It takes two to tango: hemispheric integration in pigeons requires both hemispheres to solve a transitive inference task

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A growing number of examples show that the left and right brain halves play differential roles in controlling behaviour not only in humans but also in other vertebrates and even in invertebrates (Concha et al., 2012; Frasnelli et al., 2012; Rogers et al., 2005). These cerebral asymmetries are presumably caused by differences in the preferential processing mode of the two brain halves and are based on structural variances between left- and right-hemispheric neuronal circuits (Herve et al., 2013; Ocklenburg et al., 2016). Several models suggest general encoding asymmetries that are shared by different vertebrate species and, hence, may have an evolutionary origin (Concha et al., 2012; Vallortigara & Rogers, 2005; Yamazaki et al., 2007). Hemispheric asymmetries might be traced back to a left-hemispheric specialization for routine behaviour and feeding and a right-hemispheric dominance for the detection of unexpected stimuli and control behaviour in emergency situations (Lippolis et al., 2009; MacNeilage et al., 2009; Vallortigara, 2000). In relation to this basic lateralization pattern, the left hemisphere is specialized to adopt a feature-based strategy by relying on local aspects of stimuli and extracting the common elements of individual stimulus patterns. In contrast, the right hemisphere preferentially encodes global information and responds to novelty, relying on memorized familiarity mechanisms to detect individual variations (Freund et al., 2016; MacNeilage et al., 2009; Manns & Ströcks, 2014; Yamazaki et al., 2007).

Consequently, left- and right-hemispheric networks eventually process information in their specialized relatively independent ways. These differences often result in the dominance of one hemisphere to adopt a specific function but can also lead to conflicts when both hemispheres assess information according to their preferential processing style (Manns & Ströcks, 2014; Turner et al., 2015; Vallortigara et al., 2004). This conflict entails the dominance of one hemisphere to...
control behavioural output (Van der Knaap & Van der Ham, 2011). In other situations, the hemispheres must work together, for example to acquire a complete representation of the environment, or to combine their expertise for optimal cognition (e.g., Friederici, von Cramon, & Kotz, 2007).

Interhemispheric interactions are accomplished by commissural systems that interconnect the two halves of the nervous systems in all animals (Letzner, Simon, & Güntürkün, 2016). The functional organization of the underlying neuronal processes is still under debate and may depend on the current functional requirements including transfer of information to the specialized hemisphere, inhibition of the subdominant brain side, or combination of left- and right-hemispheric processes (Bloom & Hynd, 2005; Gazzaniga, 2000; Van der Knaap & Van der Ham, 2011) and certainly differs depending on the overlap of sensory input. Thereby, the mode of interhemispheric interactions might be affected by incoming bottom-up information and/or attentional top-down mechanisms mediating current goals or expectations. In the brain of placental mammals, a large body of interhemispheric communication is mediated by the corpus callosum (Bloom & Hynd, 2005; Gazzaniga, 2000; Herve et al., 2013; Van der Knaap & Van der Ham, 2011) but lateralized interhemispheric processes do not solely depend on this major interhemispheric commissure. (Manns & Güntürkün, 2009) Information transfer via the corpus callosum rather impedes the analysis of lateralized interhemispheric processing (e.g. Bergert, Windmann, & Güntürkün, 2006). Thus, an understanding of interhemispheric interactions can profit from model systems lacking a corpus callosum. The avian brain is such a model.

Birds can master complex cognitive tasks (Clayton & Emery, 2015; Güntürkün & Bugnyar, 2016; Jarvis et al., 2005; Vallortigara, 2006, 2012) whereby the two brain halves contribute differently to cognitive challenges. Functional asymmetries in chicks, *Gallus gallus*, and pigeons, *Columba livia*, are related to structural left–right differences that develop in close gene–environment interactions (Güntürkün, Stütting, & Manns, 2014; Manns & Güntürkün, 2009; Rogers, 2014; Vallortigara & Rogers, 2005). Hemispheric specialization can be easily tested just by temporarily occluding one eye with an eye cap, i.e. by monocular testing. Since the optic nerves cross virtually completely, information from the left eye is primarily directed to the right brain side and vice versa. Nevertheless, hemispheric-specific information can be exchanged (Letzner, Patzke, Verhaal, & Manns, 2014; Skiba, Diekamp, Prior, & Güntürkün, 2000; Valencia-Alfonso, Verhaal, & Güntürkün, 2009) and combined (Manns & Römling, 2012) via subcortical commissural systems (Letzner et al., 2016). Interhemispheric cooperation is indicated by quantitative advantages of using both eyes compared to monocular performances (Güntürkün et al., 2000; Watanabe, Hodos, & Bessette, 1984).

