

## Research report

# The dog nose “KNOWS” fear: Asymmetric nostril use during sniffing at canine and human emotional stimuli



Marcello Siniscalchi\*, Serenella d'Ingeo, Angelo Quaranta

Department of Veterinary Medicine, Section of Behavioral Sciences and Animal Bioethics, University of Bari “Aldo Moro”, Italy

## HIGHLIGHTS

- Dogs sniffing at emotional odours showed asymmetric nostril use.
- Sniffing canine and human arousal odours produce higher emotional response.
- Chemosignals cues seem to be relevant for dogs social communication.

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## ABSTRACT

Previous studies have reported striking asymmetries in the nostril use of dogs during sniffing at different emotive stimuli. Here we report, for the first time, that this asymmetry is also manifested during sniffing of both human and canine odours collected during different emotional events. Results showed that during sniffing of conspecific odour collected during a stressful situation (e.g. an “isolation” situation in which a dog was isolated from its owner in an unfamiliar environment) dogs consistently used their right nostril (right hemisphere). On the other hand, dogs consistently used the left nostril to sniff human odours collected during fearful situations (emotion-eliciting movies) and physical stress, suggesting the prevalent activation of the left hemisphere. The opposite bias shown in nostril use during sniffing at canine versus human odours suggests that chemosignals communicate conspecific and heterospecific emotional cues using different sensory pathways.

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## 1. Introduction

Functional asymmetries in dogs have been reported in various sensory modalities [1]. For instance, an advantage of the left visual field (right hemisphere activation) in attending to visual stimuli of high emotional valence has been observed [2]. In the auditory sensory domain, dogs tend to turn their head with the left ear leading (right hemisphere) in response to threatening sounds (e.g. thunderstorm playbacks) and with the right ear leading (left hemisphere) in response to conspecific vocalizations [3]. Behavioural asymmetries, which directly reflect asymmetries of brain function, have also been observed in what is considered to be the most significant canine sensory domain, namely olfaction [4]. Previous research suggests the presence of a lateralized process in the analysis of olfactory stimuli in both vertebrate and invertebrate species [5]. In domestic chicks, for example, stronger head shaking was observed

in response to a noxious odour presented under the right nostril [6]. In mammals, horses show a population bias to using the right nostril first in response to both stallion faeces [7] and arousal odours [8].

Studies on dogs' free sniffing behaviour have reported asymmetric nostril use in processing odours that differ in terms of emotional valence [4]. Specifically, a right nostril bias has been observed during sniffing of clear arousal odours (e.g. adrenaline and veterinary sweat), confirming the main role of the right hemisphere in attending to threatening and alarming stimuli (contrarily to other organ senses, olfactory pathways ascend ipsilaterally in mammals: right nostril → right hemisphere). On the other hand, a shift from the right to the left nostril occurs following repetition of non-aversive stimuli (e.g. food, lemon, vaginal secretion). This particular pattern has been reported in a variety of animal models (e.g. birds: [9]; fish: [10]), supporting the hypothesis of initial right hemisphere involvement in the analysis of novel stimuli followed by prevalent control of behaviour by the left hemisphere when a stimulus becomes familiar (left hemisphere categorization reviewed in [11,5]).

\* Corresponding author.

E-mail address: [marcello.siniscalchi@uniba.it](mailto:marcello.siniscalchi@uniba.it) (M. Siniscalchi).

