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Asymmetrical Processing of Olfactory Input in the Piriform Cortex Mediates "Activation" of the Avian Navigation Circuitry

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Abstract

The role of odors in the long-distance navigation of birds has elicited intense debate for more than half a century. Failure to resolve many of the issues fueling this debate is due at least in part to the absence of controls for a variety of non-specific effects that odors have on the navigational process. The present experiments were carried out to investigate whether the olfactory inputs are involved only in "activation" of neuronal circuitry involved in navigation or are also playing a role in providing directional information. Experienced adult pigeons were exposed to controlled olfactory stimuli during different segments of the journey (release site vs. displacement + release site). Protein levels of IEGs (immediate early genes used to mark synaptic activity) were analyzed in areas within the olfactory/navigation avian circuitry. The results indicate that 1) exposure to natural odors at the release site (and not before) elicit greater activation across brain regions than exposure to filtered air, artificial odors, and natural odors along the entire outward journey (from home to the release site, inclusive); 2) activation of the piriform cortex in terms of odor discrimination is lateralized; 3) activation of the navigation circuitry is achieved by means of lateralized activation of piriform cortex neurons. Altogether, the findings provide the first direct evidence that activation of the avian navigation circuitry is mediated by asymmetrical processing of olfactory input occurring in the right piriform cortex.

Key words: brain circuitries, neuronal activation, olfaction, piriform cortex, vertebrates

Introduction

For more than half a century, homing pigeons have been used as model organisms to investigate the navigational ability of free-flying birds. Although behavioral studies have shed light on the compass mechanisms used by birds (Papi et al. 1971; Phillips 1986; Walcott 1992; Lohmann et al. 2004; Jorge et al. 2008) reviewed in (Papi 2001; Wallraff 2005; Wiltschko and Wiltschko 2015) there is a long-standing controversy concerning the type(s) of sensory information underlying the "map" or geographic position sense required for true navigation (Phillips and Jorge 2014; Wallraff 2014), and the neuronal networks involved (Patzke et al. 2011; Jorge et al. 2014). Among the geophysical factors proposed to be involved in the navigational map, the distribution of environmental odors (Papi et al. 1971; Wallraff and Andreae 2000), spatial variation in the earth's geomagnetic field (Walcott 1992; Lohmann et al. 2004), natural sources of infrasound that are detectable over hundreds of kilometers (Hagstrum 2013),

and angular differences in the gravity vector (Blaser et al. 2013), have been proposed to play a role in this process. Nevertheless, to date, no irrefutable evidence exists in favor of any of these hypotheses (Freake et al. 2006; Deutschlander et al. 2012), and many authors have suggested that the process involves multiple cues (Wiltschko et al. 1987; Freake et al. 2006; Jorge et al. 2008). Only proponents of the olfactory map hypothesis, contend that a single sensory modality (olfaction) is both necessary and sufficient to provide map information (Papi 2001; Wallraff 2005). According to the olfactory map hypothesis, the spatial distribution of olfactory gradients is used by animals to derive their geographic location.

In the 1970's, Papi and his co-workers found that pigeons were disoriented when deprived of olfactory information by a variety of methods, including nerve sectioning (Papi et al. 1971), anesthesia of the olfactory mucosa (Del Seppia et al. 1996), nasal plugs (Papi et al. 1980), or exposure to synthetic or filtered air (Wallraff and Foa 1981). At the time, the findings were interpreted as evidence that odors carry information about geographic position used to derive "map" location (Papi 2001; Wallraff 2005; 2014). However, findings from other labs were not always in accordance with the existence of an olfactory map, and alternative hypotheses were suggested (Wiltschko 1996; Phillips et al. 2006; Hagstrum 2013; Phillips and Jorge 2014). In particular, to explain the importance of olfactory cues in vertebrate navigation, it was proposed that exposure to non-home odors could act as a trigger that activates the navigation circuitry; "olfactory activation hypothesis" (Phillips et al. 2006; Jorge et al. 2009; 2010; 2014; Phillips and Jorge 2014; but see Gagliardo et al. 2011). This suggestion is consistent with evidence from studies of other vertebrates. For example, mice failed to keep track of a displacement when transported in the presence of their home-nest odors (Alyan 1996), possibly because the absence of non-home odors prevented activation of their path integration system, and salmon during the freshwater phase of their migration fail to exhibit rheotactic behavior when chemical cues from the imprinted olfactory signature of the natal stream are not detected (Stabell 1992).

Although odors clearly play an important role in vertebrate navigation, experiments carried out by proponents of the olfactory map hypothesis have consistently failed to include adequate controls to rule out a variety of alternative roles that odors can play in spatial behavior (Phillips et al. 2006; Jorge 2011; Phillips and Jorge 2009, 2014). Behavioral studies, in which pigeons were exposed to artificial odors to control for non-specific effects of odors either during displacement to a release site (Jorge et al. 2009, 2014) or at the release site (Jorge et al. 2010), showed that the initial homeward orientation of free-flying birds' was indistinguishable from that of birds exposed to natural air containing odors from the local environment (Jorge et al. 2009, 2010, 2014). The manner in which artificial odors were presented to the birds in these experiments ruled out their use in deriving geographic position. The artificial odors were presented continuously (with odor composition changing every 5 min) and at a low concentration to avoid startling the birds with sudden changes in odor concentration. As in earlier experiments, the homeward orientation of birds exposed to artificial odors contrast with that of birds exposed to filtered air (i.e. air containing no odors) that failed to exhibit homeward orientation (Jorge et al. 2009, 2010).These findings indicate that the homeward orientation of birds exposed to artificial odors resulted from olfactory activation of the neuronal circuits responsible for processing non-olfactory navigational information.