Hemispheric cooperation in pigeons can be investigated in a transitive inference paradigm (Manns & Römling, 2012). Like several animal species (Vasconcelos, 2008), pigeons are able to infer a relation between two items that have not been presented together before (Lazareva, Kandray, & Acerbo, 2015; Siemann, Delius, & Wright, 1996). After learning to discriminate overlapping pairs of stimuli (A>B−, B>C−, C>D−, D>E−) whereby + indicates rewarded and − unrewarded stimuli, pigeons rank the items by transitive inference logic (A>B>C>D>E). When each hemisphere learned only half of the premise stimulus pairs (i.e. one hemisphere learns A>B−/B>C− and the other learns C>D−/D>E−), information from both brain halves must be combined to establish the transitive line. Pigeons can successfully master this problem when seeing with both eyes after monocular learning (Manns & Römling, 2012). How the two brain halves solve this complex problem is still unclear but an answer to this question helps us understand the functional organization of interhemispheric cooperation. In the present study, we explored hemispheric-specific contributions by training and testing pigeons under monocular seeing conditions. Monocular and therefore hemisphere-specific choices when pigeons are confronted with critical test pairs should indicate whether and which hemisphere responds by transitive inference logic and, hence, is able to integrate interhemispheric information. Superior performances of one hemisphere can result from the dominance in adopting a transitive inference strategy, or from better access to transfer information. Relational learning in chickens indicates a right-hemispheric superiority in transitive reasoning (Daisley, Mascalzoni, Rosa-Salva, Rugani, & Regolin, 2009; Daisley, Vallortigara, & Regolin, 2010). In pigeons, in contrast, the left hemisphere has better access to interhemispheric information (Letzner et al., 2014; Valencia-Alfonso et al., 2009). Moreover, cells within the left visual forebrain differentiate to a higher degree between rewarded and unrewarded stimuli after associative learning, indicating a leading role in reward-associated feedback systems (Verhaal, Kirsch, Vlachos, Manns, & Güntürkün, 2012). The left hemisphere generally dominates visuomotor processing (Manns & Güntürkün, 2009; Manns & Ströcken, 2014), stores memories on sensorimotor integration tasks (Nottelmann, Wohlschläger, & Güntürkün, 2002) and tends to dominate decisions in conflict situations (Adam & Güntürkün, 2009; Freund et al., 2016; Unver & Güntürkün, 2014). Therefore, the left hemisphere might be better prepared to combine information from both brain sides and/or to control choice behaviour during critical transitive tests. On the other hand, both hemispheres may contribute to the solution of this problem. In this case, unihemispheric performances should differ from bihemispheric ones.

**METHODS**

We obtained 28 adult domestic pigeons from local breeders and split them into two groups for two consecutive, independent experiments (first group: 12 birds; second group: 16 birds; one pigeon had to be excluded due to learning impairments). The birds were housed in individual cages (45 × 40 cm and 40 cm high) where they were also trained and tested. They were kept food deprived to approximately 80–90% of their free-feeding weight throughout the experiment. Individual mass was kept within an ecologically relevant level and welfare was not affected by this level of food restriction (e.g. Kangas & Branch, 2006). Water and grit were freely available whereas food was provided daily after training or testing and over the weekend. During the monocular sessions, one eye was temporarily covered with an opaque cardboard cap that was fixed around the eye with Velcro tape. To this end, the hard side of a Velcro ring was glued onto the feathers around the eyes using a nontoxic, solvent-free adhesive (UHU Bastelkleber) while the smooth side was fixed to the cardboard cap.

The study was carried out in compliance with the European Communities Council Directive of November 24, 1986 (86/609/EEC) and the specifications of the German law for the prevention of cruelty to animals, and was approved by the animal ethics committee of the Landesamt für Natur, Umwelt und Verbraucherschutz NRW, Germany. All efforts were made to minimize the number of birds used and to minimize suffering.

**Rationale of the Task**

The transitive inference task was designed as simultaneous colour discriminations in which pigeons were first trained to discriminate four overlapping pairs of stimuli, A>B−B>C−C>D−D>E−, that represent a linear hierarchy (A>B>C>D>E). Letters
stand for five varieties of nontoxic aquarium grit differing in particle colour and size. A grain of maize buried under the positive grit (S+) rewarded a correct choice. To achieve hemispheric-specific knowledge, each eye and, hence, the contralateral hemisphere (LH: left hemisphere; RH: right hemisphere) learnt to discriminate only two of the premise pairs that represented the beginning (A+ B−, B+ C−) or end (C+ D−, D+ E−) of the transitive line (Manns & Römling, 2012). To consider relational learning, the pigeons were later confronted with stimulus combinations that had not been presented together before (Fig. 1).

We tested two experimental groups: one group learnt the beginning of the transitive line with the left hemisphere (LH-B/RH-D) and the other group with the right hemisphere (RH-B/LH-D, Fig. 1). The first experiment (12 birds: six LH-B/RH-D and six RH-B/ LH-D) indicated that some pigeons of the RH-B/LH-D group (and none of the LH-B/RH-D) made choices by transitive inference logic when tested with the left hemisphere. To further explore the critical left-hemispheric performance, we trained a second group of 16 biasing the number of experimental birds (six LH-B/RH-D and 10 RH-B/LH-D). In the Results, we present the combined data of both experiments (compare individual data in Appendix Table A1).