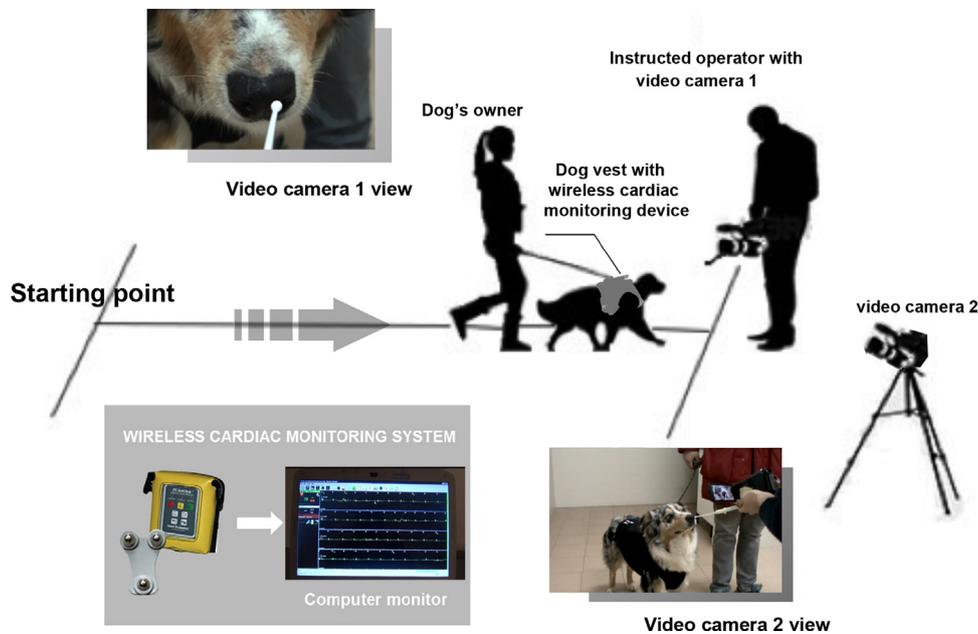


Fig. 1. Experimental set-up. Schematic representation of the testing apparatus.

In dogs, olfaction also plays a pivotal role in social relational systems [12,13]. For example, conspecific odours play a fundamental role in signalling reproductive status in bitches, and both male and female dogs are able to discriminate amongst pheromones contained in organic secretions [14,15].

Regarding interspecific relational systems, although very little is known about the informative role of human odour for dogs, there is evidence that canine species prefer sniffing specific areas of children's bodies for olfactory exploration (e.g. the face, upper limbs) indicating that odours produced at different anatomical parts could provide different specific olfactory cues [16,17].

In the light of this evidence, the aim of the present study was to investigate whether dogs show nostril asymmetries in processing both human and canine odours collected during different emotional events. In addition, dogs' behavioural and physiological reactivity to different odours was evaluated.

## 2. Materials and methods

### 2.1. Subjects

Subjects were 31 domestic dogs of various breeds. The dogs' ages ranged from 1 to 12 years ( $4.8 \pm 2.91$ ; mean  $\pm$  S.D.). All dogs (11 males and 20 females) were pets living in households. Two of the males and thirteen of the females had been neutered. The group was made up of a mixture of different body sizes.

### 2.2. Odour stimuli

Four healthy voluntary non-smoker male subjects between the ages of 25 and 27 years and three domestic male dogs between the ages of 2 and 8 years participated as donors.

Human donors were informed about the aim of the study. Sweat samples in different emotional conditions were collected: "joy" (H-joy), "fear" (H-fear), "physical stress" (H-running) and "neutral" (H-neutral). Subjects were advised to abstain from heavily flavoured foods, spices, asparagus, onions, garlic, and not to use deodorants, antiperspirants and scented products for two days before the sweat

donation session and until after the collection of samples was over. Donors were instructed to use only scent-free products provided by the experimenter for personal hygiene and for washing sheets and clothing. Human donors were also asked to take a shower on the morning of each session and to wear an odourless T-shirt to prevent odour contamination of their regular clothes. The sweat samples were collected at the same time of the day (9 a.m.) over four consecutive days (one session per day) and in each session a total of 24 odour samples per emotional state were collected.

Each donor individually watched a 15-min video in two different sessions aiming to elicit emotions of fear and joy, respectively. Three sterile cotton swabs were placed under each armpit during the viewing and heart rate was simultaneously recorded using an electrocardiograph. At the end of each session, donors indicated on a five-point visual analogue scale how scared or happy they felt (see Table S1 in the Supplementary materials). In addition, heart rate was examined in order to see if the donors' emotional response to the movies was accompanied by increased cardiac activity. The "physical stress" samples were collected after a 15-min run, keeping three cotton swabs under each armpit for 2 min. Finally, "neutral" samples were collected after the morning shower, by keeping three cotton swabs under each armpit for 2 min.

Dogs' emotional odours were collected from perianal, interdigital and salivary secretions soon after the end of different emotional events: a "play" situation (D-play), in which the dog played together with its owner; an "isolation" situation (D-isolation), in which the dog was left alone for 5 min in an unfamiliar environment; a "disturbance" situation (D-disturb) caused by a stranger approaching the car where the dog was resting and finally, the "neutral" (D-neutral) olfactory stimulus was collected while the dog was sleeping. Sterile cotton swabs were gently rubbed for about 10 s against the inside of dogs' cheeks (saliva samples) and the skin surface of perianal and interdigital areas (perianal and interdigital secretions).