Attempts by proponents of the olfactory map hypothesis to identify the neural regions in the central nervous system (CNS) responsible for processing map information has led to the suggestion that homing pigeons derive olfactory map information from odors that activate the right olfactory epithelium (Gagliardo et al. 2005; Patzke et al. 2010). From here, olfactory information required for homing is proposed to follow a pathway through the right olfactory bulb and contra-lateral piriform cortex, before reaching higher order navigational processing centers, predominantly in the left hemisphere (Gagliardo et al. 2005; Patzke et al. 2010, 2011). One such structure is the dorsolateral area of the hippocampal formation (Gagliardo et al. 2005; Nardi and Bingman 2007; Patzke et al. 2010), commonly thought to be homologues of the mammalian entorhinal cortex (Colombo and Broadbent 2000), and considered to play a central role in processing spatial information (Nardi and Bingman 2007; Jorge et al. 2014). Importantly, dorsolateral hippocampal neurons are activated by the artificial odors used in earlier behavioral experiments (Jorge et al. 2014), as well as by natural odors, suggesting that activity in this region of the brain could be involved *either* in an olfactory map or in olfactory activation of a non-olfactory map system(s) (i.e., magnetic, infrasound, etc, see refs. Wallraff 2005; Phillips et al. 2006; Burger et al. 2010; Hagstrum 2013). In fact, pigeons exposed at the home loft to unfamiliar artificial odors showed consistent activation of dorsolateral hippocampal neurons despite the familiar surroundings (Jorge et al. 2014).

Because experienced adult birds have been shown to preferentially use map information obtained at the release site to derive their geographic position relative to the home loft, we used immunocytochemical techniques to investigate which role olfactory inputs play in the avian navigation circuitry: only activating the neuronal circuitry involved in navigation and/or also playing a role in providing directional information during the initial phase of the navigational process. Adult pigeons were given exposure to olfactory stimuli either only at the release site or during the displacement from the home loft as well as at the release site. The olfactory treatments included exposure to natural odors (NA; NA*), to a sequence of artificial "nonsense" odors (NS), or to filtered air (PA). Protein levels of immediate early genes were then compared in areas within the olfactory/navigation avian circuitry, more specifically in the olfactory bulbs, piriform cortex, and hippocampal formation (dorsolateral, dorsomedial and triangular areas); (Figure 1A).

Materials and methods

Subjects, behavioral procedures and stimulus conditions

The animal husbandry and all of the experimental procedures are in accordance with the EU and the Portuguese Law for animal welfare. All experimental protocols were approved by the Portuguese Veterinarian Commission under the project reference: PTDC/BIA-BEC/99416/2008.

Forty adult homing pigeons of both sexes were randomly assorted into 1 of 4 groups. Three of these groups were transported to the release site inside airtight boxes supplied with filtered air (filters remove 99.9% of the atmospheric odors), while the fourth group (NA*) was transported inside an airtight box supplied with natural air from the outside environment (n = 6). The single release site was located 81 km ESE from the home loft in an unfamiliar area (pigeons had not been released previously within 50 km of the release site). Upon reaching the release site, the NA* group was kept with full access to natural air, while the 3 groups exposed to filtered air during transport were exposed to one of the following treatments: the Natural Odors group (NA) was exposed to natural air from the release site (n = 8); the Purified Air group (PA) was maintained in filtered air without odors (n = 7); and the Artificial Odors