**Colour Discrimination Training**

We conducted only one session per day per bird under one seeing condition. A session consisted of 30 trials while vision was restricted to the left or right eye. Left- and right-eye seeing alternated daily for each bird. The coloured grits were presented in two plastic cups set side-by-side in a plastic trough that was placed below the central opening of the home cages (Manns & Römling, 2012). The trial-to-trial left–right position of the colour stimuli varied quasi-randomly. Pecking into the positive grit was classified as a correct choice and the pigeon was allowed to find the hidden grain. Searching among the nonbaited grit represented an error and the plastic boxes were immediately removed. During the early training phase, a pigeon could switch to the positive grit to find the rewarding grain. In accordance with previous experiments, training started with the more difficult overlapping colour pairs B+ C− and C+ D (Manns & Römling, 2012; Siemann, Delius, & Wright, 1996). After a pigeon reached the learning criterion of 80% correct choices in three consecutive sessions for an individual eye, the second pairs (A+ B− or D+ E−) were introduced. Colour pairs were presented in runs and presentation frequencies of the training pairs were biased (see Siemann, Delius, & Wright, 1996), first consisting of 20x (B+ C−/C+ D−) + 11x (A+ B−/D+ E−), then 2x (10x B+ C−/C+ D− + 5x A+ B−/D+ E−) repeated trials. During the last three training sessions, 20% of the training trials remained unrewarded.

**Testing**

After a pigeon reached the learning criterion of 80% correct choices for all colour pairs after six consecutive sessions (three for the left- and three for the right-eye seeing condition), six monocular test sessions with each eye were conducted. Within a session, birds were confronted with a set of three different stimulus types that were randomly mixed. There was again only one session per day and the seeing condition switched daily. One session comprised the following stimulus pairs (Fig. 1): (1) 22 learned colour pairs (rewarded): discrimination was learnt with the tested eye/hemisphere (11x end (A+ B−/D+ E−) + 11x middle (B+ C−/C+ D−) pairs); (2) two transfer colour pairs (unrewarded): discrimination was learnt with the contralateral eye/hemisphere and, hence, reward contingencies had to be transferred (1x end AB/DE, 1x middle (BC/CD) pair); (3) six transitive colour pairs (unrewarded): discrimination of colour pairs that were not presented during training (AC, AD, AE, BD, BE and CE; 1x each).

After finishing the monocular tests, the birds were retrained to learning criterion and six binocular sessions were conducted whereby learnt colour pairs were interspersed with the six transitive pairs (Manns & Römling, 2012).

Choices were recorded when the pigeon pecked into the grit closest to the beak. Correct responses indicated discrimination

![Figure 1. Design of the experimental steps. Pigeons learnt to discriminate two colour pairs with each eye. We compared two experimental groups with reversed hemispheric-specific allocation of the premise pairs. We trained pigeons using the five-item transitive inference task (A > B > C > D > E) with four overlapping pairs of stimuli (where pluses represent reinforced stimuli and minuses nonreinforced stimuli). After reaching the learning criterion, pigeons took part in monocular test sessions in which they were confronted with a mixture of training, transfer and transitive pairs. For discrimination of transfer pairs, information about reward contingencies had to be transferred; for discrimination of the transitive pairs, interhemispheric information had to be integrated within the tested hemisphere.](image-url)
accuracy for the learnt and transfer stimulus pairs. Transitive choices were evaluated by transitive inference logic. Traditionally, test pair BD is regarded as the critical one because the component stimuli are presented equally often as rewarded and as unrewarded stimuli in the premise training pairs (Lazareva et al., 2015; Lazareva & Wasserman, 2012; Vasconcelos, 2008). Thus, their value can only be estimated in relation to the other colours and, hence, after correctly ranking along the transitive line. Accordingly, a correct decision for B indicates successful transitive inference which in turn depends on integration of knowledge from both hemispheres in our paradigm.

Data Analysis

Statistical analysis was carried out using the program Statistica 10 (StatSoft, Tulsa, OK, U.S.A.). Normal distribution was evaluated by Kolmogorov–Smirnov and Shapiro–Wilk tests. Then data sets were analysed with mixed repeated measures ANOVA. Dependent and independent t tests were conducted when there were significant factor effects. When data were not normally distributed, nonparametric tests were conducted. This was only the case for the analysis of transitive responses.