Swabs of the same emotion were gathered and stored at  $-20^\circ\text{C}$  [18] and defrosted 30 min before the session. After the presentation, cotton swabs were refrigerated to prevent the quality and the intensity loss of odours; at the end of the session, swabs were re-stored and used for a maximum of ten times.

### 2.3. Experimental setup

The experiment was carried out at the Department of Veterinary Medicine of Bari University, Italy, in an isolated room (4.5 × 1.5 m) to avoid any noise interference. The cotton swab impregnated with different odours was installed under a digital video camera (video camera 1) being held by an experimenter placed in the centre of the testing area, aligned with the entrance of the room. Another video camera (video camera 2) was also located on a tripod at a distance of about 3 m in order to avoid any possible interference with dogs' behaviour during the experiment (see Fig. 1). Once in the testing area, the owner and the dog followed a pathway mapped out on the floor (see below).

### 2.4. Procedures

Each dog was tested three times, with a 2-day inter-session interval. Each session consisted of presenting all eight different olfactory stimuli in random order (first from one species and then from the other, alternately) with a 1-min interval between each stimulus and a 10-min interval between the two species. Each presentation lasted for a maximum of 2 min.

Before the beginning of the session, dogs were allowed to explore the room and become familiar with the experimenter. Owners were asked to follow the pathway mapped out on the floor in the testing area with their dog on the leash: they were asked to stand 3 s on the start line, go straight on and stop on the second horizontal line where the experimenter with the video camera was positioned (see Fig. 1). The owner stood either to the right or to the left of the dog to prevent his position from affecting the dog's performance [19]. Owners were asked not to influence their dogs' behaviour (e.g. either to indicate the swab or to force sniffing behaviour).

During odour presentation, the experimenter stood in the mid-line of the pathway and used both hands together (i.e. without a left or right bias) to hold the video camera at the dog's head height. The experimenter had received the same instructions as the human donors with regard to personal hygiene and eating habits during the experimental period. In addition, the experimenter's hands and the floor of the testing room were washed with baking soda soon after the end of each session.

### 2.5. Cardiac activity

The cardiac activity of the dogs was recorded using the PC-Vetgard + tm Multiparameter wireless system for telemetric measurements (see Fig. 1). The apparatus weighed about 150 g and no signs of distress were observed during device application or use. Four vests were used (according to different dog size, DogLeggs®) to keep the three integrated electrodes in contact with the dog's chest and to fix the wireless ECG data-transmitting unit on the dog's back.

Digital informations (e.g. real time ECG waveforms and trending) were displayed through a wireless link on a computer monitor.

Dogs grew accustomed to the vest during weekly visits to the Department before the experimental tests. During the visits, the vest was gently fixed to the dog's chest by the experimenter and the dog-owner dyad was conducted either to a large open area adjacent to the department for a walk or to a room where some pet food toys were placed.

Each visit lasted about 40 min, twice a week over a minimum period of 1 week for dogs that were already accustomed to wear a vest during their daily life and a maximum of 4 weeks (dogs not accustomed to wearing a vest); subjects were considered adapted to vest when no behavioural signs of stress (see Table S3, Supplementary materials) were manifested.

### 2.6. Questionnaire

The dogs' owners were asked to complete a questionnaire in order to gather information about their dogs' temperament.

The questionnaire was derived from the Hsu and Serpell [20] owner-rated temperament assessment validation study (see Table S2). Briefly, the questionnaire contains 11 items asking owners to rate their dog's typical behaviour in a given situation using a four-point scale, where a score of zero represents no reaction to a particular stimulus and a score of four represents a strong reaction to it (see Table S2).

### 2.7. Data analyses

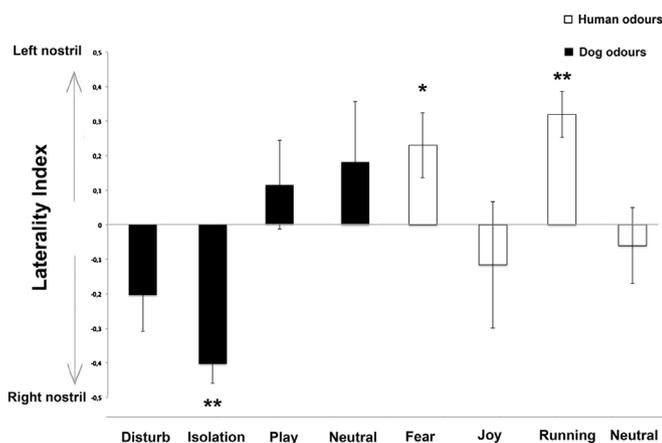
Lateral asymmetries in nostril use were computed using the index:  $LI = (L - R) / (L + R)$ , where L and R indicate, respectively, the total time (in s) spent sniffing with the left and the right nostril during the three stimulus presentations. Hence a score of 1.0 indicated exclusive use of the left nostril and a score of -1.0 indicated exclusive use of the right nostril. An LI score of 0 indicated equal left and right nostril use.

