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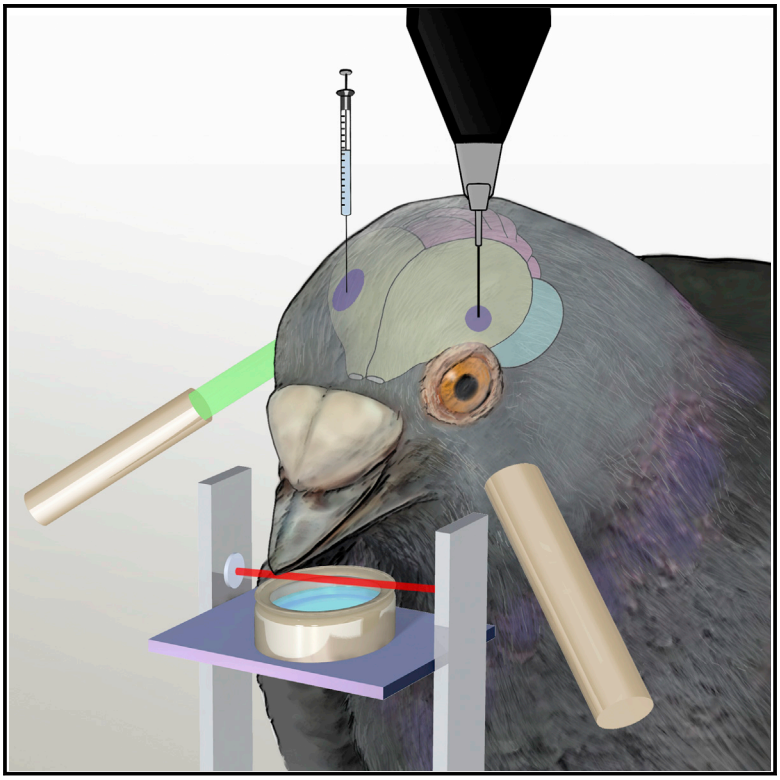
Asymmetrical Commissural Control of the Subdominant Hemisphere in Pigeons

Graphical Abstract



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In Brief

The neural mechanisms of functional brain asymmetries are mostly unknown. Birds are visually lateralized. In this work, Xiao and Güntürkün discovered that left hemispheric visuomotor forebrain neurons in the pigeon can trigger the animal's response faster by adjusting the spike time of right hemispheric neurons via asymmetrical commissural interactions.

Highlights

- Avian left hemispheric visual dominance is modified by commissural interactions
- More neurons in the left sensorimotor arcopallium are excited by visual Go-stimuli
- The left arcopallium adjusts neuronal spike time of the right arcopallium via commissures
- Lateralized activation of forebrain visuomotor areas regulates hemispheric dominance

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Asymmetrical Commissural Control of the Subdominant Hemisphere in Pigeons

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SUMMARY

Functional brain asymmetries depend both on hemisphere-specific factors and lateralized commissural interactions, but their detailed neural mechanisms are mostly unknown. Because birds are visually lateralized, we tested pigeons monocularly in a color discrimination task while recording from single visuomotor forebrain neuron. All birds learned faster and responded quickly with the right eye and left hemisphere. This asymmetry depended on three factors. First, Go-stimulus onset resulted in a higher left hemispheric proportion of excited relative to inhibited neurons such that, second, left-sided visuomotor neurons could trigger the animal's response faster. Third, the left hemisphere was able to adjust the timing of individual activity patterns of right hemispheric neurons via asymmetrical commissural interactions, such that the right hemisphere came too late to control the response. These results imply that hemispheric dominance in birds is realized by both lateralized activation of forebrain motor areas and shifts of the contralateral spike time.

INTRODUCTION

Hemispheric asymmetries are ubiquitous and can be found in diverse species, from honeybees (Rigosi et al., 2015) to killer whales (Karenina et al., 2016). In humans, they profoundly modify neurocognitive systems from perception to action and are associated with most mood- and cognition-related neural pathologies (Brandler and Paracchini, 2014). This wide spectrum is due to the fact that neural asymmetries develop very early in ontogeny (Güntürkün and Ocklenburg, 2017) and alter gray and white matter (Ocklenburg et al., 2016). In addition, a single hemisphere can control entire choice patterns. This is not only visible in split-brain patients (Gazzaniga, 2000) but also in meta-control tasks with healthy subjects in which two hemispheres are brought into conflict (Urgesi et al., 2005). Despite this relevance, we know little about the neural fundaments of brain asymmetries.

This lack of knowledge is because animal models for brain asymmetries were discovered only recently (Ocklenburg and Güntürkün, 2012; Rogers et al., 2013). Visual asymmetries in birds represent a promising animal model to approach these questions. Pigeons and chicks reveal complementary hemispheric asymmetries for different tasks. Whereas the left hemisphere excels in visual categorization of patterns and colors (Yamazaki et al., 2007; Rogers, 2014), the right hemisphere is specialized for emotionally charged stimuli (Vallortigara et al., 2011) and spatial attention (Diekamp et al., 2005; Vallortigara and Rogers, 2005). And as in humans, the hemisphere that is specialized for a stimulus class can control the entire response (Ünver and Güntürkün, 2014; Freund et al., 2016).

In pigeons, color discrimination relies mostly on the ascending tectofugal pathway of the left hemisphere (retina → contralateral optic tectum → thalamic nucleus rotundus → telencephalic entopallium), which is anatomically equivalent to the mammalian extrageniculocortical system (Mouritsen et al., 2016) (Figure 1A). The entopallium projects via surrounding visual-associative structures to the arcopallium, a structure that functionally corresponds to mammalian premotor areas (Shanahan et al., 2013). The arcopallium closes the tectofugal visuomotor loop via the descending tractus occipitomesencephalicus (TOM) to the optic tectum and further brainstem structures that control head and beak movements during visually guided ingestive behavior (Wild et al., 1985; Hellmann et al., 2004). But the arcopallium is also the critical hub for interhemispheric crosstalk via the commissura anterior (Letzner et al., 2016) (Figure 1B). In addition, this whole system is lateralized with a left hemispheric dominance for discriminating visual stimuli and producing the response (Ocklenburg and Güntürkün, 2017).

Because the arcopallium receives indirect visual input, controls descending motor pathways, and connects both hemispheres, we set out to identify how commissural interactions shape visuomotor choice behavior in a lateralized way. To this end, we trained pigeons to perform different Go or NoGo color discriminations with each eye while recording from the left or right arcopallium. We show that three main factors drive left hemispheric dominance. First, Go-stimuli activate a higher proportion of excited visuomotor neurons relative to the inhibited visuomotor neurons in the left arcopallium. Second, this enables faster left hemispheric control of brainstem motor structures and thus drives the animals' response. Third, the left hemisphere controls via commissural projections the timing of right-sided arcopallial neurons. Thereby, the left hemisphere can delay the activity of right hemispheric neurons such that they come too late to determine the animal's behavior.

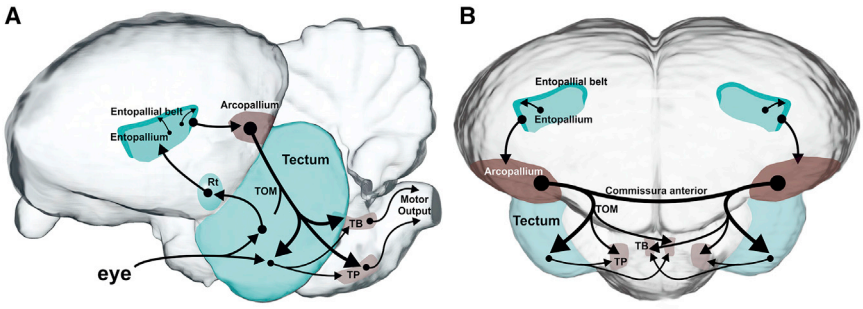

Figure 1. Ascending and Descending Pathways of the Visual Tectomotor Pathways in Pigeons

Schematic sagittal (A) and frontal (B) overview of the tectomotor visual loop in the pigeon brain. Most retinal ganglion cells project to the contralateral tectofugal pathway (optic tectum, thalamic nucleus rotundus [Rt], telencephalic entopallium), which projects via the associative entopallial belt to the premotor arcopallium. From there, the tractus occipitomesencephalicus (TOM) descends and closes the loop by contacting both ascending visual as well as descending motor neurons of the tectum. Overall, these descending projections activate premotor neurons of the tectobulbar (TB) and tectopontine (TP) systems that control ingestive behavior.