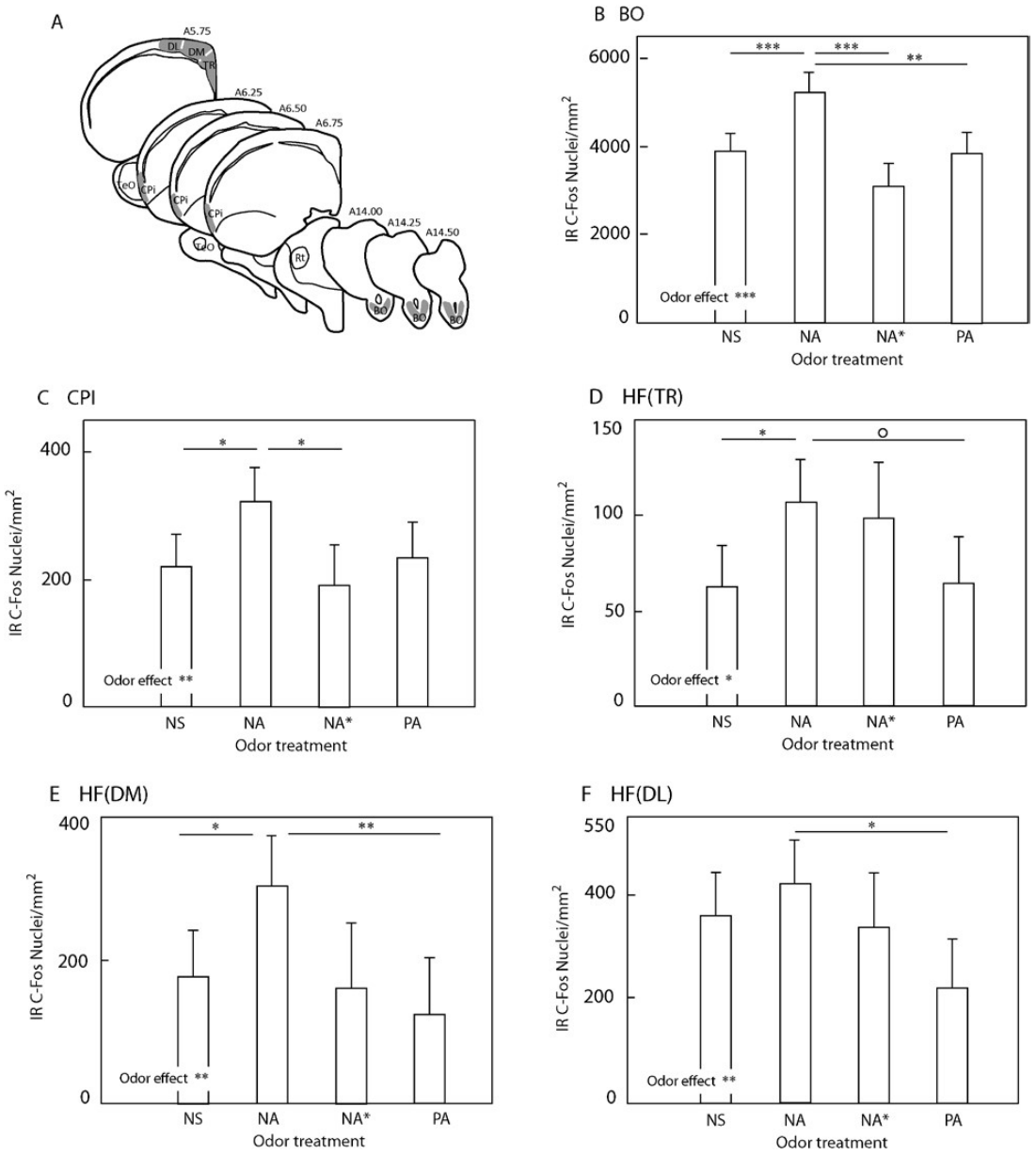

Figure 1. Neuronal activation for different olfactory treatment groups. NS, pigeons exposed to artificial odors at the release site; NA, pigeons exposed to natural air at the release site; NA*, pigeons exposed to natural air during the outward journey and at the release site; and PA, pigeons exposed to filtered air containing no odors during the outward journey and at the release site. (**A**) Histological reconstruction of brain areas analyzed (grey areas). BO, olfactory bulbs; CPi, piriform cortex; HF, hippocampal formation subdivided in: DL, dorsolateral, DM, dorsomedial, and TR, triangular areas; Rt, nucleus rotundus; TeO, optic tectum. (**B–F**) Mean numbers of c-Fos-IR neurons (bars) and the 95% confidence interval (whiskers) in (B) the olfactory bulb, (C) the piriform cortex, (D–F) the hippocampal formation: (D) the triangular area, (E) the dorsomedial area and (F) the dorsolateral area. Significance of the "Main Effect" is given by the repeated-measure ANOVA. Significance between groups is given by the HSD Post Hoc test for unequal sample sizes. Significance levels: $^{\circ}P < 0.08$; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

group (NS; referred to as nonsense odors in earlier experiments; refs. Jorge et al. 2009, 2010, 2014) was exposed to filtered air into which was introduced a fixed sequence of artificial odors ($n = 8$). The artificial odors were administered according to previously published protocol (Jorge et al. 2009, 2010, 2014). For additional information on the odor sequence and odor concentration, see refs Jorge et al. 2009, 2014.

Olfactory exposure at the release site lasted 36 min. At the end of the olfactory exposure, and 8 min prior to release, the nostrils of all pigeons were anesthetized with a Xylocaine spray (for further details see Jorge et al. 2014). Then all pigeons were released in a single flock composed of 1–2 individuals from each group plus 4 additional individuals with no treatment. This procedure helped to minimize loss of experimental (PA, NS, NA, NA*) pigeons, and to assure that most of the pigeons arrived home in an appropriate time window to be perfused (i.e. between 60 and 120 minutes); due to the time required to perfuse individual birds, we could only perfuse 4 pigeons from each release.

Immunohistochemistry

Sixty to one hundred twenty minutes after the end of the olfactory stimulation (i.e. when nostrils were anesthetized 8 min prior to release), pigeons were deeply anesthetized with an intra-peritoneal injection of sodium pentobarbital (0.5 ml/pigeon) and transcardially

perfused with phosphate-buffered saline (PBS; 0.9% NaCl in 0.1M phosphate buffer, pH 7.4) followed by fixative (PFA; 4% paraformaldehyde in phosphate buffer—PB, pH 7.4). Brains were dissected and postfixed for additional 24h in the same fixative. For sectioning, the brains were first cryoprotected in sucrose buffer (30% sucrose in PB) and then embedded in sucrose-gelatin (30% sucrose, 10% gelatin in distilled water). The embedded brains were sectioned on a freezing microtome in the coronal plane at a thickness of 60 µm. Free-floating sections were stored in PB containing 0.001% of sodium azide, at 4 °C until they were stained. Immunohistochemical detection of c-Fos was performed with free-floating sections according to a previously published protocol (Terleph and Tremere 2006; Jorge et al. 2014). Every sixth section was counterstained with cresyl violet and used for general orientation following the pigeon atlas of Karten and Hodos (1967).

Analyses

Three tissue sections from each subject (sections selected according to Gagliardo et al. 2005) were photographed for the olfactory bulbs (BO; atlas sections A14.50, A14.25, and A14.00) and the piriform cortex (CPi; atlas sections A6.75, A6.50, and A6.25). In these sections, c-Fos IR nuclei were counted in a total of 54 representative counting frames (i.e., 27 frames/each brain hemisphere; frame area 0.018714 mm^2). One tissue section from each subject (sections selected according to ref. 12) was used for the 3 subdivisions of the hippocampal formation (TR, DM, and DL; atlas section A5.75). Here, the number of c-Fos-IR nuclei was quantified in a total of 18 counting frames (i.e., 9 frames/each brain hemisphere; frame area 0.040221 mm^2).

Photographs were taken at equal light intensity for all sections by a technician blind to the experimental conditions. They were converted to 8-bit grayscale photographs and the number of c-Fos-immunopositive cells was quantified using the ImageJ program by 2 technicians blind to the experimental conditions (i.e., each section was counted twice, once by each technician, and averaged). A threshold was defined manually based on background staining; the number of cell nuclei that had higher optical density than the threshold were counted.

Statistical comparisons were made with a general linear model repeated-measures analysis of variance (GLM, Statistica). Factors included the olfactory stimulus ("Odor:" Natural air, Artificial Air, Purified Air and Full air) and brain hemisphere ("Hemisphere:" left and right). Brain regions were treated as a repeated measure (5 levels). The *post hoc* honestly significant difference (HSD) test was used for multiple comparisons with unequal sample sizes (Zar 1999).

Results

Findings from the analysis with general linear models showed that the overall treatment affected neuronal activation in the analyzed brain areas (ANOVA: $F_{(5, 34)}$ = 312,14; $P < 0.0000001$), with olfactory exposure (main effect: "odor," $F_{(15,94)}$= 3.91; $P < 0.00005$; Figure 1), but not brain hemisphere (main effect: "hemisphere," $F_{(5, 34)}$= 0.75; $P > 0.59$; Figure 2) or the interaction between both (main effect: "odor × hemisphere," $F_{(15,94)}$= 0,65; $P > 0.64$; Figure 2), being responsible for the observed variability.

