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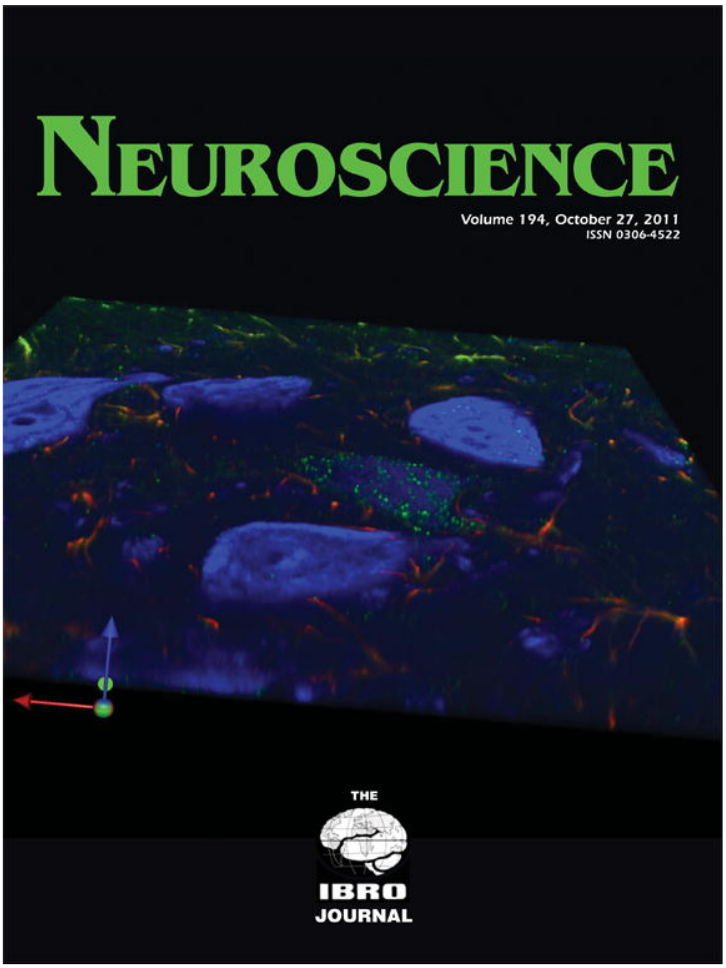


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# TELENCEPHALIC ORGANIZATION OF THE OLFACTORY SYSTEM IN HOMING PIGEONS (*COLUMBA LIVIA*)

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**Abstract—Pigeons use olfactory cues to navigate over unfamiliar areas, and any impairment of the olfactory system generates remarkable reduction of homing performance. Lesion and deprivation studies suggest a critical involvement of the right nostril and thus, the right olfactory bulb (OB) and the left piriform cortex (CPi) for initial orientation. This functional pattern suggests that OB and CPi are asymmetrically connected with a stronger projection from the right OB to the left CPi. However, the structural organization of the olfactory system is not unequivocally clarified yet. Thus, we re-analyzed the system by antero- and retrograde tract tracing with biotinylated dextran amine and choleratoxin subunit B, and we especially evaluated quantitative differences in the number of cells in the OB innervating the left and right CPi. Our anterograde tracing data verified a strong bilateral input to the CPi, and the prepiriform cortex (CPP), as well as small projections to the ipsilateral medial septum and the dorsolateral corticoid area and the nucleus taeniae of the amygdala in both hemispheres. Apart from the bilateral bulbar afferents, CPi in turn receives unequivocal input from the ipsilateral CPP, hyperpallium densocellulare, dorsal arcopallium, and from a cluster of cells located within the frontolateral nidopallium. Thus, an indirect connection between OB and CPi is only mediated by the CPP. For quantitative analysis of bulbar input to the CPi, we counted the number of ipsi- and contralaterally projecting neurons located in the OB after injections into the left or right CPi. Retrogradely labeled cells were found bilaterally in the OB with a higher number of ipsilaterally located cells. The bilaterality index did not differ after left- or right-sided CPi injections indicating that the functional lateralization of the olfactory system is not simply based on differences in the number of projecting axons of the major processing stream.** **© 2011 IBRO. Published by Elsevier Ltd. All rights reserved.**

**Key words: brain lateralization, choleratoxin subunit B, olfactory bulb, piriform cortex, homing pigeons.**

Olfaction plays an important role in many representatives of the animal kingdom. In vertebrates, the structural organization of the olfactory system is highly conserved. This also applies to birds, which have been regarded as microsmatic for a long time (for review see Roper, 1999). The external component of the avian olfactory system consists of paired nostrils that are constituted by a series of nasal chambers of which the most caudal one is lined up with the olfactory epithelium with its olfactory receptor cells. Their axons constitute the olfactory nerves, which terminate ipsilaterally in the olfactory bulbs (OBs). The efferent mitral cells of the OBs in turn project bilaterally to several forebrain areas with the piriform cortex (CPi) as one of the major targets (Reiner and Karten, 1985; Ebinger et al., 1992; Bingman et al., 1994). Electrophysiological studies and molecular analyses of olfactory receptor genes suggest the avian olfactory system to be a highly differentiated sense that is of higher complexity than previously assumed (McKeegan et al., 2002; Sieck and Wenzel, 1969; Tucker, 1965).

Pigeons use olfactory cues for their extraordinary navigation abilities (for review seeWallraff, 2005). Accordingly, the OBs of homing pigeons are enlarged compared with other non-homing pigeon breeds (Rehkämper et al., 1988, 2008). Manipulation of the olfactory system, such as plugging the nostrils (Gagliardo et al., 2007, 2011), anesthetizing the olfactory mucosa (Wallraff, 1988), transecting the olfactory nerve (Gagliardo et al., 2006, 2009; Papi et al., 1971), or ablating the CPi (Papi and Casini, 1990) generate remarkable disruptions of initial orientation and homing performance in pigeons (for review see Wallraff, 2005). Moreover, analysis of neuronal ZENK expression revealed neuronal activation in the olfactory system during homing (Patzke et al., 2010).