RESULTS

We conducted two experiments. The aim of the first experiment was to reveal the possibly asymmetrical activity patterns of arcopallial neurons during color discrimination. We hypothesized that these asymmetries were due to the differential level of activity and/or the relative timing of arcopallial neurons. During test sessions, we recorded from one arcopallium (e.g., the left), while the animals discriminated the stimuli with their contralateral eye (e.g., the right).

Pigeons Learn Faster with Their Right Eye and Left Hemisphere

Six head-fixed adult pigeons were trained to activate an automatic water release via an infrared light barrier by opening the lower jaw. The water-deprived animals quickly learned to operate this system. Subsequently, they learned a Go or NoGo task for water reward. To this end, each eye and hemisphere learned its own pair of isoluminant Go and NoGo colors (Figures 2A and 2B). Because pigeons show right eye and left hemisphere superiority for color discrimination (Güntürkün, 1997), we use the terms ‘‘dominant’’ and ‘‘subdominant’’ to label the left and right hemispheres, respectively.

All six pigeons reached the learning criterion faster with the right eye (right eye, 25.7 ± 8.4 sessions; left eye, 48.5 ± 14.9 sessions; $p = 0.03$, Wilcoxon test) (Figure 2C). During subsequent recording sessions, animals strongly responded to Go-stimuli and rarely to NoGo-stimuli with both monocular viewing conditions (right eye, 85.5% ± 0.45%; left eye, 86% ± 0.41%; $n = 115$ sessions). On average, pigeons responded faster with the right eye (right eye, 1.15 ± 0.17 s; left eye, 1.31 ± 0.18 s; $p = 0.03$, Wilcoxon test) (Figure 2C). We now report behavioral and physiological findings after stimulation of the contralateral eye. In all birds, we recorded from both hemispheres.

Left and Right Arcopallial Neurons Show Different Proportions of Excitation and Inhibition

After reaching criterion with both eyes, we recorded 457 arcopallial neurons while the animals discriminated colors (Figure S1). Two hundred eighty-six neurons were task related (139 left, 147 right). Most arcopallial neurons were excited by stimulus onset, while some were inhibited. In the dominant left hemisphere, 123 neurons (88.5%) were excited and 16 neurons (11.5%) were inhibited. The comparable numbers for the subdominant right arcopallium were 73 excited (49.7%) and 74 inhibited (50.3%) neurons . Thus, two arcopallia displayed a significantly different proportion of excited and inhibited neurons ($p < 0.001$, chi-square test; Figure 3).

Arcopallial neurons showed different kinds of response patterns to the Go-stimulus. The largest group (109 neurons) were activated after Go-stimulus onset and returned to baseline after the animals’ response. These neurons did not respond to the NoGo-stimulus (Figures 4A and 4B). Because these neurons bridged the time between visual stimulus onset and the animal’s behavior, we called them visuomotor neurons. As we were especially interested in the possibly lateralized translation of a visual choice stimulus into a binary response, we concentrate our analyses on this cell type. Visuomotor neurons were found in roughly the same frequency in all subjects. Briefly described, four further cell types were observed (Figure S2). Response-phase neurons started to ramp up at the animals’ response onset (Figure S2A). Pre-reward, reward-phase, and post-reward neurons were activated before, during, and after reward delivery, respectively (Figures S2B,S2C, and S2D).

Twenty-four left arcopallial visuomotor neurons (66.7%) were excited, while 12 neurons (33.3%) were inhibited at Go-color onset. The comparable numbers in the right arcopallium were 28 (38.4%) and 45 neurons (61.6%) (Figure 3A). Thus, although the overall proportion of inhibited arcopallial neurons was 31.5%, this proportion increased to 52.3% for visuomotor neurons ($p < 0.001$, chi-square test). In addition, a majority of left but only a minority of right arcopallial visuomotor neurons were excited by Go-stimulus onset ($p = 0.005$, chi-square test) (Figure 3B).

Response Patterns of Visuomotor Neurons Reflect Hemispheric Differences in Behavior

Once learning baseline was reached, the pigeons did not show any asymmetry in discrimination accuracy, although right eye and left hemisphere superiority in response speed prevailed. To unravel if arcopallial neurons reflect a corresponding pattern, we first analyzed the receiver-operating characteristic (ROC) curve of visuomotor neurons. The area under the ROC curve (AUROC) gives the strength of firing selectivity, which varies

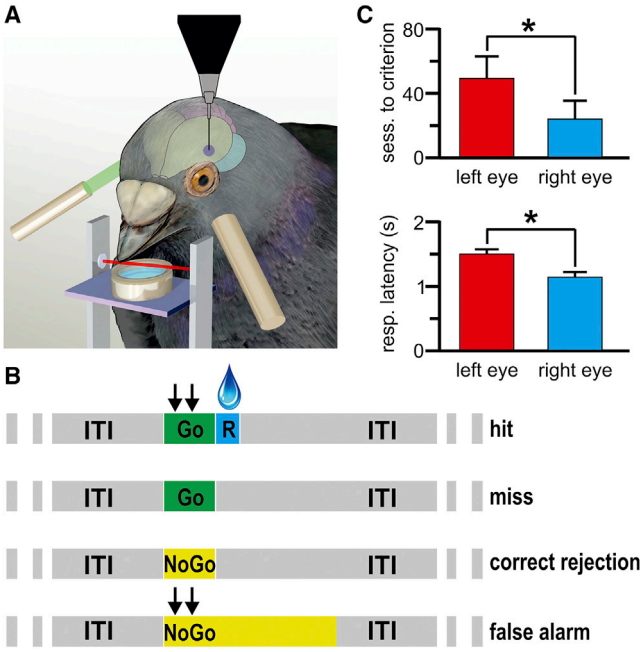

Figure 2. Color Discrimination Task Using Mandibulation as an Operant in Pigeons

(A) Opening the lower jaw activated a release of 0.24 mL water via an infrared light barrier when a Go color was shown. Here it is green on the right eye while recording from the left arcopallium. Water-deprived animals learned this Go or NoGo task for water reward. Each eye learned its own pair of Go and NoGo colors. Colors and left-right positions were balanced across animals.

(B) Schematic drawing of the discrimination paradigm. The color stimuli (green, yellow, red, and blue) were presented in pseudorandomly interleaved trials after a 15 s inter-trial interval (ITI). Then, animals had a 3 s response time to either respond to the Go-stimulus (hit) or withhold responses to the NoGo-stimulus (correct rejection). Hits were rewarded with a drop of water in the 1.5 s reward period (R). False alarms prolonged stimulus presentation to 9 s. Only one eye was stimulated per trial. Vertical arrow indicates mandibulation.

(C) Pigeons reached the learning criterion faster (mean ± SEM) with the right eye and left hemisphere and also responded faster to the Go-stimuli when viewing with the right eye and left hemisphere. *p < 0.05.