In adult pigeons, the olfactory bulbs were significantly more affected by exposure to natural odors when this exposure was restricted to the release site (NA) than by any other olfactory stimulation (Figure 1B), including stimulation with natural odors during the entire outward journey to the release site as well as at the release site (NA*). Interestingly, findings show that it is not simply access to natural odors at the release site that contributes to the increased neuronal activation in the olfactory bulbs of NA pigeons, because NA* pigeons exposed to the same natural odors in addition to natural odors from the displacement (Figure 1B) had significantly *lower* neuronal activation (see discussion below). A similar pattern was found in the piriform cortex (Figure 1C), with the NA group showing significantly higher levels of activation than the other olfactory treated groups (NS and NA*).

In the hippocampal formation, levels of neuronal activation in the triangular and dorsomedial hippocampal areas for birds in the NA, NS, and PA groups were in line with previous findings from immunocytochemical analyses of young inexperienced homing pigeons exposed to the same treatments during the displacement to the release site (Jorge et al. 2014). NA pigeons showed higher neuronal activation in the triangular and dorsomedial hippocampal areas than NS and PA pigeons (Figure 1D, E, respectively). While in the dorsomedial hippocampal area, NA* pigeons exhibited patterns of neuronal activation that are more in line with patterns of neuronal activation observed in NS and PA pigeons (Figure 1E), in the triangular hippocampal area NA* pigeons exhibit patterns of neuronal activation that were similar to those observed in the NA pigeons (Figure 1D). Neuronal activation patterns in the dorsolateral hippocampal area were very similar to the ones observed in the dorsomedial hippocampal area, although NS and NA* pigeons were more intermediate between NA and PA pigeons (Figure 1F).

Although we did not find evidence for overall lateralization of the olfactory input to the olfactory bulbs, or to any of the 3 areas of the hippocampal formation (i.e. overall neuronal activation in one side of the brain generally matched the neuronal activation in the opposite side; Figure 2), differences in neuronal activation in the olfactory bulbs among treatment groups were more exaggerated in the left hemisphere, with the NA pigeons significantly different from all groups (Figure 2A). In the right hemisphere, a significant difference only occurred between the NA and NA* groups.

The piriform cortex was the only region out of 5 analyzed that provided evidence of overall lateralization (main effect: "odor × hemisphere," $F_{(3,38)}$= 3.00; $P < 0.05$; Figure 2B) although, no significant differences among groups were reported. The one exception was levels of neuronal activation between NA and NA* pigeons that approached significance (right hemisphere, HSD post hoc test: $P = 0.058$; Figure 2B). Interestingly, further analysis suggests that both the olfactory bulbs and the piriform cortex may be involved in processing olfactory input with relevance to the navigational process—see below.

Further analyses (Figure 3–5) revealed several interesting patterns showing: 1) that neuronal activation patterns in both the dorsomedial and dorsolateral hippocampal areas are responses to the presence or absence of odors (filtered air vs. other odors; Figure 3D and E), rather than to odor discrimination (Figure 4D and E) or to odor context (Figure 5D and E); 2) that neuronal activation in the triangular hippocampal area is primarily a response to odor discrimination (natural vs. artificial; Figure 4C) rather than to odor presence (Figure 3C) or to odor context (Figure 5C); 3) that neuronal activation in the olfactory bulbs and piriform cortex is a response to odor context (release site vs. displacement and release site; Figure 5A, B) and in the piriform cortex is also a response to odor discrimination (natural vs. artificial; Figure 4B) but not to odor presence (Figure 3B); and 4) that neuronal activation that is dependent on odor context in the piriform cortex is processed asymmetrically in the brain, with emphasis to the right hemisphere (Figures 2B and 5B).