Behavioral studies have indicated that the left and right hemispheric systems differentially contribute to olfactory-dependent navigation. Pigeons with a lesioned right CPi behaved similar to controls and oriented significantly towards their home direction, whereas pigeons with left CPi lesions were heavily impaired in their initial orientation skills (Gagliardo et al., 2005). This argues for a dominance of the left CPi in processing olfactory cues during navigation. In addition, unilateral nostril occlusions revealed that the right nostril/OB is more important during initial orientation than the left nostril/OB (Gagliardo et al., 2007, 2011).

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*Abbreviations:* A, antero posterior coordinate; AD, dorsal acropallium; BDA, biotinylated dextran amine; BI, bilaterality index; BSTL, bed nucleus of the stria terminalis lateral part; CA, anterior commissure; CDL, dorsolateral corticoid area; CPi, piriform cortex; CPP, prepiriform cortex; CtB, choleratoxin subunit B; DM, dorsomedial portion of the hippocampal formation; E, entopallium; GP, globus pallidus; HD, hyperpallium densocellulare; HF, hippocampal formation; HL, hyperpallium laterale; M, mesopallium; MSt, medial striatum; N, nidopallium; NC, caudal nidopallium; NDB, nucleus of the diagonal band; NFL, frontolateral nidopallium; OB, olfactory bulb; PoAb, basal division of the nucleus posterioris amygdalopalli; PoAc, compact division of the nucleus posterioris amygdalopalli; SM, medial septum; SMd, stria medullaris; SpA, subpallial amygdala; St, striatum; TnA, nucleus taeniae of the amygdala; TPO, temporo-parieto-occipital area; TuO, olfactory tubercle; Va, vallecula.

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Accordingly, plugging only the right nostril altered neuronal ZENK expressions during homing (Patzke et al., 2010). Since the OB projects bilaterally to the CPi (Reiner and Karten, 1985; Bingman et al., 1994), it is conceivable that the functional lateralization is based on an asymmetrical projection from the OB to CPi with a stronger projection from the right OB to the left CPi. A larger bilateral innervation of the dominant brain hemisphere is also known from visual pathways in birds (Güntürkün et al., 1998; Rogers and Deng, 1999; Manns and Güntürkün, 2009). However, findings regarding the general projection patterns of the olfactory bulb are remarkably inconsistent (Rieke and Wenzel, 1978; Reiner and Karten, 1985). While Rieke and Wenzel (1978) found bulbar projections to the ipsilateral prepiriform cortex (CPP), mesopallium, medial striatum (MSt), nucleus acumbens, and the contralateral globus pallidus (GP) crossing via the anterior commissure (CA). In contrast, Reiner and Karten (1985) demonstrated a completely different projection pattern with the exception of the CPP. They found that the OB projects to the septum, the CPP, the olfactory tubercle, the nucleus taeniae of the amygdala and the CPi, entering the contralateral hemisphere via the habenular commissure. These available data on the olfactory projections in pigeons are based on degeneration or autoradiographic techniques. The limited sensitivity of these methods could explain some of the contradictions. Therefore, we re-analyzed OB projections using biotinylated dextran amine (BDA). Quantitative differences in the projection pattern between OB and CPi were assessed by injections of choleratoxin subunit B (CtB) unilaterally into the left or right CPi.

## EXPERIMENTAL PROCEDURES

A total of 20 adult homing pigeons (*Columba livia*) of both sexes from local breeding stocks were used in this study. For the quantitative determination of the projection from the OB to the CPi, 16 birds successfully received unilateral injections of the retrograde tracer CtB(1% in deionized water; Sigma, Deisenhofen, Germany) either into the left ($n=8$) or to the right ($n=8$) CPi. For anterograde pathway tracing, successful BDA (10,000 MW; 10% in 2% DMSO; Molecular Probes, Leiden, The Netherlands) injections into the OB of four pigeons (left $n=2$; right $n=2$) were performed. These studies were carried out in compliance with the European Communities Council Directive of November 24, 1986 (86/609/EEC) and were 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 animals used and to alleviate their suffering as much as possible.

Before surgery, pigeons were anesthetized with equithesin (0.33 ml per 100 g body weight) and secured in a standard stereotaxic apparatus (Karten and Hodos, 1967). For CPi injections, a modified device was used that allowed lateral rotation of the head around the longitudinal axis over 100° to the left or right side (Hellmann and Güntürkün, 1999). After opening the skull with a dental drill, the tracers were injected via a glass micropipette (outer tip diameter 15–20 $\mu$m for CtB and 25–30 $\mu$m for BDA) mounted to a mechanic pressure device (WPI Nanoliter injector; World Precision Instruments, Berlin, Germany). The micropipette was inserted either into the CPi or the OB. To estimate potential asymmetries in the projection pattern, we had to ensure a complete filling of the CPi. Therefore, we made six injections along the entire antero-posterior elongation of the CPi between level A 7.5, and 4.5 according to stereotaxic coordinates of the pigeon brain atlas by Karten and Hodos (1967), with an injection depth of 0.15 mm. Placing of OB injections was visually controlled since the OB is a clearly delimited area on which six injections with two depths (0.2 mm and 0.4 mm) were performed. At each depth, about 60/90 nl, CtB/BDA were applied.

After two (for CtB injection) or fourteen days (for BDA injection) of survival time, animals received an injection of 2000 U heparin, and were then deeply anesthetized with equithesin (0.45 ml per 100 g body weight). The pigeons were perfused through the left ventricle with 0.9% saline (40 °C), followed by 4% paraformaldehyde in 0.12 M phosphate-buffered saline (PBS, 4 °C, pH 7.4). The brains were removed and postfixed in 4% paraformaldehyde+30% sucrose for 2 h at 4 °C, cryoprotected in 0.12 M PBS+30% sucrose at 4 °C for 48 h. The brains were cut in frontal plane at 40 $\mu$m on a freezing microtome. The left or the right side of the brain was marked by a hole stuck with a small needle. Sections were collected in five parallel series for the OB and 10 parallel series for the rest of the brain and stored in 0.12 M PBS containing 0.1% sodium azide at 4 °C until they were subjected to immunohistochemistry.