RESULTS

Training Performance

The pigeons learned with each eye to discriminate two premise colour pairs representing the beginning or end of the transitive line. They needed a mean ± SD of 82 ± 24 days to reach the learning criterion of 80% correct choices during the last three training sessions (Fig. 1). A mixed repeated measures ANOVA with dependent factors ‘time point’ (training–test), ‘pair type’ (end–middle), and ‘hemisphere’ (left–right). On average, discrimination accuracy was reduced during testing \( F(2,26) = 14.90, P < 0.001, \) partial eta-squared \( \eta^2_p = 0.364 \) but depended on ‘pair type’ and ‘hemisphere’ (three-way interaction: \( F(1,26) = 6.76, P < 0.05, \eta^2_p = 0.206 \)). It was intriguing that especially the discrimination accuracy of the middle pairs (B+–/C+–/D+–) was significantly decreased (left hemisphere: \( t_{26} = 3.992, P < 0.001 \); right hemisphere: \( t_{26} = 3.103, P < 0.01 \)) although these pairs were originally better learnt. Concerning the end pairs (A+B+/–/D+/–), a slight increase in the left- and a decrease in the right-hemispheric discrimination accuracy (\( t_{26} = 2.104, P < 0.05 \)) led to an asymmetry that was not present at the end of training (left–right difference: \( t_{26} = 3.880, P < 0.001 \)).

Transfer Colour Pairs

Impaired discrimination of ambiguous middle pairs

Differences in the discrimination performances of end and middle pairs were also evident when the pigeons were confronted with the transfer colour pairs (Fig. 3). A 2 × 2 MANOVA with dependent factors ‘pair type’ (end–middle), and ‘hemisphere’ (left–right) showed that the mean performances did not differ between the hemispheres \( (F_{1,26} = 0.41, P = 0.528, \eta^2_p = 0.016) \) whereas discrimination accuracy of the end and middle pairs varied remarkably \( (F_{1,26} = 46.007, P < 0.0001, \eta^2_p = 0.639) \). Discrimination of the end pairs (A+B+/–/D+/–) was significantly above chance level \( (t \text{ test for single means against reference value 50\%}) \).
for single means against reference value 50%: \( t_{26} = -0.238, \ P = 0.815 \).

The differential discrimination scores of transfer end and middle pairs indicated that associative memory was not simply recalled but evaluated in relation to the hemispheric-specific learning experience (see Discussion). End pairs (A–B/–D–E) combined colours with which the tested hemisphere had no direct experience and, therefore, the transferred reward contingencies did not interfere with intrahemispherically conditioned stimulus values. Middle pairs (B–C–/C–D–), on the other hand included overlapping colour C whose reward value was contrasting between the learnt and transfer stimulus pair. Thus, when the tested hemisphere was confronted with the transfer middle pair, it had to choose the opposite colour to the directly learnt reward contingency. As such, the middle pairs (B–C–/C–D–) interfered with intrahemispherically conditioned stimulus values. The left hemisphere performed at chance level irrespective of whether it was confronted with transfer pair B+C− or C+D− (\( t \) test for single means: \( P > 0.05 \); Fig. 3). This suggests that the learnt associative values influenced the discrimination accuracy but the performance was independent of the direction of the learnt reward contingency. Right-hemispheric choices, on the other hand, differed depending on the direction of the conflict. When intrahemispheric learning associated C with a negative value (C–), transfer pair C+D− was discriminated at chance level (\( t \) test for single means: \( t_b = 0.139, \ P = 0.891 \)) and discrimination scores did not differ from left-hemispheric ones (\( t \) test for independent groups: \( t_{25} = 1.32, \ P = 0.200 \)). But when learning experience associated C with a positive value (C+), discrimination scores for B+C− were significantly below 50% (\( t \) test for single means: \( t_b = 6.860, \ P < 0.001 \)). This means that the right hemisphere clearly chose the stimulus with learnt appetitive association, disregarding information from the contralateral brain side. This was distinct from choices made by the left hemisphere when confronted with B+C− (\( t \) test for independent groups: \( t_{25} = 3.149, \ P < 0.01 \)). Thus, the hemispheres displayed a differential decision pattern when there was a conflict between learnt appetitive and transfer aversive encoding of the conflict stimulus C. This points to a differential relative evaluation of appetitive and aversive stimulus associations when accessing conflicting reward contingencies. These differences also affected transitive responding as described in the following section.

Transitive Colour Pairs

**Impaired and hemispheric-specific monocular choices**

Confronted with the transitive test pairs, the tested hemisphere had only experience with one of the presented colour stimuli; information about the other colour had to be accessed from the contralateral brain side. Therefore, pecking responses could, in principle, be based on two different strategies. On the one hand, the tested hemisphere could choose the known colour with the highest associative value. This ‘default’ strategy did not require relational encoding and integration of interhemispheric information. It was nevertheless transitive correct when the hemisphere learnt the beginning of the transitive line. When, on the other hand, the tested hemisphere learnt the end of the transitive line; it had to adopt a ‘relational’ strategy for correct transitive responding. In this case, the hemisphere had to choose the unknown colour of the test pair with which it had no direct experience. This strategy required access and integration of interhemispheric information and thereby relational evaluation of reward contingencies of the learnt and transfer stimuli.