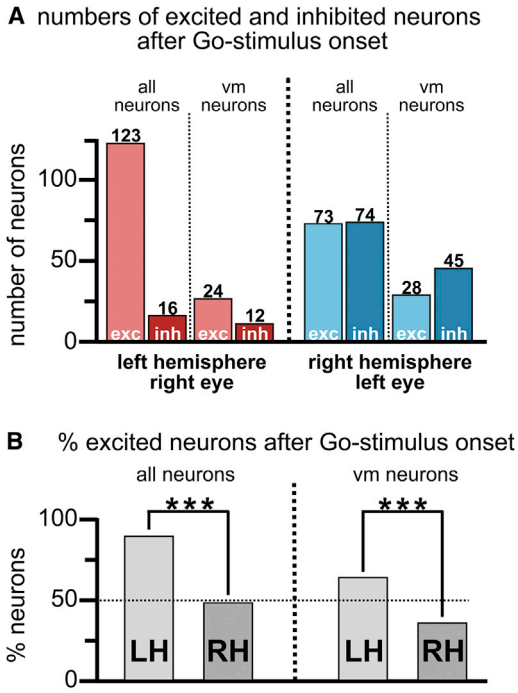

Figure 3. Numbers of Excited and Inhibited Neurons in Left and Right Arcopallia

(A) Numbers of excited (exc) and inhibited (inh) neurons in the left and right arcopallia after Go-stimulus onset (see Figure S1). Depicted are all recorded task-related neurons as well as visuomotor (vm) neurons.

(B) Percentages of excited neurons for all recorded task-related neurons (left) and only for visuomotor neurons (right) in left hemisphere (LH) and right hemisphere (RH). ***p < 0.001.

between 0 and 1. A value of 0.5 indicates no discrimination, while values of 1 and 0 indicate perfect separation with selectivity for Go-stimuli or against Go-stimuli, respectively. Neither excited (left, 0.82 ± 0.02 [n = 19]; right, 0.83 ± 0.03 [n = 21]; p = 0.18) nor inhibited (left, 0.38 ± 0.01 [n = 10]; right, 0.33 ± 0.01 [n = 40]; p = 0.08) neurons showed any discrimination asymmetry between Go and NoGo stimuli.

We then analyzed the response speed of visuomotor neurons, bearing in mind that arcopallial neurons of the left dominant hemisphere could produce faster responses by either responding more quickly to the Go-stimulus and/or by a faster activation of the motor output. To discern between these two options, we first calculated the firing onset time of each excited visuomotor neuron to contralateral Go-stimuli: a Poisson spike train analysis was applied trial by trial to detect the first burst prior to the animal's first mandibulation across all correct response trials. Mean stimulus-induced spike onset times of excited neurons (ExS_t) were comparable between left (0.74 ± 0.06 s, n = 19) and right (0.74 ± 0.08 s, n = 21) arcopallia (p = 0.97) (Figure 5A, top). Also inter-trial variances showed no left-right difference (left, 0.19 ± 0.04 s [n = 19]; right, 0.31 ± 0.08 s [n = 21]; p = 0.28). We then conducted the same analyses with the inhibited visuomotor neurons, expressed as InS_t (mean stimulus-induced inhibition onset time). Left arcopallial neurons evinced shorter response times to the Go-stimulus than those on the right (InS_t left, 0.15 ± 0.03 s [n = 10]; right, 0.49 ± 0.07 s [n = 40]; p = 0.03) (Figure 5A, bottom). Again, there was no hemispheric difference of inter-trial variance (left, 0.11 ± 0.02 s [n = 10]; right, 0.55 ± 0.2 s [n = 40]; p = 0.27). Overall, inhibited neurons responded faster to the Go-stimulus than the excited ones (p < 0.0001).

Because there was no evidence of a faster left hemispheric excitatory response to the Go-stimulus, we analyzed if the time from cellular onset to behavioral activation was lateralized. To this end, we aligned, trial by trial, all spikes of excited or inhibited visuomotor neurons to the animal's first response, expressed as ExR_t or InR_t. ExR_t values of excited neurons and InR_t values of inhibited neurons depict the mean time lag between the initial cellular response onset times and the animals' first behavioral response across all correct response trials. Our results revealed shorter left arcopallial time lag (ExR_t left, 0.57 ± 0.06 s [n = 19]; right, 0.8 ± 0.06 s [n = 21]; p = 0.02) (Figure 5B, top). Inter-trial

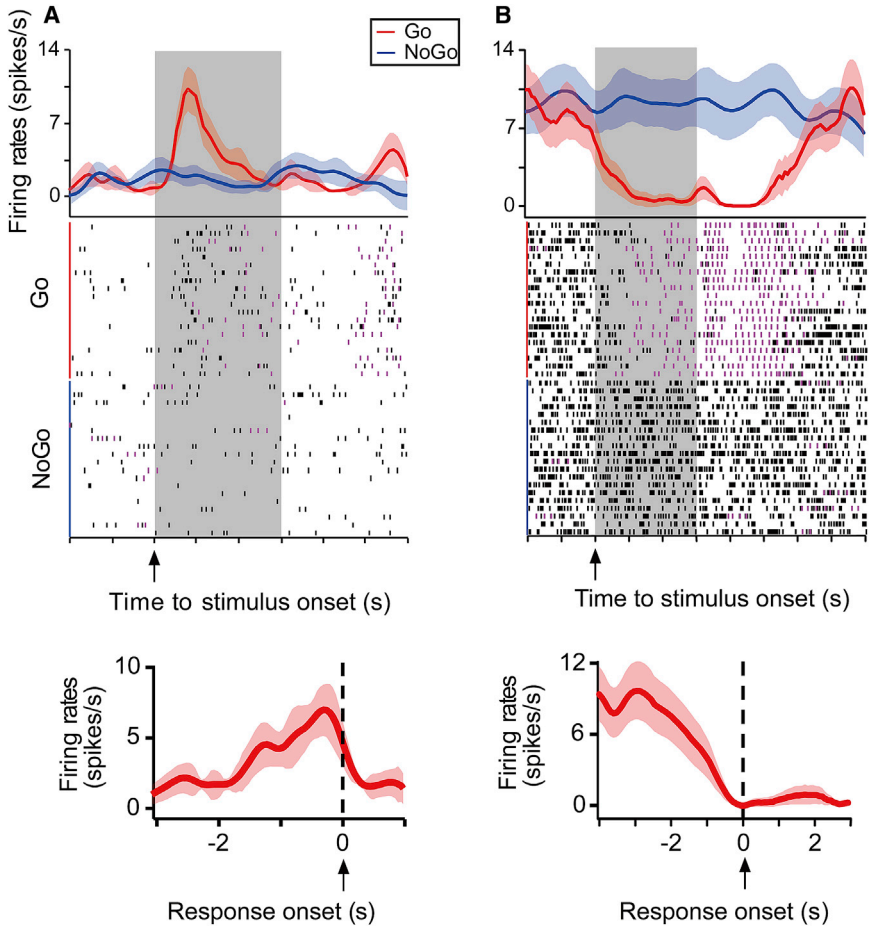

Figure 4. Response Properties of Arcopallial Visuomotor Neurons

(A) Excited arcopallial visuomotor neuron. Top: spike trains were aligned to stimulus onset. The spike frequency of this neuron increased at the beginning of 3 s Go-stimulus display time (gray shading). The firing rate (spikes/s, mean ± SEM) after Go-stimulus is shown in red, and that after NoGo-stimulus is depicted in blue. Spikes are shown as black ticks in the raster plot, while mandibulations are shown as magenta ticks. Bottom: same neuron after trial-by-trial aligning all spikes during 6 s before and after stimulus onset to the animal's first response (zero).

(B) Visuomotor arcopallial neuron was inhibited by Go-stimulus. All further details are as in (A).

For further cell types see Figure S2.

variances did not differ between two hemispheres (left, 0.38 ± 0.07 s [n = 19]; right, 0.57 ± 0.09 s [n = 21]; p = 0.14). No significant interhemispheric differences were found for lnR_t scores (left, 0.93 ± 0.11 s [n = 10]; right, 1.13 ± 0.09 s [n = 40]; p = 0.29) and corresponding inter-trial variances (left, 0.97 ± 0.22 s [n = 10]; right, 1.62 ± 0.23 s [n = 40]; p = 0.18) (Figure 5B, bottom). Thus, excited left arcopallial visuomotor neurons were faster in bridging cellular and behavioral response onsets.