Brain sections were treated free-floating according to the immuno-ABC-technique (Hellmann and Güntürkün, 2001). The sections of one serial set were incubated in 0.3% hydrogen peroxide in distilled water for 30 min to reduce endogenous peroxidase activity. For CtB immunostaining, the sections were incubated in 10% normal goat serum for 1 h in order to block unspecific binding sites. Sections were incubated overnight at 4 °C in the primary antibody solution (rabbit anti-CtB; 1/10,000 in 0.12 M PBS 0.3% Triton X-100 [PBST], Sigma-Aldrich, Munich, Germany). After being rinsed, the sections were incubated for 60 min at room temperature in the biotinylated secondary antibody solution (goat anti-rabbit1/250 in PBST; vectastain, Vector, Camon, Wiesbaden, Germany). After additional rinsing, the sections were incubated for 60 min in avidin–biotin–peroxidase solution (1/100 in PBST, Vectastain Elite ABC kit, Vector, Burlingame, CA, USA). For the BDA staining, one series of sections was incubated for 60 min in avidin–biotin–peroxidase solution. The peroxidase activity was detected using a heavy metal intensified 3,3'-diaminobenzidine (DAB; Sigma-Aldrich, Munich, Germany) reaction, modified by the use of $\beta$ D-glucose/glucose-oxidase (Sigma-Aldrich; Hellmann and Güntürkün, 2001). The sections were mounted on gelatin-coated slides, dehydrated, and coverslipped with Permount (Fisher Scientific, Hampton, NH, USA).

The number of ipsi- and contralaterally labeled CtB cells within the OB were counted with 40×1.6 magnification at a Leica DML microscope (Leica Microsystems, Wetzlar, Germany) in one series of sections. It is important to note that we were not interested in the "true number" of OB-neurons with projections to the CPi, but in the relative proportion of ipsi- and contralaterally projecting cells in the left and the right hemisphere. Since the CPi is a thin superficially located laminar structure, the path of our injection pipette often distorted the shape of this area (Fig. 3). This made our attempts to reconstruct the dimensions of our injections unreliable. Thus, we could not correct absolute labeled cell numbers by injection volume. But we estimated the relation between ipsi- and contralateral projections expressed as the bilaterality index (BI) to evaluate an asymmetry of CPi input (Fig. 3b), which is independent of possible variances in injection volume. The BI was calculated according to Güntürkün et al. (1998):

$$\mathrm{BI}=\frac{\text{cell number ipsi}-\text{cell number contra}}{\text{cell number ipsi}+\text{cell number contra}}$$

Statistical analysis was performed with the statistics program Statistica (StatSoft, Tulsa, OK, USA). As a measure of variability, the standard deviations (SD) together with the mean values were given. Photographic documentation was carried out using a digital camera system (Zeiss Axiocam; Zeiss, Jena, Germany) attached

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to the microscope. Images were processed with Zeiss AxioVision 3.0. Color balance, contrast, and brightness were adjusted with Photoshop CS2 software (Adobe, Frankfurt am Main, Germany).

## RESULTS

### Anterograde tracing of bulbar telencephalic efferents

In all four pigeons injected with BDA, tracer application was successfully confined to the OB (Fig. 1a) since no spread into adjacent brain areas was observed.

As known from previous tracing experiments in reptiles and partly analyzed by Reiner and Karten (1985) in pigeons, there seem to be three major olfactory projections from the OB in pigeons. In our tracing experiment, we observed a continuum of a small number of fibers that extended dorsally from the OB to the ventral and then to the dorsomedial walls of the ipsilateral telencephalon, representing most likely the medial olfactory tract in pigeons. In addition to that, a large compact bundle of fibers was