On average, response patterns indicated that neither hemisphere successfully adopted a transitive strategy but there were intriguing differences between the hemispheres. For a better understanding of the complex data sets, we confine the description of results to test pairs that combine eventually conflicting reward values most clearly (AC = combining the two colours with the highest reward value for the two hemispheres, BD = critical transitive test pair, CE = combining the two colours with the lowest reward value for the two hemispheres, AE = combining the colour with the highest (A) and the lowest (E) reward value). For an overview of all data, see Fig. A1, and of individual data see Table A1.

When the tested hemisphere learnt the beginning of the transitive line (i.e. the left hemisphere of the LH-B/RH-D group and the right hemisphere of the RH-B/LH-D group), left- as well as right-hemispheric discrimination scores were well above chance for all colour pairs (Fig. 4a) as indicated by a comparison between correct and false dwitinas (Fig. 4a, Fig. A1). All responses could be explained by a choice for the known colour with the higher conditioned stimulus (except for CE) and, hence, by the adoption of the default strategy.

**Figure 4.** Left- (IH) and right-hemispheric (rH) transitive responding expressed as the mean ± SE choice percentage for the transitively correct colour. Dependent on the learnt stimulus pairs, the hemisphere can respond successfully with different strategies: (a) when the tested hemisphere learnt the beginning of the transitive line, decisions for the known colour are correct (default strategy), (b) when the tested hemisphere learnt the end of the transitive line, decisions for the transfer colour are correct (relational strategy). Letters within the columns indicate the learnt (−known) colour. Dashed lines indicate 50% chance level. \( *P = 0.07; \ P < 0.05; \ **P < 0.01; \ ***P < 0.001 \); Wilcoxon signed-rank tests.
When the tested hemisphere learnt the end of the transitive line (i.e. the left hemisphere of the RH-B/LH-D and the right hemisphere of the LH-B/RH-D group), left- as well as right-hemispheric discrimination scores differed profoundly (Fig. 4b). The left hemisphere responded transitively correct when confronted with pairs AE and CE (Fig 4b, Fig. A1), simply avoiding the most negative stimulus E. In contrast, choices for pairs AC and BD (Fig 4b, Fig. A1) were at chance level. Unclear choices indicated interference between intra- and interhemispheric information preventing a definite transitive response. The right hemisphere also responded correctly when confronted with AE and CE (Fig 4b, Fig. A1). When confronted with pairs AC and BD (Fig 4b, Fig. A1), the pigeons chose the transitively incorrect but known colours C and D, respectively. Thus, the right hemisphere clearly used the ‘default’ and not the ‘relational’ strategy.

Relational responding during binocular discrimination

Monocular performances indicated that neither the left nor the right hemisphere alone adopted a transitive strategy. This response pattern differed profoundly from binocular choices. When pigeons used both hemispheres, discrimination scores differed between the transitive colour pairs (Friedman’s ANOVA: $\chi^2 = 46.225, N = 27, P < 0.000$; Fig. 5). Comparison between correct and false decisions yielded a well above chance transitive responding for colour pairs AC, AD, AE, BE, CE (Wilcoxon signed-ranks test: $P < 0.0001$ for all colour pairs), a nonsignificant tendency to peck on B in test pair BD (Wilcoxon: $Z = 1.810, P = 0.07$) and no significant difference in binocular performances between the two experimental groups.

DISCUSSION

The present study shows that pigeons do not integrate hemisphere-specific knowledge to adopt a transitive inference strategy when using only one hemisphere. This is presumably caused by a differential encoding of conflicting stimulus values whereby the two hemispheres differ in the evaluation of associative information. Since the birds, on the other hand, display transitive whereby the two hemispheres differ in the evaluation of associative information. We work out the details of this conclusion step by step in the following discussion. Here, we take the perspective of the hemispheres so that contralateral input means afferents from the contralateral eye while ipsilateral input represents transfer information from the ipsilateral eye and/or contralateral hemisphere. Accordingly, intrahemispheric information is directly learnt while interhemispheric information requires transfer from the ipsilateral eye/contralateral hemisphere.

Even discrimination accuracy of learnt colour pairs changed during critical testing as also observed in previous experiments (e.g. Letzner et al., 2014; Manns & Römling, 2012). Diminished performances might be caused by the more complex test situation; it was however, intriguing that especially discrimination of the overlapping middle pairs was decreased. This was particularly surprising since these pairs were significantly better learnt at the end of training presumably due to overtraining (Siemann, Delius, & Wright, 1996). Middle pairs included colour C, which had two opposite associative values because of its different reward contingencies in the two trained colour combinations (B+C−/C+D−). Although discrimination of the intrahemispherically learnt reward contingencies should be unaffected by information from the contralateral brain side, the decreased performance points to a distracting influence and provides a first hint that associative memory is not just passively accessed, at least when contralateral experience provides divergent information. Interhemispheric interference, however, was more obvious when the pigeons were discriminating transfer colour pairs.