We then compared the cellular peak response times relative to the animal's first mandibulation. Although left and right excited neurons had comparable peak responses (left, 19.57 ± 3.48 spikes/s; right, 18.79 ± 2.81 spikes/s; p = 0.86), left visuomotor neurons reached their peak responses earlier (left, −0.23 ± 0.06 s; right, −0.09 ± 0.03 s; p = 0.03). To compare the neuronal activities between two hemispheres, the firing rates of left and right arcopallial neurons were measured in time windows of 200 ms. The time window was moved in steps of 50 ms along the time line from 3 s before first mandibulation until 200 ms after the animal's first response. We found that a significantly larger number of excited left arcopallial neurons reached their peak responses prior to the animal's first mandibulation (left, 74% [14 of 19]; right, 38% [8 of 21]; p = 0.03, chi-square test) (Figure 5C, top). Nothing comparable was visible for the inhibited neurons (Figure 5C, bottom).

Taken together, arcopallial visuomotor neurons revealed highly asymmetrical activity patterns. As a result, Go-stimuli activated a majority of excited visuomotor neurons in the visually dominant left hemisphere, while most of those in the subdominant right were inhibited. In addition, left-sided excited neurons were overall faster in activating the motor response after spike onset. They also peaked faster and earlier before the animal's response onset, relative to neurons on the right. These data might imply that the animals' behavior is controlled mostly by left arcopallial neurons, as they are faster to activate the response. In the second experiment, we used the temporal perturbations of the functionality of left or right arcopallium to test this preliminary conclusion. This was the aim of the second experiment.

Unilaterally Inactivating the Arcopallium during Color Discriminations

Letzner et al. (2016) showed that arcopallial neurons constitute the bulk of the commissura anterior and project homotopically to the contralateral side. The aim of the second experiment was to test if the observed left-right differences result from asymmetrical interactions via this commissure. To this end, we temporarily inactivated left or right arcopallium during the discrimination task, while simultaneously recording from the non-anesthetized contralateral arcopallium (Figure S1). For example, when the left arcopallium was anesthetized, we recorded from the right arcopallium while the birds discriminated colors with the left eye. Our hypothesis was that the dominant left arcopallium could inhibit and/or delay the activity of right visuomotor neurons more than the other way round. Discrimination sessions with saline injections served as controls. Because one bird of the first experiment had died before the second study started, we used five animals with left and five with right arcopallium cannula injections.

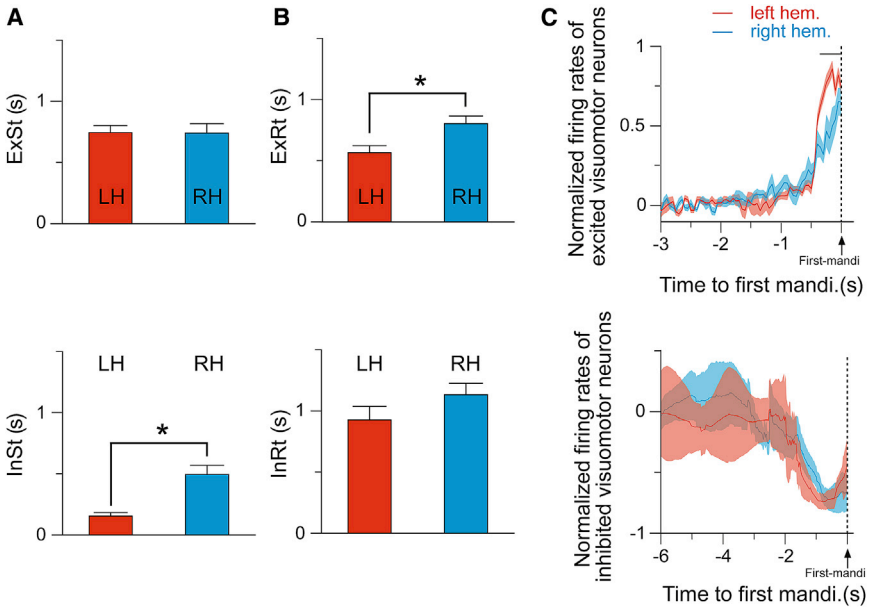

Figure 5. Time-Related Response Properties of Left and Right Arcopallial Visuomotor Neurons

(A) Analyses of response latencies to stimulus onset (mean ± SEM) of excited (top; ExS_t) and inhibited (bottom; InS_t) arcopallial visuomotor neurons in left hemisphere (LH) and right hemisphere (RH).

(B) Latency from cellular spike onset to the animal's response onset (mean ± SEM) for excited (top; ExR_t) and inhibited (bottom; InR_t) arcopallial visuomotor neurons.

(C) Neuronal responses of all recorded visuomotor neurons that were excited (top) or inhibited (bottom) after Go-stimulus onset. The spike frequency function of each neuron relative to the animal's response onsets was normalized to its maximal excited (top) and inhibited (bottom) firing rates. Mean values (±SEM) of normalized neuronal responses of all neurons are shown. The short bar indicates a significant difference ($p < 0.05$) between responses of left and right arcopallial neurons in this time window.

*$p < 0.05$.

The five new animals also reached criterion faster with the right eye (right eye, 19.8 ± 6.8 sessions; left eye, 34.6 ± 9.23 sessions; p = 0.04, Wilcoxon test) and responded faster with right eye (right eye, 1.09 ± 0.11 s; left eye, 1.37 ± 0.11 s; p = 0.04).

Left-Sided Lidocaine Injections Drastically Impair Discrimination Performance

In five pigeons, lidocaine was injected into the left arcopallium while the animals worked on the color discrimination task, and neuronal responses were recorded from the right arcopallium. Lidocaine is a local anesthetic with a rapid onset of action and intermediate duration. After injection, the discrimination accuracies of the left eye (contralateral to recording, ipsilateral to lidocaine injection) dropped to chance level when viewing the Go-stimulus (pre, 87% ± 3%; post, 48% ± 4%; n = 25 sessions; p = 0.04, Wilcoxon signed rank test) (Figure 6A). In addition, response times to the Go-stimulus increased from 1.15 ± 0.15 s to 1.54 ± 0.17 s (p = 0.04) (Figure 6A). Lidocaine injections did not affect NoGo-stimulus performance (pre, 86% ± 2%; post, 85.5% ± 2%). Left arcopallial saline injections had no effects on the animal's correct responses (pre, 84% ± 1.4%; post, 83% ± 2%) and response speed (pre, 1.05 ± 0.17 s; post, 1.1 ± 0.2 s).

The effects of right arcopallial lidocaine injections (five animals, n = 26 sessions) were far less pronounced. Correct responses to Go-stimuli dropped from 88% ± 4% to 73% ± 4% (p = 0.04, Wilcoxon signed rank test) but were still higher than chance level (p < 0.01, Wilcoxon test; Figure 6B). Neither response speeds to Go-stimuli (pre, 0.99 ± 0.2 s; post, 1.13 ± 0.15 s; Figure 6B) nor correct responses to NoGo-colors were affected (pre, 86.5% ± 2.2%; post, 85.8% ± 2.1%). Saline injections also had no effects on the animal's response accuracies (pre, 84% ± 1.5%; post, 83.6% ± 1.1%) and response speed (pre, 0.89 ± 0.1 s; post, 1 ± 0.16 s; n = 6 sessions).

Thus, the animal's correct responses were more reduced by left-sided (38% ± 4%) compared with right-sided injections (15% ± 2%) (p = 0.008). Moreover, reaction times increased significantly after left-sided (0.43 ± 0.08 s) compared with right arcopallial injections (0.14 ± 0.06 s) (p = 0.03).