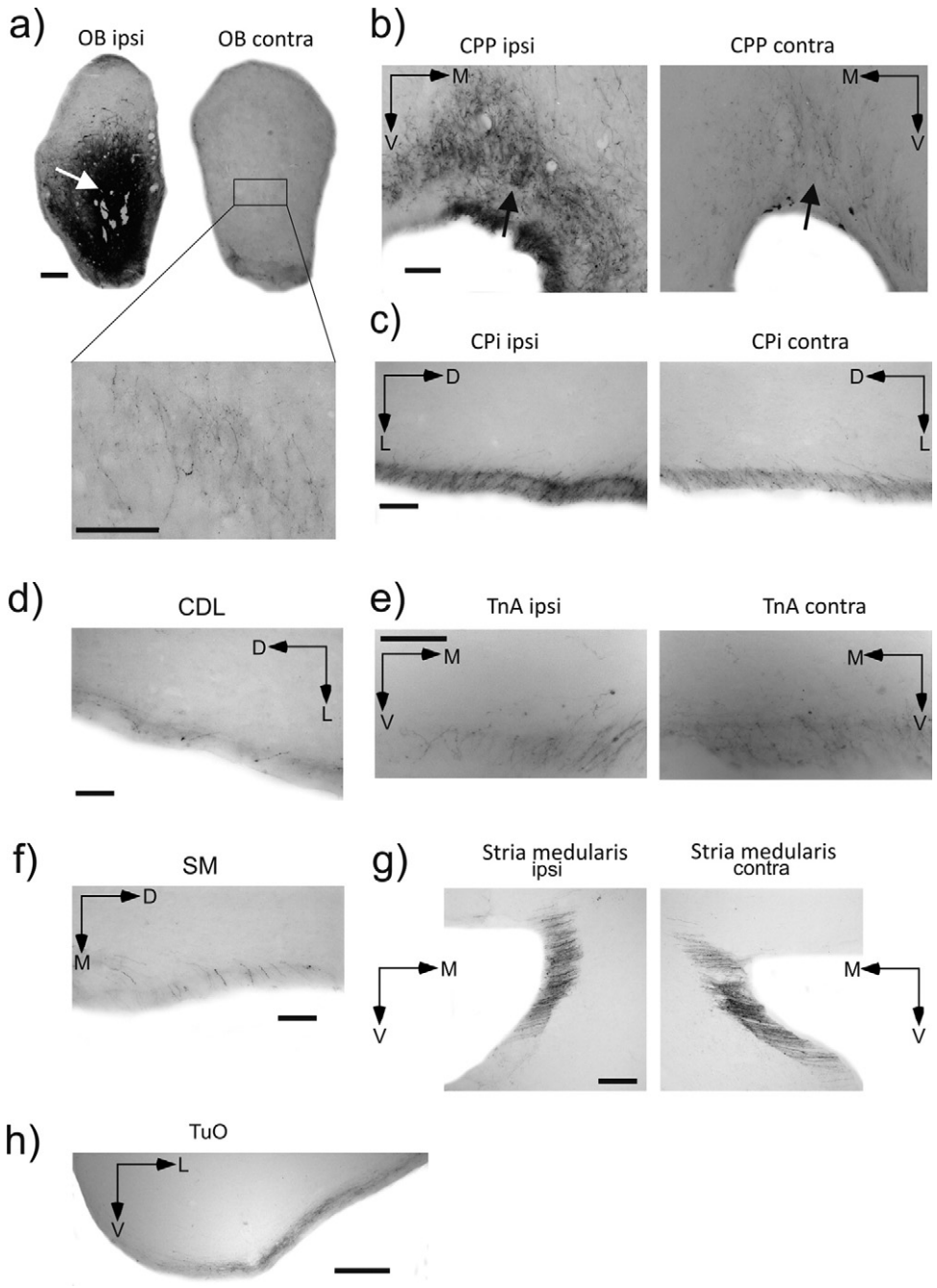


**Fig. 1.** Anterograde BDA labeling of OB projection targets. (a) Injection site of the OB and the corresponding contralateral OB. Fibers ascend via TuO (h) into several forebrain areas: ipsilaterally into the SM (f), bilaterally into the CPP (b), CPi (c), CDL (d), TnA (e). The fibers cross via the stria medularis bridging (g). Dordal (D), lateral (L); medial (M), ventral (V); scale bar (a)=200 $\mu$m and 20 $\mu$m, (b)=100 $\mu$m, (c–g)=50 $\mu$m, (h)=200 $\mu$m.

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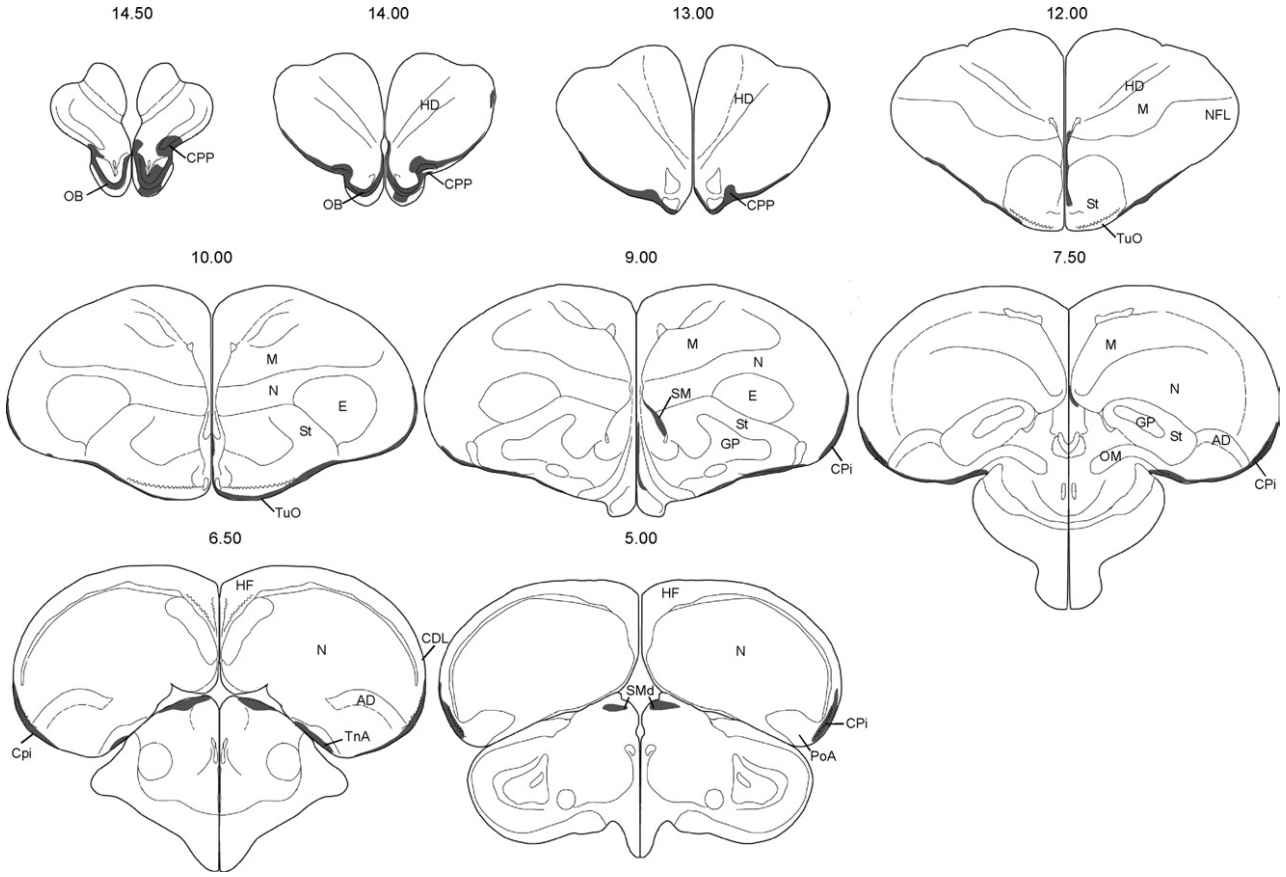