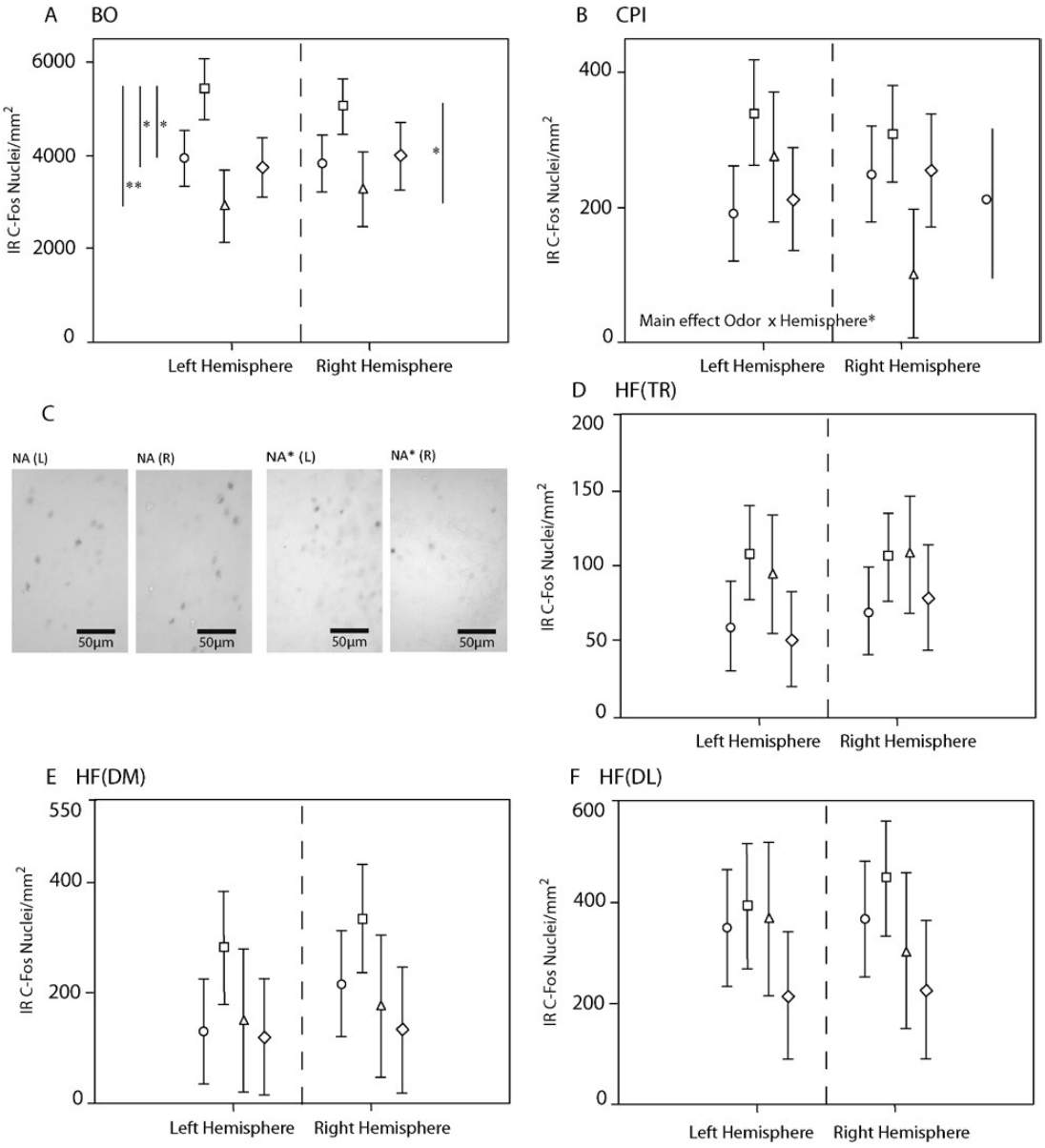

Figure 2. Lateralization of responses to olfactory stimuli. Neuronal activation in each brain hemisphere in (**A**) the olfactory bulb, (**B**) the piriform cortex, (**C**) Photomicrographs of the piriform cortex showing c-fos immune reactive nuclei. NA, pigeons exposed to natural odors only at the release site; NA*, pigeons exposed to natural odors during the displacement and at the release site (**D–F**) the hippocampal formation (HF): (D) the triangular area (TR), (E) the dorsomedial area (DM) and (F) the dorsolateral area (DL). Mean numbers of c-Fos-IR neurons are indicated for pigeons exposed to artificial odors at the release site (circles); pigeons exposed to natural air at the release site (squares); pigeons exposed to natural air during the outward journey and at the release site (triangles); pigeons continuously exposed to filtered air containing no odors (diamonds). Additional symbols and abbreviations as in Figure 1.

Discussion

The prevailing view that odors provide an olfactory gradient map that can be used at unfamiliar sites at any distance from the home loft (Papi 2001; Wallraff 2005, 2014) stands on a lateralized neuronal network (Gagliardo et al. 2005; Nardi and Bingman 2007; Patzke et al. 2010). The neuronal network involved in navigation is proposed to include the right olfactory bulb, the left piriform cortex and the left hippocampal formation and, in particular, the left dorsolateral hippocampal area. While our findings show that odors play an important role in the neuronal activation of these areas (Figures 1–5), there is no evidence in the present study for lateralization that provides support for the use of an olfactory map (Figure 2). The one exception is the piriform cortex where neuronal activation patterns suggest an interaction between the context in which odors are presented (release site vs. displacement + release site) and the hemisphere where processing occurs (Figures 2B and 5B). This contrasts with an earlier study using young homing pigeons exposed to olfactory stimuli during the outward journey to a familiar release site, which did not find evidence for lateralized processing of olfactory information in the piriform cortex (Jorge et al. 2014). Because distinct context-dependent olfactory information (e.g. familiar/unfamiliar—or outward journey/release site—olfactory information) was being processed in the 2 studies, differences between the 2 studies may reflect differences in olfactory context processing by the piriform cortex (Cohen et al. 2015).