Left Hemispheric Inactivation Decreases Discrimination Performance of Right Arcopallial Neurons

We recorded 29 excited arcopallial visuomotor neurons from the subdominant right arcopallium before and after injecting lidocaine into the left arcopallium. Left-sided lidocaine injections significantly reduced the mean discrimination ability of right arcopallial neurons to contralateral colors (AUROC values: pre, 0.85 ± 0.01; post, 0.71 ± 0.02; p < 0.0001, paired t test; n = 29) (Figure 6C). This was due to the response reduction of both the Go (pre, 18.86 ± 2.49 spikes/s; post, 11.92 ± 1.65 spikes/s; p = 0.00003) and the NoGo (pre, 8.82 ± 1.32 spikes/s; post, 6.55 ± 0.99 spikes/s; p = 0.003) stimuli. Two neurons (2 of 29 [7%]) stopped responding to Go-stimuli, while 8 neurons shifted their response times from before to after the animal's first response (8 of 29 [27%]). Thus, only 19 right arcopallial neurons (19 of 29 [66%]) kept their pre-injection activation patterns, albeit with reduced discrimination values.

We then analyzed the activity changes of the 27 right arcopallial neurons that still responded to Go-stimuli after injection. At the first look, nothing seemed to have changed for these neurons. Neither their average peak response times (relative to Go-stimulus: pre, 2.26 ± 0.24 s; post, 2.5 ± 0.27 s [p = 0.1]; relative to mandibulation: pre, −0.12 ± 0.03 s; post, −0.21 ± 0.07 s [p = 0.21]) nor their peak responses (relative to Go-stimulus, 49.35 ± 4.38 spikes/s; post, 53.99 ± 4.23 spikes/s [p = 0.24]; relative to mandibulation: pre, 27.01 ± 5.82 spikes/s; post, 31.79 ± 5.49 spikes/s [p = 0.31]) were affected. Similarly, left-sided injections had not affected mean ExS_t (pre, 0.46 ± 0.03 s; post, 0.59 ± 0.07 s; p = 0.05) or ExR_t (pre, 0.71 ± 0.06 s; post, 0.83 ± 0.12 s; p = 0.47) values (Figure 6E). However, the variance of ExS_t and ExR_t values of these right arcopallial neurons had increased

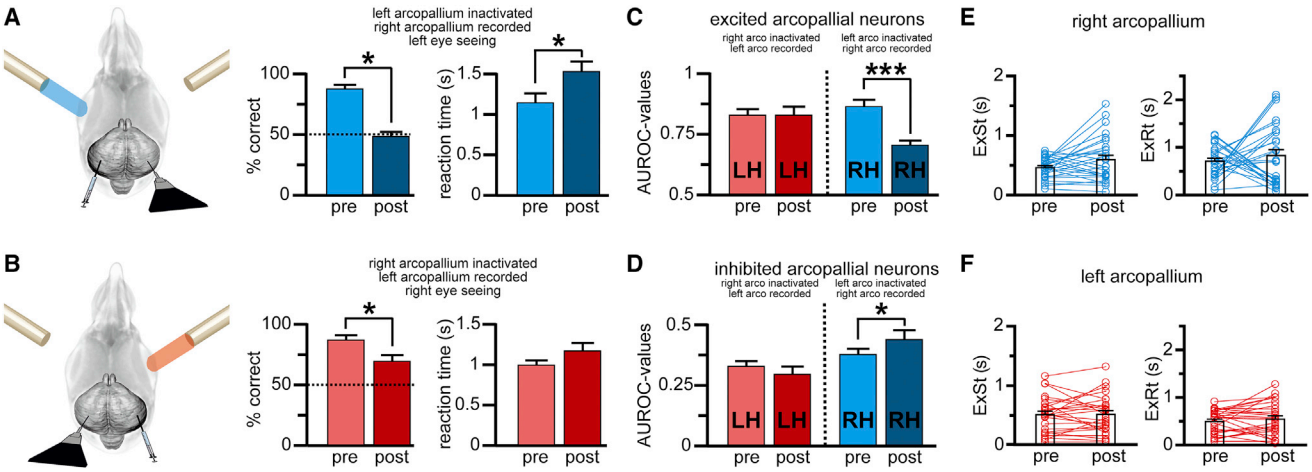

Figure 6. Left Hemisphere Arcopallial Inactivation Results in Severe Discrimination Deficits

(A and B) Color discrimination performance after (A) left or (B) right arcopallium lidocaine injection. Recording site was contralateral to both injection site and tested eye (see Figure S1). The experimental condition is depicted on the left. Accuracies and reaction times mainly reflect the performance of the hemisphere that was not directly affected by lidocaine. Correct responses (percentage of correct) and response times (s) are depicted as mean ± SEM.

(C and D) Discrimination ability (AUROC values, mean ± SEM) of left hemispheric (LH) and right hemispheric (RH) (C) excited and (D) inhibited visuomotor neurons before and after inactivation contralateral arcopallium. Each recorded side was tested with Go and NoGo stimuli delivered to the contralateral eye.

(E and F) Neuronal activity changes of excited (E) right and (F) left visuomotor neurons after unilateral inactivation of the contralateral arcopallium. ExS_t and ExR_t values are shown as individual and population mean value (±SEM) before and after injection.

*p < 0.05, ***p < 0.001.

dramatically after left-sided unilateral inactivation (pre versus post, p < 0.001 for both, F test). Thus, left-sided lidocaine injections drastically altered the individual ExS_t and ExR_t values of right arcopallial neurons. Some of these values dropped, while others increased. These parallel changes prevented a change of the overall population values (Figure 6E). It is likely that the temporal structure of activity patterns of right arcopallial neurons was under prominent control by the left arcopallium via the commissura anterior.

Next, we analyzed the 15 inhibited right arcopallial neurons that were recorded before and after left hemisphere injections. Their AUROC scores were increased from 0.38 ± 0.01 to 0.44 ± 0.02 (p = 0.03) because of elevated responses to Go-stimuli (pre, 4.29 ± 1.14 spikes/s; post, 5.29 ± 1.33 spikes/s; p = 0.01) (Figure 6D). Three neurons (3 of 15 [20%]) were no longer inhibited by Go-stimuli after injection. The 12 remaining right arcopallial neurons showed no changes of their InS_t (pre, 0.43 ± 0.13 s; post, 0.22 ± 0.07 s; p = 0.13) or InR_t (pre, 0.87 ± 0.18 s; post, 0.8 ± 0.18 s; p = 0.62) or their variances (pre versus post, p = 0.06 and p = 0.97, F test). There were also no significant changes in the inhibitory duration (pre, 2.13 ± 0.35 s; post, 1.28 ± 0.42 s; p = 0.09). However, six neurons (6 of 15 [40%]) shifted their inhibitory responses to a time after animal's first mandibulation.

Right-Sided Lidocaine Injections Leave Left Arcopallial Neurons Mostly Unaffected

We recorded 26 excited visuomotor neurons from the dominant left hemisphere before and after right arcopallium inactivation. These neurons were mostly unaffected by the contralateral lidocaine injection. AUROC (pre, 0.82 ± 0.018; post, 0.82 ± 0.02; p = 0.87; n = 26), ExS_t (pre, 0.5 ± 0.06 s; post, 0.51 ± 0.06 s; p = 0.9), and ExR_t (pre, 0.49 ± 0.05 s; post, 0.54 ± 0.07 s; p = 0.27) values (Figures 6C and 6F) were not changed, and ExS_t and ExR_t variances (pre versus post, p = 0.75 and p = 0.08, F test), mean peak responses (pre, 44.37 ± 5.37 spikes/s; post, 39.57 ± 5.03 spikes/s; p = 0.09), and peak response times (pre, 2.08 ± 0.3 s; post, 1.96 ± 0.31 s; p = 0.15) were also not affected. Except for three neurons, most left arcopallial neurons (23 of 26 [88%]) still started firing prior to the animal's first mandibulation. The only significant effect of a right arcopallial lidocaine injection was that the excited visuomotor neurons of the left arcopallium reached peak responses earlier (pre, −0.22 ± 0.05 s; post, −0.37 ± 0.06 s; p = 0.03; n = 26).