Significant departures from chance level (0) were estimated by two-tailed one-sample *t* tests.

HR basal average (baseline) was calculated from the heart rate (HR) curve obtained during the pre-experimental phase (ECG-RR intervals during the recording period). The highest (HV) and lowest values (LV) of the HR response to different olfactory stimuli were scored. Furthermore, the area delimited by the HR curve and the baseline was computed for each dog and each odour separately using Microsoft Excel®. The Area Under Curve (above baseline and under curve, AUC) was then graphically separated from the Area Above Curve (under baseline and above curve, AAC). Each area value was then calculated and expressed as number of pixels (Adobe Photoshop Elite®). HR changes for each dog during presentations of different odours were then analysed by comparing different area values with the corresponding baseline.

Subjects' behaviours were video recorded continuously throughout the experiment and then analysed frame by frame by two trained observers who were blind to the odour stimulus presented to the dogs.

Behavioural score was then calculated, allocating a score of 1 for each behaviour shown; a total of 46 behaviours were recorded



**Fig. 2.** Nostril use during sniffing at different odours. Laterality index in the total time spent sniffing with the right and left nostril during inspection of different odours (group means averaged across all three trials with SEM are shown). Asterisks indicate significant biases (\* $P < 0.05$ , \*\* $P < 0.01$ , two-tailed one-sample *t*-tests).

which were then split into three categories (neutral/relaxed, stress/anxiety, and alerting/targeting; see Table S3, Supplementary materials [20,21].

The experiments were conducted according to the protocols approved by the Italian Minister for Scientific Research in accordance with EC regulations and were approved by the Department of Veterinary Medicine (University of Bari) Ethics Committee EC (Approval Number: 4/15); in addition, before the experiment began, the procedure was explained to owners and written informed consent was obtained.

### 3. Results

#### 3.1. Heart rate and VAS score of donors

Donor heart rates when watching emotion-eliciting films was above basal levels: fear ( $108.60 \pm 6.92$ ) ( $m \pm s.d.$ ) vs. basal levels ( $70.09 \pm 7.71$ ) ( $m \pm s.d.$ ) ( $t_3 = -5.624$ ,  $P < 0.05$ ); joy ( $86.75 \pm 4.18$ ) vs. basal levels ( $73.80 \pm 10.62$ ) ( $t_3 = -3.600$ ,  $P < 0.05$ ); VAS scores were  $3.75 \pm 0.95$  (fear) and  $4.00 \pm 0.00$  (joy) ( $m \pm s.d.$ ).

#### 3.2. Nostril use

Results for nostril use are shown in Fig. 2. The analysis of variance revealed a significant main effect of the type of odour on the laterality index in the total time spent sniffing with the right and left nostril ( $F_{7,49} = 2.395$ ,  $P = 0.034$ , GLM analysis for repeated measures). Dogs consistently used their right nostril to sniff the dog-isolation (D-isolation) odour (two-tailed one-sample  $t$  test:  $t_{27} = -7153$ ,  $P < 0.001$ ) and the left nostril to sniff the human-fear (H-fear) ( $t_{29} = 2.446$ ,  $P < 0.05$ ) and human-running (H-running) ( $t_{28} = 4.798$ ,  $P < 0.001$ ) stimuli. No statistically significant bias in nostril use was apparent for the other odour stimuli (two-tailed one-sample  $t$  test:  $P > 0.05$ ).