**Fig. 2.** Distribution of anterogradely labeled fiber and terminals (gray area) following injections of BDA into the right OB. These drawings were done representatively from one case.

traced running from the OB along the ventral telencephalic wall. From this large bundle, one part splits off and enters the diencephalon via the bridge of the stria medullaris (SMd) in the habenular commissure and ascends along the contralateral telencephalic wall to the nucleus taeniae of the amygdala (TnA), CPi, and the OB (Fig. 1g). The other part of the bundle runs laterally along the telencephalic wall to the ipsilateral CPi and dorsolateral corticoid area (CDL). These two tracts seem likely to correspond to the intermediate and the lateral olfactory tract as observed in reptiles (Reiner and Karten, 1985).

BDA-labeled fibers could be detected in several telencephalic areas. A great number of BDA-positive fibers were found in the entire ipsilateral OB (A 14.50–14.00; Fig. 1a). Moreover, some fibers were observed in the contralateral OB where they were mostly confined to the mitral cell layer (A 14.50–14.00; Fig. 1a), indicating that the OBs are directly interconnected. Fiber terminals were massively present bilaterally in the CPP (A 14.50–13.00, Fig. 1b), with more fibers on the ipsilateral side. The medial olfactory tract seems to project to the ipsilateral medial septum (SM, A 9.00; Fig. 1f, h) where few BDA-positive fibers were observed. In the CPi a great number of fibers terminated in both hemispheres with more fibers on the ipsilateral side (A 7.50–5.00; Fig. 1c). Few fibers ended slightly above the ipsilateral CPi in the CDL, A 6.00; Fig. 1d). Some fiber terminals were also observed bilaterally in the TnA(A 6.50; Fig. 1e). A bundle of fibers running through the ventral olfactory tubercle (TuO) to the SM was identified (Fig. 1h), with no obvious arborizing terminals in the TuO as found by Reiner and Karten (1985). In sum, our tracing experiment largely verified the observations of Reiner and Karten (1985), although differing in one important detail (Fig. 2).

### Retrograde tracing of telencephalic afferents to the CPi

All CtB injections were successfully placed into the CPi (Figs. 3c and 4). After each injection, CtB-positive cell could be observed in the OB (Fig. 3a), spanning its complete dorsoventral extent. Since the CPi is a thin structure on the surface of the telencephalon, it was inevitable that some tracer spread into the nearby acropallium and amygdaloid nuclei. Accordingly, we could not avoid labeling of cells along the lateral edge of the forebrain. Nevertheless, the injection site was mostly restricted to the CPi in four birds (left $n=2$, right $n=2$). These cases were used to describe the qualitative telencephalic projection pattern of the CPi.

In principle, our CtB injections confirmed the results of an earlier study by Bingman et al. (1994) but with some exceptions (Fig. 4). Retrogradely labeled cells were found

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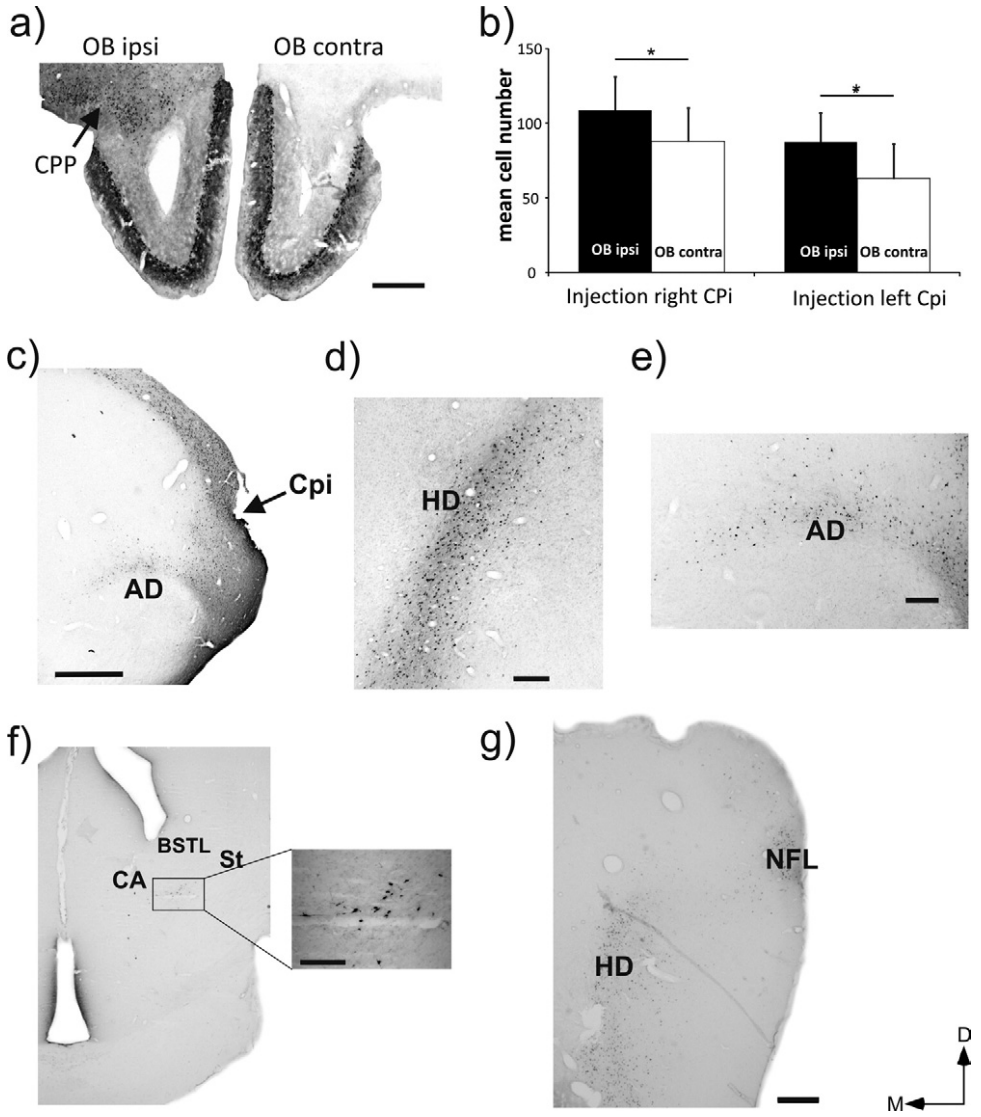