Similarly, unilateral right-sided arcopallial inactivation had no significant effects on the inhibited neurons in the left arcopallium. Before injection, we separated 14 inhibited neurons from the left arcopallium. None of them lost their preceding inhibitory responses before the animal's first response, and there were no changes in their AUROC (pre, 0.33 ± 0.02; post, 0.32 ± 0.02; p = 0.69) (Figure 6D), InS_t (pre, 0.18 ± 0.05 s; post, 0.27 ± 0.07 s; p = 0.26), and InR_t (pre, 0.84 ± 0.11 s; post, 0.88 ± 0.16 s; p = 0.79) values. Also the firing rates and variances of InS_t and InR_t values were unaltered (pre versus post, p = 0.15 and p = 0.25, F test).

Lidocaine Inactivation Effects Were Compared between Two Hemispheres

Unilateral arcopallial inactivation affected the neuronal responses of each injection group, but dramatic effects were observed only after inactivating neuronal activity in the left hemisphere. To compare this asymmetry of injection effects, we subtracted for each neuron the post-injection ExS_t and ExR_t values

from those before injection and then compared the resulting values between right and left arcopallia. This was done for the neurons that were still spiking after lidocaine injections and thus could contribute to the animal's response. The same was done with the InS_t and InR_t values. The lidocaine-induced ExS_t value changes of excited neurons were comparable between hemispheres (post-pre: right, 0.27 ± 0.05 s [n = 27]; left, 0.18 ± 0.03 s [n = 26]; p = 0.15). However, lidocaine injections into the contralateral arcopallium increased ExR_t values of right arcopallial neurons to a larger extent than those of the left arcopallium (post-pre: right, 0.68 ± 0.12 s [n = 19]; left, 0.18 ± 0.04 s [n = 23]; p = 0.0001). For inhibited neurons, unilateral inactivation had comparable effects between left and right hemispheres (InS_t: post-pre: right, 0.38 ± 0.08 s [n = 12]; left: 0.24 ± 0.04 s [n = 14]; p = 0.13; InR_t: right, 0.28 ± 0.15 s [n = 6]; left, 0.47 ± 0.09 s [n = 14]; p = 0.28).

Furthermore, the same analyses were applied to compare variance changes of ExS_t and InS_t values and ExR_t and InR_t values between the right and left arcopallia. No significant variance changes of ExS_t values were observed between two injection groups (post-pre: right, 0.19 ± 0.06 s [n = 27]; left, 0.19 ± 0.05 s [n = 26]; p = 0.97), but the variance changes of ExR_t values of right arcopallial neurons were larger (post-pre: right, 0.54 ± 0.11 s [n = 19]; left, 0.2 ± 0.04 s [n = 23]; p = 0.0005). For inhibited neurons, the variance changes of InS_t (post-pre: right, 0.31 ± 0.09 s [n = 12]; left, 0.32 ± 0.1 s [n = 14]; p = 0.91) and InR_t values were comparable between two groups (post-pre: right, 0.29 ± 0.07 s [n = 6]; left, 0.35 ± 0.07 s [n = 14]; p = 0.59).

The AUROC score reduction of excited neurons in the right arcopallium was larger than those of left arcopallial neurons (post-pre: right, 0.11 ± 0.02 [n = 29]; left, 0.04 ± 0.01 [n = 26]; p = 0.001). For inhibited neurons, AUROC score changes were comparable between two injection groups (post-pre: right, 0.07 ± 0.02 [n = 15]; left, 0.07 ± 0.01 [n = 14]; p = 0.91).

Taken together, unilateral arcopallial lidocaine injections revealed that the dominant left and the subdominant right arcopallia interact asymmetrically via the commissura anterior. As a result, inactivating the left arcopallium drastically reduced the animals' correct responses and prolonged their reaction times. Right-sided inactivation produced only minimal effects. Similarly, left arcopallial lidocaine injections reduced right arcopallial AUROC values, prolonged ExR_t times, and increased their variability. Our results might imply that the left hemispheric visual dominance in pigeons could in part result from the ability of the left hemisphere to modify the activity of right arcopallial visuomotor neurons, thereby gaining control of the animal's behavior.

DISCUSSION

Asymmetries of brain functions are assumed to depend both on hemisphere-specific factors and inhibitory commissural interactions by which the subdominant hemisphere is inhibited during task execution (Ocklenburg et al., 2016). Lateralized commissural inhibition is thought to ensure that the dominant side takes control of the response such that the subdominant hemisphere has no chance to produce competing actions (Gazzaniga, 2000). Our results are the first that tested these decade-old assumptions at the single-unit level in an animal model and discovered a partly different picture. In short, the results of two experiments make it likely that the neural fundaments of brain asymmetries, at least in pigeons, are not about inhibition or excitation but mostly about time differences between two hemispheres.

Hemisphere-specific mechanisms unfolded at three levels. First, left and right arcopallial neurons responded equally fast to stimuli, but the left hemisphere had a clear time advantage at triggering reactions. Second, the Go-stimulus activated more visuomotor neurons in the left premotor arcopallium such that this hemisphere dominated the animal's behavior. Third, the left arcopallium modulated the spike time of the right arcopallium via the commissura anterior and thus controlled the timing of the activity patterns of the right hemispheric visuomotor neurons. Below, we argue that these three factors are interrelated aspects of a larger picture. We first discuss a few basic facts about our animal model.

The Avian Left Hemisphere Dominates Visual Feature Discrimination

Many non-human animals are lateralized (Ocklenburg et al., 2013; Ströckens et al., 2013), but the number of species in which the neural foundations of these asymmetries could be delineated is limited (Güntürkün and Ocklenburg, 2017). Avian species such as pigeons and chicks belong to these few animal models. Birds reach higher accuracy and speed when using their right eye and left hemisphere to discriminate objects on the basis of visual features such as color, shape, and pattern (Güntürkün and Kesch, 1987; Prior and Güntürkün, 2001; Valenti et al., 2003; Yamazaki et al., 2007; Rogers, 2014). This left hemispheric superiority is task dependent. Indeed, the right hemisphere is superior when birds engage in visually guided behavior that is based on spatial, social, or affective cues (Vallortigara et al., 2011; Rosa Salva et al., 2012). Thus, our findings do not reflect a static hemispheric dominance but the dynamics of hemisphere-specific circuits while processing specific cues. Thus, the phrase "dominant hemisphere" as we used here always implies a temporal left hemispheric superiority during color discrimination.

As expected, the pigeons of the present study learned faster, reached higher discrimination scores and showed shorter reaction times using their right eyes while discriminating colors. Only after reaching learning criterion, left-right accuracy differences vanished, possibly because of ceiling effects, while response speed asymmetries prevailed. This is typical for visual discrimination tasks with pigeons (Güntürkün, 1997).

Visual Asymmetries Depend on Lateralized Proportions of Excited and Inhibited Neurons

The proportion of excited relative to inhibited neurons was about 2:1 in the left arcopallium but only 0.6:1 in the right arcopallium. Thus, most left arcopallial visuomotor neurons were activated by the Go-stimulus, whereas most comparable neurons on the right were inhibited. Because the specificities of the task determine whether the left or right hemisphere dominates (Vallortigara and Rogers, 2005), this arcopallial ratio must depend on the current processes of upstream visual structures. Indeed, the primary visual structures in pigeons already show left-dominant activity patterns during color discriminations (Verhaal et al.,