Differential Encoding of Ambiguous Intra- and Interhemispheric Memory

In principle, transfer of conditioned colour discriminations was possible, as indicated by the well above chance performance of all pigeons when they were confronted with the transfer end pairs. This was in line with previous data, although we did not observe the reported left-hemispheric superiority (Letzner et al., 2014; Valencia-Alfonso et al., 2009). End pairs (A=B−/D+/E+) were composed of colours with which the tested hemisphere had no direct experience so that no conflict with intrahemispheric memory arose. This was different when pigeons were confronted with a middle pair (B+C−/C+D−). Owing to the two opposite reward values of C, intrahemispheric learning experience contrasted with the transfer information. Accordingly, the tested hemisphere had to ignore the directly conditioned reward value of C when discriminating the transfer pair. But the poor performances suggest that intrahemispheric memory affected the discrimination. Thereby the hemispheres differentially dealt with the ambiguous intra- and interhemispheric information (Fig. 6). The left hemisphere performed at chance level when confronted with transfer middle pairs supporting the hypothesis that there was interference between directly experienced and transferred associative memory. The right hemisphere displayed differential response patterns depending on the directly experienced reward value of C. As already discussed in the Results, association with a negative value (i.e. after learning B+C−) led to uncertain choices when pigeons were confronted with the transfer pair C+D−. Positive association of C (i.e. after learning C+D−) resulted in pigeons choosing C when discriminating transfer pair B+C−. Thus, when associative learning led to an appetitive coding of a stimulus within the right hemisphere, neuronal processes comparing intra- and interhemispheric information weighted the intrahemispheric information more strongly or even inhibited information from the contralateral side (Fig. 6b).

![Figure 5](image-url) Transitive responding expressed as the mean ± SE choice percentage for the transitivity correct colour when using both hemispheres. Dashed line indicates 50% chance level. ***P = 0.07; ****P < 0.001; Wilcoxon signed-rank tests.
In sum, impaired discrimination of the ambiguous middle pairs indicates that transfer information is differentially processed depending on the kind of conflict between intra- and interhemispheric reward values. This means that access to associative memory is the result of an active process whereby the two hemispheres differ in their strategies to integrate conflicting information. Poor performances might be related to neuronal mechanisms avoiding conflicts between the hemispheres in animals with laterally placed eyes (see below). As discussed in the following section, the underlying encoding strategies prevent relational encoding of interhemispheric stimulus values and therefore impair transitive responding.

**Hemispheric-specific Evaluation of Conflicting Information and Transitive Responding**

Since the discrimination of middle transfer pairs was impaired, it was not surprising that neither the left nor the right hemisphere displayed relational responding during monocular tests. Aligning stimuli along a transitive line is based on considering relational reward values of the premise stimulus combinations (Lazareva & Wasserman, 2012; Vasconcelos, 2008) and requires integration of intra- and interhemispheric knowledge in our paradigm. Thereby C is the critical stimulus linking left- and right-hemispheric knowledge but representing ambiguous associative values for the left and right hemispheres (highest value for one, lowest value for the other hemisphere; Manns & Römling, 2012). Therefore, only relational not associative encoding (Lazareva & Wasserman, 2012; Vasconcelos, 2008) of intra- and interhemispheric stimulus values allows the establishment of a complete transitive line. Response patterns during monocular tests in our experiment show that neither the left nor the right hemisphere based its choices clearly on relational computations. Stimulus pairs were rather considered pair by pair relying on a differential relative evaluation of conflicting intra- and interhemispheric stimulus values. As described in the Results, the hemispheric choices can, in principle, be explained by two strategies that require a different degree of interhemispheric interaction. When the tested hemisphere (the left hemisphere of the LH-B/RH-D group and the right hemisphere of the RH-B/LH-D group) learnt the beginning of the transitive line (A+B−, B+C−), responses were transitively correct when the hemisphere chose simply according to its direct learning experience and, hence, according to a default strategy. This means that the observed superior performances can be explained by an associative reinforcement-driven mechanism that does not require relational encoding of associative values (Lazareva & Wasserman, 2012; Wynne, 1997). An associative mechanism even explains responding to BD, the generally accepted critical test pair indicative of transitive reasoning (Lazareva & Wasserman, 2012; Vasconcelos, 2008). The tested hemisphere had only direct experience with B and it clearly chose this stimulus although B was reinforced in one (B+C−) and not in the other (A+B−) training pair. Owing to the overtraining of B+C−, B was presumably associated with a positive reward (Lazareva et al., 2015; Lazareva & Wasserman, 2012) and therefore was chosen. The hemispheres also successfully discriminated pairs that included color E and, hence, the color with the lowest reward value that was, however, not directly experienced. This is especially remarkable for stimulus pair CE since it combined...
E with C, the stimulus with the lowest reward value for the tested hemisphere. This choice pattern supports the hypothesis that each hemisphere can integrate interhemispheric reward information when no conflict with intrahemispheric learning experience arises.

