, 61–69.
- Shimizu, T., Patton, T.B. & Husband, S.A. (2010) Avian visual behavior and the organization of the telencephalon. *Brain Behav. Evol.*, **75**, 204–217.
- Skiba, M., Diekamp, B., Prior, H. & Güntürkün, O. (2000) Lateralized interhemispheric transfer of color cues: evidence for dynamic coding principles of visual lateralization in pigeons. *Brain Lang.*, **73**, 254–273.
- Skiba, M., Diekamp, B. & Güntürkün, O. (2002) Embryonic light stimulation induces different asymmetries in visuoperceptual and visuomotor pathways of pigeons. *Behav. Brain Res.*, **134**, 149–156.
- Theiss, C., Hellmann, B. & Güntürkün, O. (1998) The differential distribution of AMPA-receptor subunits in the tectofugal system of the pigeon. *Brain Res.*, **785**, 114–128.
- Treue, S. & Maunsell, J.H. (1996) Attentional modulation of visual motion processing in cortical areas MT and MST. *Nature*, **382**, 539–541.
- Treue, S. & Maunsell, J.H. (1999) Effects of attention on the processing of motion in macaque middle temporal and medial superior temporal visual cortical areas. *J. Neurosci.*, **19**, 7591–7602.
- Tsushima, Y. & Watanabe, T. (2009) Roles of attention in perceptual learning from perspectives of psychophysics and animal learning. *Learn. Behav.*, **37**, 126–132.
- Valencia-Alfonso, C.E., Verhaal, J. & Güntürkün, O. (2009) Ascending and descending mechanisms of visual lateralization in pigeons. *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, **364**, 955–963.
- Vallortigara, G. & Andrew, R.J. (1994) Differential involvement of right and left-hemisphere in individual recognition in the domestic chick. *Behav. Process.*, **33**, 41–57.
- Vallortigara, G., Regolin, L., Bortolomiol, G. & Tommasi, L. (1996) Lateral asymmetries due to preferences in eye use during visual discrimination learning in chicks. *Behav. Brain Res.*, **74**, 135–143.
- Vischer, A., Cuenod, M. & Henke, H. (1982) Neurotransmitter receptor ligand binding and enzyme regional distribution in the pigeon visual system. *J. Neurochem.*, **38**, 1372–1382.
- Von Fersen, L. & Güntürkün, O. (1990) Visual memory lateralization in pigeons. *Neuropsychologia*, **28**, 1–7.
- Wannig, A., Rodriguez, V. & Freiwald, W.A. (2007) Attention to surfaces modulates motion processing in extrastriate area MT. *Neuron*, **54**, 639–651.
- Xiao, Q. & Güntürkün, O. (2009) Natural split-brain? Lateralized memory for task contingencies in pigeons. *Neurosci. Lett.*, **458**, 75–78.
- Yamazaki, Y., Aust, U., Huber, L., Hausmann, M. & Güntürkün, O. (2007) Lateralized cognition: asymmetrical and complementary strategies of pigeons during discrimination of the “human concept”. *Cognition*, **104**, 315–344.