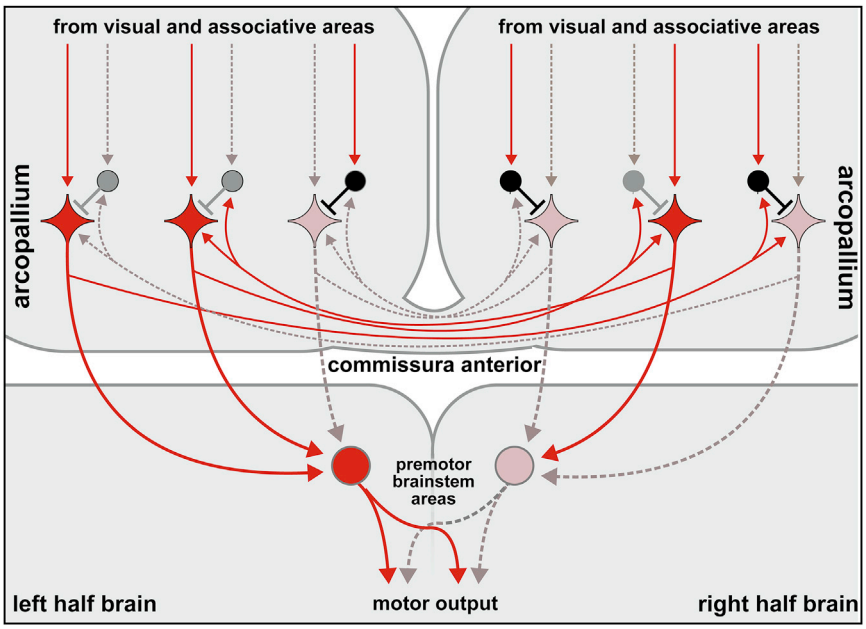

Figure 7. Model of the Neural Foundations of Avian Visual Asymmetry

Hypothetical schema of arcopallial visual afferents, brainstem projections, and commissural interactions. Excited neurons and axons are shown in red; inhibited ones are pink and with dotted lines. Arcopallial visuomotor neurons are star shaped, while inhibitory neurons are round. During color discrimination, visual areas of the left hemisphere are highly active and consequently activate downstream left visuomotor neurons. Most right-sided arcopallial visuomotor neurons are not activated by descending input but inhibited via local inhibitory neurons. Arcopallial neurons also project to homotopic targets via the commissura anterior and thereby adjust contralateral activity patterns by delaying or accelerating spike time. As a result, the left hemisphere can delay right arcopallial neurons such that they come too late to gain control over the animal's response. Consequently, brainstem premotor neurons and the animals' behavior are mostly controlled by left arcopallial neurons.

2012; Freund et al., 2016). These stimulus-dependent visual asymmetries could activate different proportions of arcopallial visuomotor neurons and GABAergic interneurons. Letzner et al. (2016) demonstrated that arcopallial principal neurons are surrounded by GABAergic interneurons. If left-right differences of visual input into the arcopallium would primarily drive principal neurons or inhibitory interneurons, they could mainly activate or inhibit the descending motor output.

Descending arcopallial neurons drive brainstem premotor structures via the TOM to control head, beak, and body movements during ingestive behavior (Wild et al., 1985; Dubbeldam and Den Boer-Visser, 1994; Hellmann et al., 2004). These downstream projections are bilateral and thus control whole-body movements. Therefore, the inactivation of the left arcopallium could indeed reduce overall response probabilities and reaction speed. Accordingly, transection of left-sided TOM fibers result in similar deficits (Güntürkün and Hoferichter, 1985).

Functional Asymmetries Depend on Hemisphere-Specific Speed

We could not find any asymmetry in spike latencies to the Go-stimulus (ExS_t) but significantly shorter left hemispheric latencies between cellular spike onset times and the animal's behavioral reactions (ExR_t). Thus, the arcopallial visuomotor neurons in the left hemisphere triggered the conditioned response faster. In addition, 74% of left but only 38% of right hemispheric visuomotor neurons reached their peak firing time before the animal's first response. As a result, if both hemispheres would in parallel rush to activate downstream motor neurons of the brainstem, the right arcopallium would in most cases simply come too late to determine the action. This finding explains the results of pigeons in meta-control tasks (Ünver and Güntürkün, 2014; Freund et al., 2016) for which the left hemisphere dominates the animal's decisions, although both hemispheres could in principle contribute equally. These observations suggest that the hemispheric asymmetry rests to some extent on hemisphere-specific speeds of sensorimotor transduction.

Some of this uneven speed could result from the biased proportion of excited and inhibited neurons we discussed above. If a large number of left arcopallial neurons would jointly activate brainstem premotor areas, it is likely that they trigger a fast behavioral response. As a consequence, left arcopallial visuomotor neurons would have shorter latencies between spike onset and the animal's response (ExR_t), although the latency between stimulus and spike onset (ExS_t) evinced no asymmetry. This assumption is part of our working hypothesis on the neural mechanisms of avian visual asymmetries (Figure 7).

The Left Arcopallium Controls the Temporal Structure of the Right-Sided Activity Patterns

We observed that lidocaine injections into the dominant left arcopallium drastically increased the variance of ExS_t and ExR_t values. Thus, the temporal structure of right arcopallial neuronal response was controlled mostly by the left side. This control implies both excitation and inhibition, as left lidocaine injections slowed down some right-sided neurons while accelerating others. This mechanism gives both hemispheres the ability to either recruit neural resources of the other hemisphere or to delay the other side when competing (Figure 7). Thus, our findings contradict the assumption that the dominant left hemisphere simply inhibits the right.

The Neural Fundaments of Asymmetries in a Comparative Context

Could these findings also shed light on the commissural mechanisms of functional asymmetries in humans? Little is known about the human commissura anterior, but the human corpus callosum is a key structure for the emergence and maintenance

of brain asymmetries (Gazzaniga, 2000). Most callosal fibers arise from glutamatergic pyramidal neurons (Kumar and Huguenard, 2001), but the main effect of callosal activation is inhibitory (van der Knaap and van der Ham, 2011). This is because callosal axons either activate contralateral inhibitory interneurons or first excite contralateral pyramidal neurons, which then induce surround inhibition by driving GABAergic interneurons (Kumar and Huguenard, 2001). This arrangement ensures that depending on task condition, the net effect of callosal activation can be either inhibitory or excitatory (Bloom and Hynd, 2005). Consequently, callosal mechanisms can recruit bilateral cortical resources when working on demanding tasks (Weissman and Banich, 2000) or inhibit the other side under conditions of hemispheric competition (Putnam et al., 2008). How these contradictory actions are realized by the same commissure is presently unclear.

Our results offer a solution to this seeming discrepancy. We suggest that the control of the action time of the other hemisphere is a key variable of any bilateral organism. Consequently, these organisms need a commissural system that enables two hemispheres to flexibly switch back and force between recruiting contralateral resources or inhibiting them. According to our results, this is realized in pigeons by adjusting contralateral spike times. The avian visual system is in many ways different from that of primates. If, however, a similar mechanism could be discovered in humans, it could solve a core riddle of brain asymmetries in our species.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

Supplemental Information includes two figures and can be found with this article online at https://doi.org/10.1016/j.celrep.2018.10.011.

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AUTHOR CONTRIBUTIONS

Q.X. performed experiments and data analyses. Q.X. and O.G. designed experiments, interpreted results, and jointly wrote the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals		
isoflurane	AbbVie, Germany	Lot# 6070961
ketamine hydrochloride	Bela-Pharm, Germany	Lot# FS 1670041
xylazine hydrochloride	Bayer, Germany	Lot# KPOBZPE
sodium heparin	Ratiopharm, Germany	Lot# S15746
pentobarbital	Merck, Germany	Lot# SLBF7349V
lidocaine	AstraZeneca, UK	Lot# T391B
Software and Algorithms		
MATLAB	MathWorks(R2009a),USA	https://www.mathworks.com; RRID: SCR_001622
Spike2 system (Micro1401-mk2)	Cambridge Electronic Design Ltd., UK	http://ced.co.uk/
Other		
Eckhorn System	Thomas recording, Germany	http://www.thomasrecording.com
guide cannula	PlasticsOne, Roanoke, USA	http://www.plastics1.com
AxioImager M1 microscope	Carl Zeiss, Germany	https://www.zeiss.de/corporate/home.html
microtome	Leica, Germany	https://www.leicabiosystems.com

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Qian Xiao (qianxiao@moon.ibp.ac.cn).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

All procedures were in compliance with the guidelines for the care and use of laboratory animals and were approved by the local committee (LANUV). Permission to keep the animals and conduct the study was obtained (file number 84-02.04.2013.A458) according to § 8 Abs. 1 TierSchG i.V.m. §§ 33 Abs. 2 5.2 TierSchVersV. The animals were not sexed because there is no evidence for sex differences in visual asymmetry in pigeons. Pigeons were young adults of 1-3 years. In total 11 homing pigeons (*Columba livia*) from local breeders were used in the experiment.