When the tested hemisphere (the left hemisphere of the RH-B/LH-D group and the right hemisphere of the LH-B/RH-D group) learnt the end of the transitive line (C→D→, D→E→), a transitively correct response had to be a choice for the unknown and against the appetitively encoded color. Only responses against intrahemispheric associative experience indicate adoption of a relational strategy that evaluates learnt and transfer stimulus values and establishes an interhemispheric transitive line. In this respect, choices were critical when pigeons were confronted with the test pairs AC and BD. Neither of these test pairs was discriminated transitively correct suggesting that the hemispheres did not base their decisions on an ordered transitive series. Thereby, the hemispheres displayed intriguing differences in their response pattern. Ambiguous choices made by the left hemisphere indicate interference between intra- and interhemispheric information preventing a definite transitive response. Right-hemispheric choices support the hypothesis that this brain side preferentially chooses the stimulus with which it has direct positive experience (see above). This is particularly illustrated in the discrimination of AC. A relational response required a choice for A although C had the highest reward value for the tested hemisphere. The right hemisphere chose C and, hence, according to its appetitive associative memory. Interference or even suppression of interhemispheric information presumably prevented relational encoding of interhemispheric stimulus values and therefore also prevented the hemispheres combining information to adopt a transitive strategy.

The monocular response patterns were however, in stark contrast to the binocular performances. Responses when discriminating AC and BD indicate that transitively correct decisions relied on mechanisms that detect an ordered hierarchy after combination of hemispheric-specific knowledge (Manns & Römling, 2012). Thus, our results clearly show that pigeons only integrate information of the two hemispheres to solve a complex cognitive task when both hemispheres are simultaneously activated by visual input. This supports the hypothesis that mono- and binocular visuomotor processing differ profoundly, as already indicated, at the behavioural level (e.g. Wilzeck, Prior, & Kelly, 2009) and physiological level (Folta, Diekamp, & Güntürkün, 2004; Schmidt & Bischof, 2001; Voss & Bischof, 2003). This also implies that associative and relational strategies are not mutually exclusive and depend not only on the trained relational rules and reinforcement history (Lazareva et al., 2015) but also on the integration of intra- and interhemispheric learning experience. Thereby, the two hemispheres obviously differ in the underlying encoding strategies.

We cannot conclude which neuronal areas mediate the neuronal processes but a recent study indicates the involvement of the hippocampus to create a representation of an ordered series of the stimuli instead of maintaining the reinforcement history of each stimulus (Lazareva et al., 2015). Impaired monocular performance indicates that the hippocampus is not able to integrate contralateral information or does not receive appropriate interhemispheric information, for example from striatal or neopallial areas that are involved in decisions between differentially valued options in mammals (Strait, Sleezer, & Hayden, 2015) and pigeons (Rose, Schiffer, & Güntürkün, 2012; Starosta, Güntürkün, & Stüttgen, 2013).

Functional Organization of Interhemispheric Visual Information Processing

Evaluation and combination of information from the left and right brain sides may emerge from differential hemispheric-specific interactions of intra- and interhemispheric visual networks. It is intriguing that recall and integration of conditioned memory were only affected in situations when intra- and interhemispheric information were at odds in our experiment. This might be related to general mechanisms avoiding processing of discrepant information from the laterally positioned eyes of pigeons. Owing to the limited binocular visual fields, the eyes mediate virtually different input to the brain. This in turn requires mechanisms that control the interhemispheric attention switch and suppression of input from one eye to avoid interocular conflicts (Güntürkün, 2000; Stacho, Letzner, Theiss, Manns, & Güntürkün, 2016). As indicated by the results of our experiments, these processes may also regulate access to interhemispheric associative memory. After monocular training, unihemispheric discrimination performance was influenced by conflicting intra- and interhemispheric memory in a hemispheric-specific manner (Fig. 6). Ambiguous choices of the left hemisphere suggest that transfer information is accessed in principle, and it points to interference in the encoding of intra- and interhemispheric information about the associative values of single stimuli. The right-hemispheric response pattern, on the other hand, indicates that conflicting transfer information is ignored or even blocked when this input contrasts with intrahemispheric appetitive memory. These differences (right versus left) were related to the differential functional role of the hemisphere. The left hemisphere dominates routine behaviour (MacNeileag et al., 2009; Vallortigara, 2000) based on categorization and the integration of experience into visual analysis (Freund et al., 2016). This processing style may also consider information from the contralateral brain side. Since the right hemisphere, on the other hand, controls quick responses in emergency situations (MacNeileag et al., 2009; Vallortigara, 2000), information processing should not be compromised by eventually conflicting information from the contralateral brain side.