**Fig. 3.** Injection site of the CPi (c). The CPi receives projection from both OBs (a), with a higher innervation from the ipsilateral OB (b), and ipsilateral projections from the CPP (a), the HD (d), and AD (e), the NFL (g), and from few large cells (f) near the juncture of the striatum (St), lateral part of bed nucleus of stria terminalis (BSTL), and the anterior commissure (CA). Dordal (D), medial (M); scale bar: (a)=500 $\mu$m, (c)=1000 $\mu$m, (d–f)=200 $\mu$m, (f)=500 $\mu$m; (b) error bars=standard error; * $P<.05$.

bilaterally throughout the entire OB (A 14.50–14.00, Fig. 3a), exclusively in the mitral cell layer, which constitutes the bulbar output lamina. A large number of ipsilateral retrogradely labeled cells were also detected within the CPP (A 14.50–13.00; Fig. 3a), the hyperpallium densocellulare (HD) (A 14.25–12.00; Fig. 3d, g), and some cells in the frontolateral nidopallium (NFL) (A 14.00–12.00; Fig. 3g) and the hyperpallium laterale (HL) (A 14.00; Fig. 4). Very few ipsilaterally labeled cells were detected near the vallecula (Va) (A 14.00, Fig. 4). Moreover, the ipsilateral dorsal arcopallium (AD) (A 6.50–5.50; Fig. 3c, e) revealed CtB labeled cells. As described by Bingman et al. (1994), few large CtB-positive cells were observed near the juncture of the MSt, the lateral part of bed nucleus of stria terminalis (BSTL), and the CA (A 9.00–7.75; Fig. 3f). But in contrast to Bingman et al. (1994), no projections from the TnA, the septum or the hippocampus were identified.

In cases where tracer spread from the injection site to adjacent brain areas, additional cell populations could be found. In these cases, CtB-positive cells were also detected ipsilaterally in the CDL(A 6.00), the temporo-parieto-occipital area (TPO) (A 7.00), the caudal nidopallium (NC, A 6.00), the basal and compact division of the nucleus posterioris amygdalopalli (PoAb, PoAc) (A 6.00), throughout the dorsomedial portion of the hippocampal formation (DM) (A 9.00–4.50), the TuO (A 12.00), the nucleus of the diagonal band (NDB) (A 9.00), and, with very few cells, in the nucleus accumbens. Moreover, CtB-positive fibers showing terminal-like labeling were found in the BSTL and in the area subpallial amygdala (SpA). Few cells were observed in the contralateral caudoventral wall

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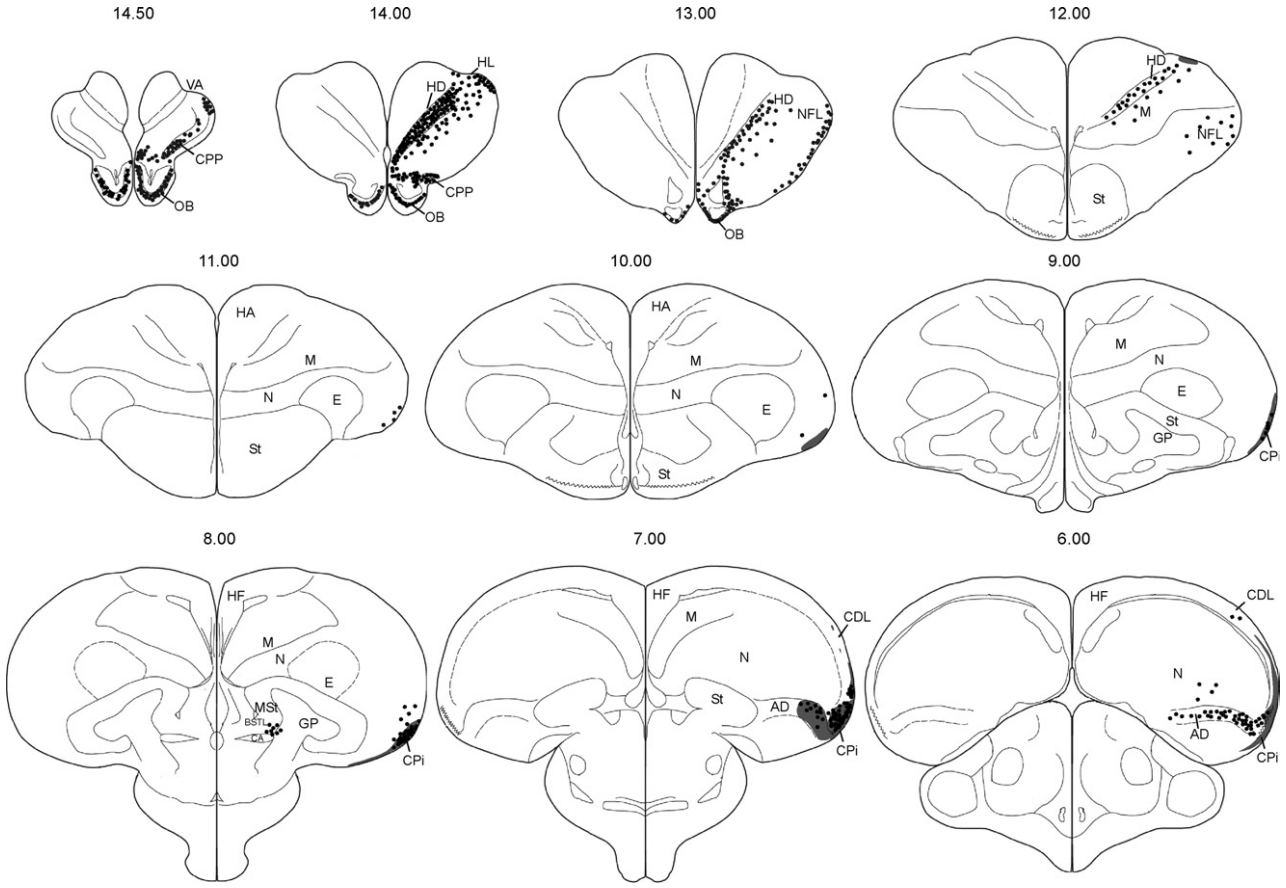