METHOD DETAILS

Animal training

Eleven adult homing pigeons (*Columba livia*) were used and kept on a 12 h:12 h cycle with food *ad libitum*. On days without training and testing, animals received unrestricted water. One day before training or recording days, cage water was removed in the afternoon. On the next day, about 4 ml water was obtained during a session by animals. Thereafter, birds could drink *ad libitum* for several hours. Weight and health of animals were monitored daily.

Before training, a metal head-fixation block (1 × 0.7 × 0.4 cm; 1.6 g) was glued onto the skull with dental cement while animals were anesthetized with isoflurane (~2% by volume in O_2) and body temperature was maintained at 40°C. A recording trough (~5 × 5 mm) was placed on the skull to access target areas. After surgery, birds recovered for at least 7 days. Experiments were run in a dark room. Stimuli were provided by color light-emitting diodes (LED) located at each side 5 cm away from the eye. LEDs were inserted in a 11 mm wide tube that pointed closely toward one eye to avoid light diffusion to the other eye. LEDs were controlled by custom software written in MATLAB. Animals were trained to discriminate four colors (blue, green, yellow, and red) with luminances of 0.6 cd/m^2. The colors were 2 × 2 paired and each pair of colors (Go and NoGo) was exclusively learned by one eye and hemisphere. Color pairs were balanced between animals. Using the custom-made water container (1 × 0.6 × 0.4 cm), each beak movement (mandibulation) was detected by an infrared light barrier and synchronically recorded with task events. The beak tip was in the middle of the water container. The pump needed ~0.5 s to fill or empty the container. After filling, water remained for 0.5 s (Figure 2A).

Our study had two experiments. In experiment one, we characterized visual responses of arcopallial neurons of both hemispheres (Figure 2A). A training session consisted of 80 trials with 20 trials for each color. Only one eye per trial was stimulated. Stimuli were presented in pseudorandomly interleaved trials with 15 s ITIs. Once the LED pointing to one eye was switched on (stimulus onset), the

animals had 3 s (response period) to either respond to the Go-stimulus or withhold responding to the NoGo-stimulus. Correct responses in Go-trials were rewarded after the response period. The stimulus switched off during reward. Correct NoGo responses (''rejections'') were not rewarded, but mandibulations during NoGo-trials (''false alarms'') prolonged stimulus presentation time from 3 s to 9 s (Figure 2B). When the animals' correct responses for each eye reached 85% on three continuous days, electrophysiological recordings started. In the second experiment (10 animals), we explored the interactions of both arcopallia by unilaterally inactivating the arcopallium of one hemisphere with lidocaine while recording from the other arcopallium (Figures 6A and 6B). Everything else was as in experiment one. After animals reached 85% correct responses on three continuous days, they were divided into 2 groups (right-injection; left-injection).

Extracellular recording

Before recording, a small craniotomy was made above the targeted area under isoflurane anesthesia. Animals recovered for 1-2 weeks and were retrained until reaching criterion. After each recording day, the recording trough was filled with dental silicone.

Neuronal responses from the arcopallium (Anterior: 5.50-7.50; Lateral: 6.0-8.0; Height: 5.5-7.5) (Herold et al., 2018; Karten and Hodos, 1967) of both hemispheres were recorded (1-2 MΩ) while the birds discriminated colors (Figure S1). Spikes were amplified (× 1,000-2,500), filtered (500-5,000 Hz), and signals were continuously acquired at 20.8 kHz on a 16-channels Spike2 system. Task events and beak movements were digitized at a sampling rate of 1 kHz.

After the last recording day, an electrolytic lesion was made by passing positive current of 50 μA for 20-30 s after anesthesia by injecting ketamine hydrochloride (initial dose of 40 mg/kg, followed by supplements of 20 mg/kg/h) and xylazine hydrochloride (initial dose of 5 mg/kg followed by supplements of 2 mg/kg/h) into the pectoral muscle. After electrolytic lesion, birds were deeply anaesthetized with equithesin (0.45 ml/100 g body weight). Brains were removed and histologically processed to determine exact positions of cannula and lesion sites.

Unilateral inactivation

To investigate interhemispheric commissural interactions between arcopallia, lidocaine inactivation was used (Figures 6A and 6B). Before training, a guide cannula (9 × 0.46 × 0.24 mm) was unilaterally implanted into the arcopallium (Herold et al., 2018) of either the left (5 animals) or the right hemisphere (5 animals) (Figure S1). Five of these ten animals were from the first experiment, five were newly recruited. One bird from the first study had died before the second study started. All surgery and postsurgery procedures were as described above. On days without recording, a dummy was inserted to prevent tissue and dust entry into the cannula.

The internal cannula was connected to a 5 μl Hamilton syringe filled with 2% lidocaine. 1 μl lidocaine (2%) was injected into the left or right arcopallium intermedium which is the main source of the commissura anterior. Lidocaine (1 μl, 2%) is known to inactivate neuronal response within 0.6 mm around the cannula tip for maximal 30 min, and reach maximal effect at 8 min after injection (Tehovnik and Sommer, 1997). The experiment was separated into three phases: before injection, after injection and slow recovery. Each phase consisted of 40 trials (10 trials for each color). Lidocaine effects were measured by comparing neuronal activities before and after injection. The same amount of saline was injected as control.

Data analyses

Using ANOVA, neurons were defined as task related when their firing rates during response or reward phase across all successful response trials were significantly different to the same length of spontaneous activities during ITI. Data were quantitatively analyzed offline by Spike2 software and custom-made MATLAB routines. Single unit was classified based on full wave templates and clustered by principle component analysis and direct waveform feature measures. Only well isolated units were included in this study. The spike density function of each neuronal response was estimated with kernel density estimation (Shimazaki and Shinomoto, 2010).

To calculate correlation of mandibulation-related excitatory or inhibitory neuronal responses and the animals' initial response, all spikes during ITI before the stimulus onset and 3 s response period were aligned trial by trial to the first mandibulation. For neurons with preceding responses to the first mandibulation, we further calculated 1) the time of first neuronal modulation after stimulus onset, 2) peak responses and 3) peak response time. The time of first neuronal modulation per trial was determined by Poisson spike train analysis (Hanes et al., 1995). Peak response times and peak responses were calculated on spike density functions across all successful response trials. After subtracting the mean spontaneous activity during ITI, the spike density function was the temporally pure firing rate changes.

To quantify response selectivity to Go and NoGo stimuli, spike density functions to Go and NoGo stimuli during the 3 s response period was compared with ROC analysis. Each point on the ROC curve depicted the proportion of bins (20 ms) on which the NoGo-responses exceeded a criterion level against the proportion of bins on which the Go-responses exceeded the same criterion. The criterion level was increased from minimum spikes/bin to maximum ones in one-spike increments. AUROC values give the strength of selectivity from 0 to 1, with 0.5 indicating equal responses to Go and NoGo stimuli, while values of 0/1 indicate perfect separation. For excited/inhibited neurons, the higher/lower AUROC value implied a better selectivity to Go than to NoGo stimuli.

QUANTIFICATION AND STATISTICAL ANALYSIS

We used t tests for unpaired or paired two-sample tests. One-way analysis of variance (ANOVA) was used for > 2 group comparisons and Wilcoxon signed rank test and chi-square test for non-parametric comparisons. All mean values are reported with standard errors of the mean.

DATA AND SOFTWARE AVAILABILITY

Dataset and software are available upon request.