The observed flexible regulation of information transfer may depend on lateralized intra- and/or interhemispheric interactions (Fig. 6). Thereby, interhemispheric mechanisms are primarily implemented within the ascending visual processing stream (Fig. 6). In pigeons, visual information is critically transferred within the tectofugal system projecting from the retina to the contralateral optic tectum from where fibres ascend to the thalamic nucleus rotundus and then to the telencephalic entopallium (Güntürkün et al., 2014; Manns & Güntürkün, 2009). Although the tectotrudinal projection is largely ipsilaterally organized, a considerable number of fibres recross within the supraoptic decussation (DSO) whereby more fibres project from the right tectum to the left rotundus than vice versa (Güntürkün, Hellmann, Melsbach, & Prior, 1998). Integrity of the DSO is a prerequisite for successful transfer of pattern, brightness or colour discrimination (e.g. Watanabe, 1985; Watanabe, Hodos, Bessette, & Shimizu, 1986), which indicates that ascending projections mediate interhemispheric exchange of associative memory. Nevertheless, transfer of colour discriminations requires at least 1 h after acquisition, suggesting that accessing transfer information does not occur automatically (Skiba et al., 2000). Electrophysiological recordings show that input from the ipsilateral eye is primarily suppressed at the rotundal (Engelage & Bischof, 1988; Folta, et al., 2004; Schmidt & Bischof, 2001) and entopallial level (Voss & Bischof, 2003). Inhibition is mediated by a tectofugal side pathway via the subpretectal nuclei (Mpdozis et al., 1996; Theiss, Hellmann, & Güntürkün, 2003; Voss & Bischof, 2003) or via an interhemispheric network between the left and right tectum comprising pretectal and thalamic structures (Stacho et al., 2016). At forebrain level, context-dependent interhemispheric interactions (Schmid & Bischof, 2001) can be regulated via the anterior commissure which is the major commissural projection of the avian forebrain (Letzner et al., 2016). This commissure does not directly mediate information
transfer but is presumably part of an active filter (Skiba et al., 2000) controlling exchange and integration of interhemispheric information (Letzner et al., 2016).

A second mechanism comprises descending projections that arise from the visual Wulst in the forebrain and exert top-down control onto tectofugal processing (Folta et al., 2004; Freund et al., 2016; Manns, Freund, Patzke, & Güntürkün, 2007; Fig. 6b). The Wulst mediates attentional processes in accordance with experience or current goals but is also relevant when changing or ambiguous stimulus values have to be considered (Freund et al., 2016; Pasternak, 1977; Shimizu & Hodos, 1989). This top-down control is primarily ipsilaterally organized and has strong impact on the left but not the right tectofugal system (Freund et al., 2016; Manns Freund, Patzke, & Güntürkün, 2007) which is in accordance with the more top-down regulated processing style of the left hemisphere dominating routine behaviour (MacNeile et al., 2009; Vallortigara, 2000). In relation to this (although speculative), enhanced top-down control may facilitate access to transfer information within the left hemisphere while contralateral input is blocked within the right brain side (Fig. 6b).

In sum, our results show that exchange and combination of intra- and interhemispheric associative memory is an active and flexible process that critically depends on the seeing conditions and differs between the two hemispheres. Our results provide some of the first evidence for behavioural consequences of neuronal mechanisms that avoid interocular conflicts in animals with laterally placed eyes. They illustrate the complex intra- and interhemispheric processes in a lateralized brain (Hervé et al., 2013; Manns & Ströcks, 2014; Ocklenburg & Güntürkün, 2012).

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References


Figure A1. Transitive responding expressed as the mean ± SE choice percentage for the transitively correct colour in test pairs when pigeons used (a) the left or (b) the right hemisphere. Dashed lines indicate 50% chance level. *P < 0.05; **P < 0.01; ***P < 0.001; Wilcoxon signed-rank tests. Left-hemispheric discrimination scores of the LH-B/RH-D group (grey bars in (a)) differed between the transitive colour pairs (Friedman’s ANOVA: $\chi^2 = 17.253, N = 9, P < 0.01$). Comparisons between correct and false decisions were significant for colour pairs AC, AE, BD (Wilcoxon signed-ranks test: N = 8, P < 0.05 for AC, N = 9, P < 0.01 for AE, BD) or approached significance for CE (Wilcoxon signed-ranks test: N = 8, P = 0.07). Left-hemispheric discrimination scores of the RH-B/LH-D group (black bars in (a)) varied between the transitive test pairs (Friedman’s ANOVA: $\chi^2 = 49.029, N = 18, P < 0.0001$); the birds chose the transitively correct colours significantly more often (Wilcoxon signed-ranks test: $P < 0.01$ for all colour pairs). Right-hemispheric discrimination scores of the RH-B/LH-D group (black bars in (b)) varied significantly between the transitive colour pairs (Friedman’s ANOVA: $\chi^2 = 22.859, N = 18, P < 0.001$); the birds chose the transitively incorrect colours C and D. Right-hemispheric discrimination scores differed significantly between the experimental groups for all colour pairs (Mann–Whitney U test: $P < 0.05$ except BE ($Z = 1.800, P = 0.07$).