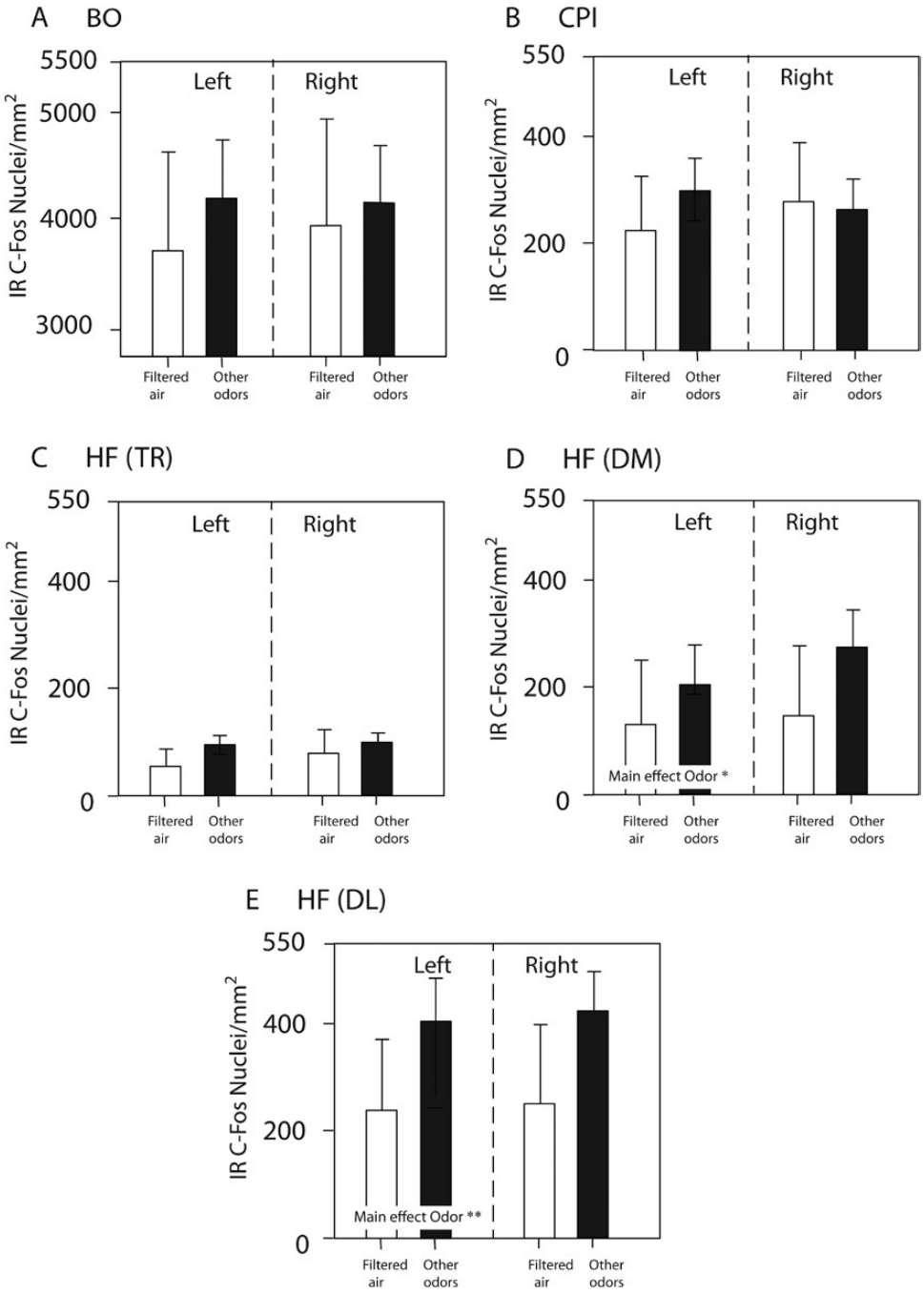

Figure 3. Responses to olfactory input. Post hoc analysis comparing neuronal activation patterns elicited by exposure to non-home odors (natural/artificial) vs. filtered air. Note; pigeons in all 4 groups were exposed to self-generated odors that were assumed to be common to the home environments. The neuronal activation is shown separately for the left and right brain hemispheres. Additional symbols and abbreviations as in Figure 1.

Importantly, the findings support the view that the piriform cortex and its lateralized response is crucial to avian navigation. However, instead of providing positioning information as proposed by the navigational hypothesis, our data supports that the right piriform cortex has a transient activation with subsequent habituation upon exposure to natural odors that is consistent with an activation role of the neuronal circuitry involved in navigation (see below; Figures 1C, 2B, and 5B).

In brain centers that previously proposed to be involved in processing navigational information (i.e. subdivisions of the hippocampal formation), different characteristics of odors appear to contribute to the neuronal activation in each subdivision (Figures 1D–F and 3C–E). In the triangular hippocampal area, odors appear to be involved in some other aspects of olfactory recognition (e.g. episodic-like memory: exposure to natural odors either during the outward journey or at the release site elicited a neuronal response distinct from exposure to artificial or filtered air; Figures 1D and 4C), while in the dorsolateral hippocampal area odors appear to have an "activation" function (i.e. exposure to either natural or artificial odors elicit a neuronal response distinct from exposure to