**Fig. 4.** Distribution of retrogradely labeled neurons (dots) following injections of CtB into the right CPi. The injection site and CtB-positive fibers are identified by the gray area. These drawings were done representatively from one case.

of the telencephalon, mostly located in the caudal PoAb, although some of these cells might have been located in the ventral CPi.

### Quantitative analysis of the OB CPi projection

For quantitative analysis of the projection from the OB to the CPi, 16 birds received unilateral injections of CtB either into the left ($n=8$) or to the right ($n=8$) CPi.

The number of CtB-positive cells in the OB ipsilateral to the injection site was significantly higher than that in the contralateral OB (right CPi injection, ipsilateral neurons: 108.68±63.06, contralateral neurons: 87.79±63.50; Wilcoxon test $Z=2.52$ $P<.05$; left CPi injection, ipsilateral neurons: 87.21±55.69, contralateral neurons: 62.83±65.66 Wilcoxon test $Z=2.24$ $P<.05$; Fig. 3b), and the absolute cell numbers did not differ after left- and right-hemispheric injections neither between the ipsilateral (Mann–Whitney *U*-test $Z=-0.05$ $P=0.958$) nor in the contralateral (Mann–Whitney *U*-test $Z=0.262$ $P=0.793$) cell populations. Furthermore, total cell numbers of ipsi-and contralaterally labeled cells within the OBs did not differ significantly between the two injection sites (injection right Cpi, total cell number OB: 176.38±133.17; injection left CPi, total cell number OB: 150.04±120.11; Mann–Whitney *U*-test $Z=-0.05$ $P=0.96$).

Although, as outlined above, these numbers could not be corrected by injection volume, the absence of asymmetries is supported by a comparison of the BIs of the projections. The BI of CtB-positive cells in the OB revealed no significant left-right differences (right injection BI: 0.18±0.13; left injection BI: 0.25±0.17; Mann–Whitney *U*-test $Z=-0.74$ $P=0.46$). This indicates that none of the CPis received a higher bilateral input from the OBs.

## DISCUSSION

This tract tracing study had two goals. First, older studies showed an inconsistency in the projection pattern of the olfactory system. Therefore, using modern tracing techniques, we re-analyzed the general projection patterns of the olfactory bulb of homing pigeons. Second, behavioral data indicated a functional superiority of the right nostril/OB and the left CPi for olfactory-dependent navigation in the pigeon. We therefore tested if functional lateralization is based on asymmetrical connections between these two structures.

### Olfactory bulb efferents

Until now, only two studies analyzed the projection patterns of the olfactory bulb in pigeons and came to partly contradictory

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conclusions. Since these studies used techniques with a highly limited resolution (anterograde degeneration: Rieke and Wenzel, 1978; autoradiography: Reiner and Karten, 1985), our first aim was to re-analyze this projection with BDA as a highly sensitive anterograde tracer.

In accordance with the results of Reiner and Karten (1985), our labeling demonstrated strong bilateral projections to several telencephalic brain areas with only the projection to the SM being confined to the ipsilateral hemisphere. Beside the bilateral projection to the CPP, the CPi is subject of strong innervation from the OB. The extent of this OB projection corresponds approximately to the CPi described previously in the atlas of Karten and Hodos (1967). The projection to the contralateral hemisphere runs via the habenular commissure (Reiner and Karten, 1985) and not via the CA as described by Rieke and Wenzel (1978). Within the amygdaloidal area, only the TnA receives olfactory input (Reiner and Karten, 1985). Accordingly, our tracing results largely confirm the data of Reiner and Karten (1985). However, a bulbar projection to the TuO is not clear. Although we cannot completely exclude that some of the fibers terminated in the TuO, our findings indicate that the TuO may not represent a primary olfactory brain area. As Reiner and Karten (1985) pointed out, they had difficulties to limit their injections to the OB due to its small size. Thus, their staining within the TuO could result from tracer spread into brain areas adjacent to the OB. Moreover, like Reiner and Karten (1985), we could not verify projections to the ipsilateral mesopallium, MSt, nucleus accumbens, and the contralateral GP as reported by Rieke and Wenzel (1978).

### Piriform cortex afferents

The projections to the CPi observed in this study largely reflect the findings by Bingman et al. (1994), with some interesting exceptions that can be explained with more localized injections in our preparations and the higher signal-to-noise ratio of our tracer. The main telencephalic areas projecting to the CPi are the OBs, the CPP, and the HD. But projections from the hippocampal formation (HF) and from the septum, as observed by Bingman et al. (1994), could not be revealed.