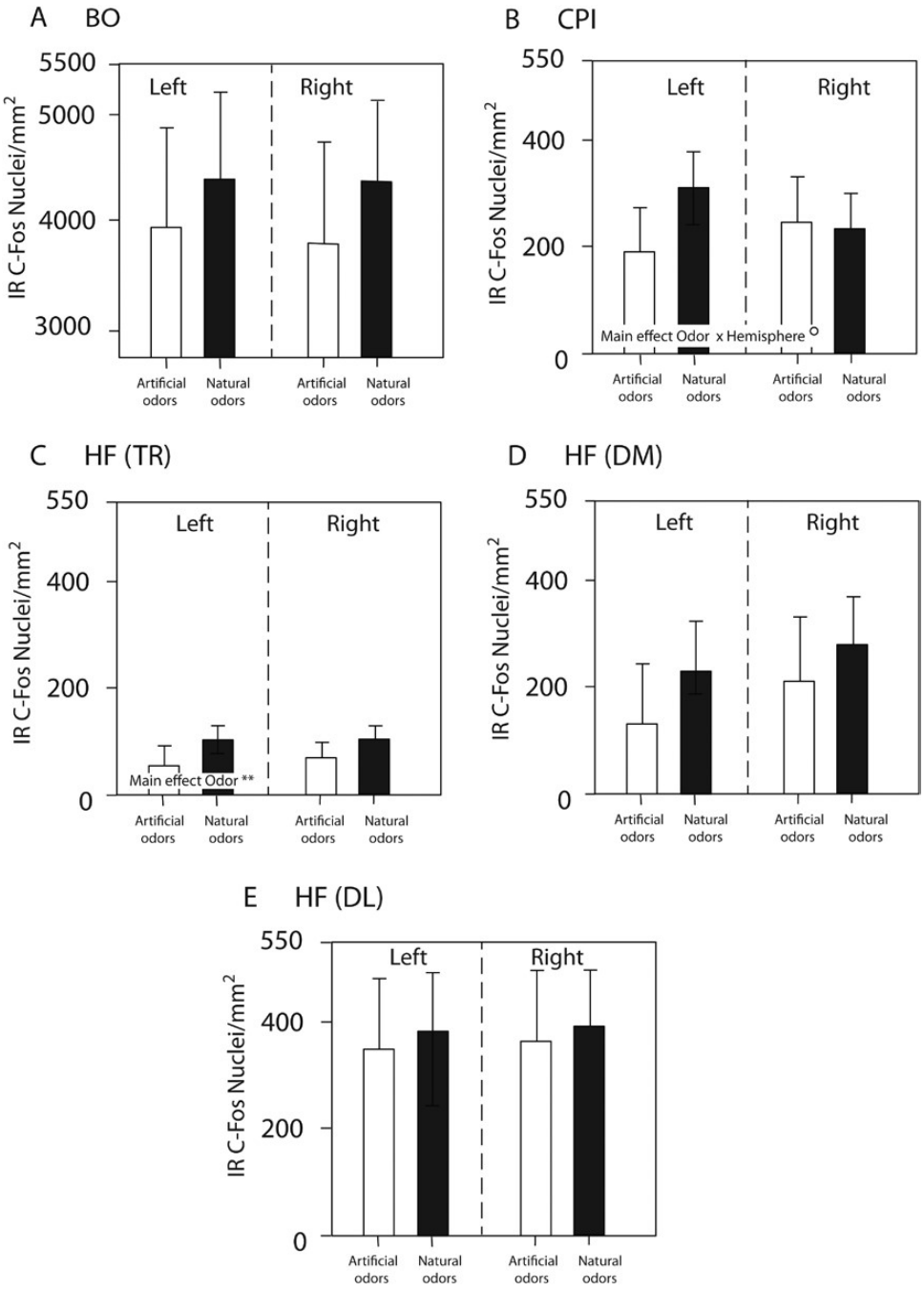

Figure 4. The role of natural odors. Differences in neuronal activation patterns elicited by natural versus artificial odors in distinct brain areas. The neuronal activation is shown separately for the left and right brain hemispheres. Additional symbols and abbreviations as in Figure 1.

filtered air with no odors; Figures 3E and 4E). The effect of odors in the dorsomedial hippocampal area is less clear (Figure 1E) although an "activation" effect may be involved because neuronal activation is primarily a response to presence rather than to discrimination of odors (Figures 3D and 4D).

Overall, the fact that olfactory manipulation greatly affect neuronal activation in the olfactory centers was to be expected (Gagliardo et al. 2005; Jorge et al. 2014; Patzke et al. 2010, 2011). The 3 olfactory treatments vary in odor type (natural vs. artificial), concentration (high vs. low), and distinctiveness (several vs. few). For example, natural air should contain more odor compounds than artificial odors, but in turn, artificial odors were more intense than natural odors (at least to a human observer), and these differences should be even greater in comparison to filtered air where neither natural nor artificial odors were present. It is not surprisingly, therefore, that, our findings showed significant differences in neuronal activation at the olfactory bulbs and piriform cortex (Figures 1B, C and 2A, B), with pigeons exposed to natural air at the release site (NA) exhibiting higher neuronal activation than the other groups (NS and PA; Figure 1). What was not expected, based on a rule of local odors in providing navigational information, was the difference between the 2 groups exposed to the same natural odors at

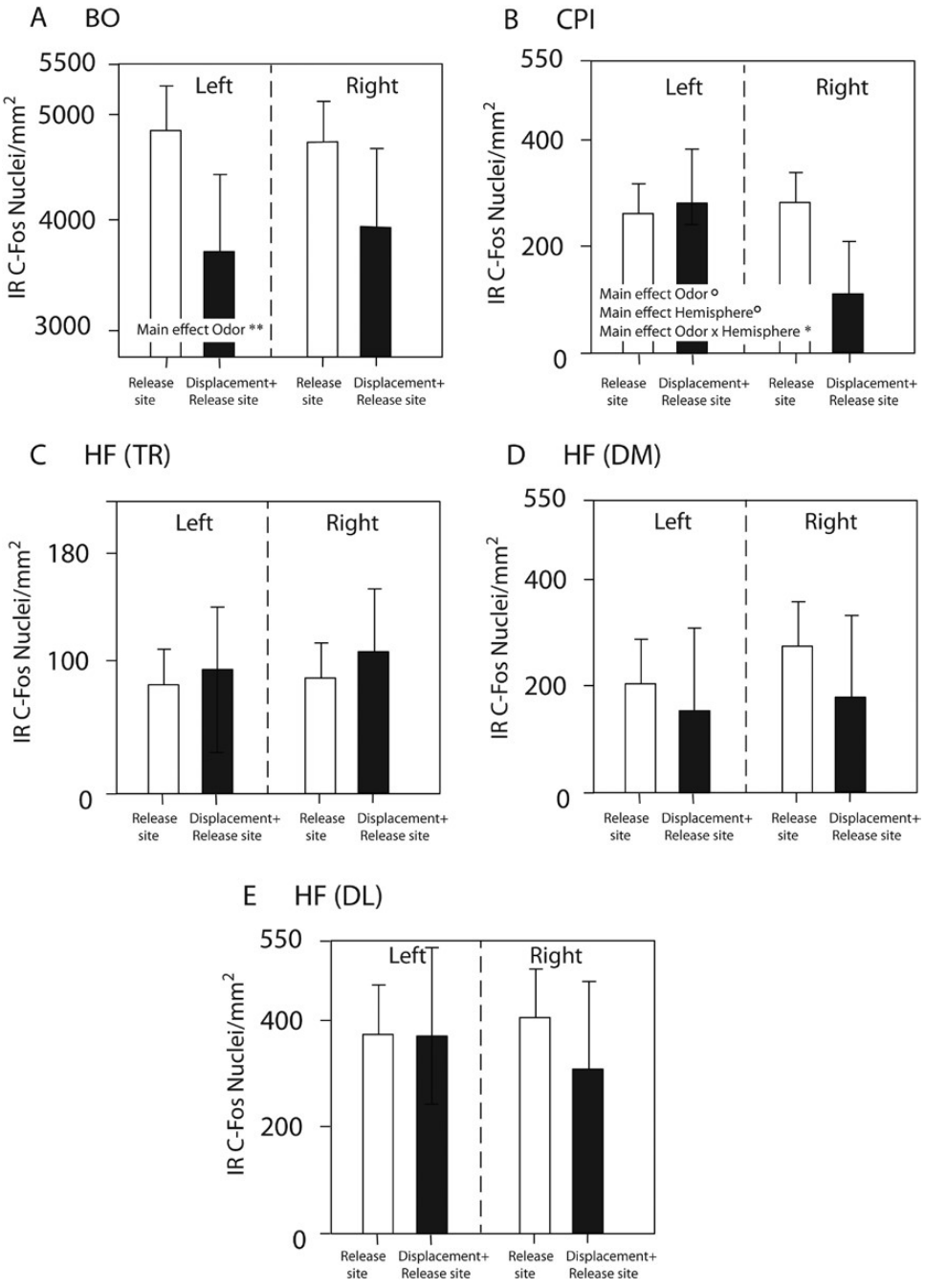

Figure 5. Timing of exposure to natural odors. Differences in neuronal activation patterns elicited by exposure to natural odors only at the release site versus during the displacement as well as at the release site in distinct brain areas. The neuronal activation is shown separately for the left and right brain hemispheres. Additional legends as in Figure 1.

the release site (NA and NA*; Figures 1B, C and 2A). The only difference between the 2 treatments was that NA pigeons were only exposed to natural odors at the release site, while the NA* pigeons were exposed to natural odors from the beginning of the journey to the release site as well as at the release site and therefore not only the context but the novelty of the odors may have differed assuming there was some degree of similarity in the types of odors the birds were exposed to during the outward journey and at the release site.