In the present study, all four cases wherein the injection site was mostly restricted to the CPi, no CtB-positive cell could be observed in the HF. Since in these cases, retrogradely labeled cells could be detected along the complete extensions of both OBs, it is unlikely that a lack of hippocampal afferents was due to incomplete CPi injections. It is more likely that the afferents from the HF that were observed by Bingman et al. (1994) resulted from tracer spread into areas adjoining the CPi. A likely candidate represents the PoAc since it shares several connections with the CPi (Atoji et al., 2006). PoAc afferents from HD, NFL, HF, and contralateral CPi and PoAb have been described, as well as input from the HF and from the septum but not from the OB and CPP (Atoji et al., 2006). The assumption that not CPi but PoAc receives input from HF is further supported by anterograde BDA tracings into the DM of the HF that demonstrate only very few fibers in the CPi (Atoji and Wild, 2004). Comparing the labeling pattern of injections limited to the CPi and the larger ones of our study with that of Bingman et al. (1994), it seems very likely that the projections from CDL, TPO, NC, PoAb, PoAc, DM, TuO, and the NDB terminate within the PoAc (Atoji et al., 2006) and not within the CPi. Moreover, in contrast to the study by Bingman et al. (1994), no projection from the ipsilateral TnA was detected in the present study. This is consistent with a tract tracing study in ringdoves that could not detect a projection from the TnA to the CPi (Cheng et al., 1999). Taken together, our results provide strong evidence that both olfactory bulbs, the ipsilateral CPP, HD, HL, NFL, and AD clearly project to the CPi (Fig. 5).

### Olfactory projections and functional lateralization

One of the aims of this study was to examine whether the functional lateralization of the olfactory system is based on an asymmetrical projection pattern between OB and CPi. Behavioral studies revealed a functional dominance of the left CPi, which appears to be triggered by the right nostril/OB, as demonstrated by plugging the left or right nostril of homing pigeons (Gagliardo et al., 2005, 2007, 2011). Following this line of thought, this functional lateralization could be based on an asymmetrical projection pattern with a stronger innervation for the right OB to the left CPi. Such a bottom-up asymmetry is known from the visual system of pigeons. Here, the left thalamic relay nucleus of the tectofugal pathway receives a stronger bilateral input from the mesencephalic optic tectum than the right one (Güntürkün et al., 1998). This connectional asymmetry could be verified by electrophysiological (Folta et al., 2004) and behavioral means (Güntürkün and Hahmann, 1999) and is in line with the left hemispheric dominance for visual feature analysis (Manns and Güntürkün, 2009).

However, our tracing experiment neither revealed left-right differences in the absolute numbers of retrogradely labeled neurons nor in the relation of ipsi- to contralateral projections of the OB onto the CPi. Although an interpretation of the absolute numbers is hampered by the absence of a correction factor for injection volume, the interpretation of the bilaterality factor is not. One always has to be extremely careful when interpreting negative data, but it seems likely that the asymmetrical behavioral (Gagliardo et al., 2005, 2007, 2011) and molecular imaging effects (Patzke et al., 2010) do not result from anatomical asymmetries in the ascending projections in the OB–CPi system. This obviously does not rule out that the functional lateralization originates from asymmetries at synaptic or cellular level, which are left undetected with our method. It is also in principle conceivable that asymmetrical input from the right olfactory system to the left CPi is mediated by an indirect projection via the CPP, which receives bilateral input from the OB. Moreover, a dominance of the left CPi can also result from higher-order differences where the left CPi may receive more pronounced input, for example, either the HD, HL, NFL, or the AD.

Quantitative analysis of the neuronal activity marker ZENK in homing pigeons revealed no left-right differences in OB activation. Further, the sensitivity to olfactory deprivation resulted in a similar downregulation of the activity in

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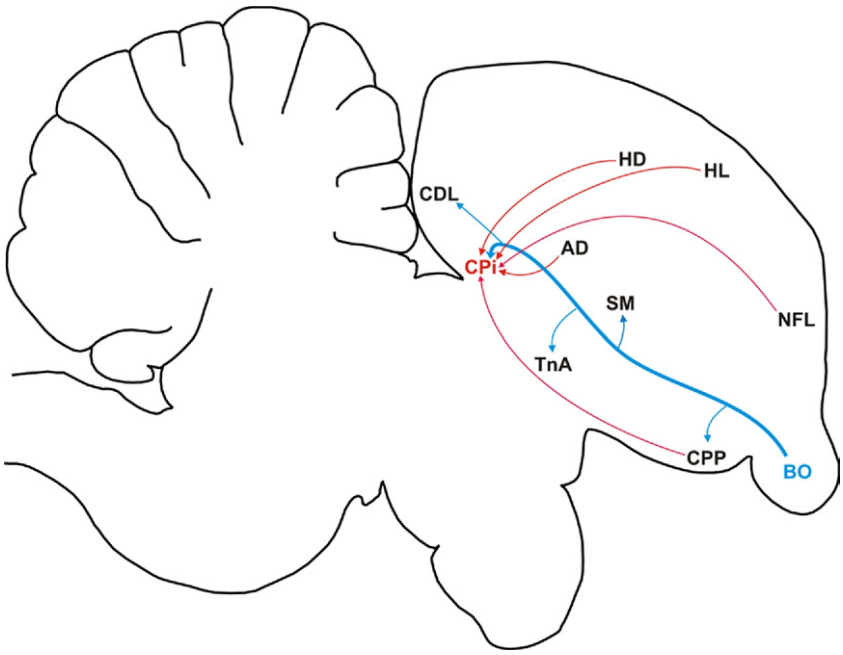


**Fig. 5.** Schematic representation of the main telencephalic olfactory projections. The OB project bilaterally to the CPP, TnA, CPi, CDL, and ipsilaterally to the SM. In addition to that, CPi receives bilateral input from CPP and ipsilateral input from AD, HD, HL, and NFL. For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.

both OBs (Patzke et al., 2010). These results indicate that it is unlikely for the functional dominance of the right nostril to be caused by the processing of olfactory information in the OB.

Taken together, first, we successfully re-analyzed the telencephalic organization of the olfactory system in homing pigeons with modern techniques demonstrating some differences to older studies. Second, we demonstrated that the functional lateralization of the olfactory system during the initial orientation in homing pigeons is presumably not based on an asymmetrical organization of the olfactory telencephalic projection.

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