Staining protein products of immediate early genes (IEG) is a widely used technique to map brain activity (Morgan and Curran 1991; Gagliardo et al. 2005; Terleph and Tremere 2006; Nardi and Bingman 2007; Burger et al. 2010; Patzke et al. 2010; Jorge et al. 2014; Lefeld et al. 2014). C-fos is instantaneously expressed in response to synaptic input and the time-course expression of their products is well known with peaks in mRNA occurring 30 min after the onset of the stimulation and in protein levels (fos) occurring 1h30-2h after the end of the stimulation (Morgan and Curran 1991; Terleph and Tremere 2006). Because the peak in protein levels is determined by the end of the stimulation (Supplementary Figure S1), the exposure of NA*, but not the NA, pigeons to natural odors during the displacement is unlikely to account for the lower levels in neuronal activation in the NA* pigeons, because the exposures ended simultaneously for both groups.

An alternative possibility is that the time course of the response of the pigeon nervous system to olfactory stimuli may explain the neuronal activation patterns observed in the different olfactory treatment groups. For example, a stimulus that activates ("switches on") the pigeon's navigation circuitry (e.g. the *transition* from home to non-home odors) could be more pronounced when it first occurs. This is consistent with the findings showing a decrease in neuronal activation patterns in pigeons that were exposed to natural odors continuously from the home site to the release site (inclusive) compared to no change in neuronal activation patterns between hemispheres in pigeons who were only exposed to natural odors at the release site (Figure 2B).

Importantly, when pigeons are displaced to distant, unfamiliar sites, as was the case in these experiments, neuronal activation in the relevant components of the pigeon's navigation circuitry would be transient, resulting in protein peaks that occur much earlier than those resulting from the termination of olfactory cues at the release site (Supplementary Figure S1). In contrast, a mechanism that uses olfactory input over the entire displacement (including the final stages in unfamiliar territory) and/or information that is derived solely from olfactory cues at the release site (where both NA and NA* spent an additional 36 min prior to release) could not account for the low levels of neuronal activation observed in NA* birds in comparison to NA birds (e.g., in the left BO and in the right CPi; Figure 2A–C; see earlier discussion). These findings, i.e., transient activation of the pigeon's navigational system, contradict the current view that odors provide geographic position information at the release site (Papi 2001; Wallraff 2005, 2014).

Therefore, the patterns of neuronal activation reported here (Figures 1B, C and 2A–C) are consistent with a stimulus that occurred earlier for NA* birds during the initial phase of the outward journey (i.e. discrimination between home vs. non-home odors that ended 2–3 h prior to pigeons' return to the home loft; Supplementary Figure S1) but would not occur until later for the other groups (i.e. when birds were exposed to odors at the release site), rather than at the end of the displacement 36 min prior to release (Supplementary Figure S1). Conversely, different patterns of neuronal activation in hippocampal areas may result from the presence or absence of olfactory input (Figures 3D and E), and in the triangular hippocampal area from processing of non-navigational aspects of spatial information, the latter including a possible involvement of odors in the retrieval of episodic-like memories (Figure 4C; and see below).

The hippocampal formation has been suggested to play a critical role in learning and retrieving of map-like representations of local landmarks (including olfactory landmarks) in familiar surroundings (i.e., an area within a ~10 km radius of the home loft) when the sun compass is used to determine the relative position of discrete stimuli in space (Bingman et al. 2005; Nardi and Bingman 2007). This might be the reason for differences observed in triangular hippocampal area neurons activated by natural and artificial odors (Figures 1D and 4C); in adult-experienced pigeons, natural odors would be expected to elicit the retrieval of a significant number of memories while artificial odors should elicit retrieval of few if any.

Olfactory input appears to strongly affect neuronal activation in the right piriform cortex (Figures 2B and 5B). Lateralized processes are relatively common in other animals (e.g. Wiltschko et al. 2002; Shipton et al. 2014; Cohen et al. 2015; Marlin et al. 2015; reviewed in Bisazza et al. 1998; Springer and Deutsch 1998; Rogers and Andrew 2002;) and suggest a highly developed neuronal processing system. Importantly, the findings suggest that the piriform cortex is involved in processing both olfactory context (i.e. exposure at the release site vs. displacement + release site; Figure 5B) and olfactory discrimination (i.e., natural vs. artificial odors; Figure 4B), but here it is the olfactory context rather than the olfactory discrimination that contributes to the activation of navigation circuitries (Figures 2B and 5B). Interestingly, transient olfactory processing in the piriform cortex for both olfactory events and context during a spatial discrimination olfactory task was recently reported in rodents (Cohen et al. 2015), suggesting that the transient processing of odors by the piriform cortex during spatial navigation might be relatively common among vertebrates and crucial for navigation.

Altogether, our findings indicate that odors are involved in a transient process mediated by the piriform cortex that activates neuronal circuitries involved in processing navigational information. Importantly, the findings suggest that the response to olfactory stimuli is occurring during the initial step of the exposure to non-home odors (here at the beginning of the displacement or at the release site), and that processing resulting in initial activation of navigational circuitries involves the right piriform cortex.

Supplementary material

Supplementary material can be found at http://www.chemse.oxfordjournals.org/

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Conflict of interests

The authors declare have no conflict of interests.

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