#### 3.3. Cardiac activity

Statistical results for the cardiac activity are summarized in Table 4S. A statistically significant main effect of odours at higher heart values was observed: overall, higher emotional odour stimuli induced a higher maximum heart rate than neutral odours: ( $F_{7,3} = 6.577$ ,  $P < 0.001$ ; post hoc analysis Fisher's protected least significant difference (LSD): "D-neutral" vs. "D-isolation", "H-fear" and "H-running" ( $P < 0.01$ ); "D-neutral" vs. "D-disturb" ( $P < 0.05$ ); "H-neutral" vs. "D-disturb", "D-isolation", "H-fear" ( $P < 0.01$ ) and "H-neutral" vs. "H-running" ( $P < 0.001$ ). Statistically significant differences were also found between "D-play" vs. "D-isolation" and "D-fear" ( $P < 0.05$ ). No statistically significant differences were observed between odour stimuli regarding LV ( $F_{7,56} = 1.397$ ,  $P = 0.225$ ; GLM analysis).

A significant main effect of odour was observed in the overall time at which heart-rate values were higher than the basal average (see Fig. 3B AUC) (i.e. the area above baseline and under curve;  $F_{7,63} = 6.218$ ,  $P < 0.001$ ); similarly to the HR results, post hoc analysis (Fisher's protected LSD) revealed that the AUC was higher for the emotional stimuli than for the neutral odours: "D-neutral" vs. "D-disturb" and "H-fear" ( $P < 0.05$ ), and "D-neutral" vs. "D-isolation" and "H-running" ( $P < 0.01$ ); "H-neutral" vs. "D-disturb", "D-isolation", "H-running" ( $P < 0.01$ ) and "H-neutral" vs. "H-fear" ( $P < 0.05$ ). In addition, AUC values during "D-isolation" were higher than for "D-play" and "H-joy" ( $P < 0.05$ ). Finally, regarding human odours, the AUC values for "H-running" were higher than for "D-play" ( $P < 0.01$ ) and "H-fear" ( $P < 0.05$ ). No differences were observed in AAC values between odour stimuli ( $F_{7,56} = 0.766$ ,  $P = 0.618$ ).

#### 3.4. Behavioural score

As to behavioural score, analysis of the stressed behavioural category revealed that there was a significant difference between odour stimuli ( $F_{7,56} = 6.597$ ,  $P < 0.001$ ) (see Table 5S, Supplementary materials). Post hoc analyses revealed that dogs were more stressed when they sniffed "D-isolation" and "H-fear" odours than for other stimuli ( $P < 0.01$ , comparisons between "D-isolation" and "D-disturb", "D-play", "D-neutral", "H-joy" and "H-running"; "D-isolation" vs. "H-neutral"  $P < 0.001$ ; "H-fear" versus "D-disturb", "D-neutral", "D-play"  $P < 0.05$ ; "H-fear" versus "H-joy", "H-running" and "H-neutral"  $P < 0.01$ ). In addition, dogs were more stressed during sniffing at "D-disturb" than at "H-neutral" ( $P < 0.05$ ). A significant main effect of odour stimuli was also observed in the targeting category ( $F_{7,56} = 6.694$ ,  $P < 0.001$ ). The results showed that dogs displayed more targeting behaviours during presentation of "D-disturb", "D-isolation" and "H-running" than during "D-neutral", "D-play", "H-joy" and "H-neutral" ("D-disturb" and "D-isolation" vs. "D-neutral", "D-play" and "H-joy"  $P < 0.05$ ; "D-disturb" vs. "H-neutral"  $P < 0.01$ ; "D-isolation" vs. "H-neutral"  $P < 0.001$ ; "H-running" vs. "D-play" and "H-fear"  $P < 0.05$ ; "H-running" vs. "D-neutral", "H-joy" and "H-neutral" ( $P < 0.01$ ); "H-fear" vs. "D-neutral", "H-joy" and "H-neutral"  $P < 0.05$ ). No significant differences were observed between odour stimuli in terms of eliciting a relaxed response by dogs ( $F_{7,56} = 0.676$ ,  $P = 0.692$ ) (Fig. 4).

#### 3.5. Correlations between LI (nostril use), cardiac activity, questionnaire and behavioural scores

A negative and statistically significant correlation was found between the LI "D-disturb" and dog fear/aggressiveness to other dogs (item 5 of the questionnaire) ( $r_{25} = -0.498$ ,  $P = 0.011$ ); i.e. the stronger the aggressiveness/fear to other dogs, the more likely the right nostril was to sniff the "D-disturb" odour (see Fig. 5A). Furthermore, the scores for predatory behaviour (section 9 "chasing", see Table S2) of the temperament questionnaire were correlated with LI "H-running", and a significant positive relationship was found, ( $r_{29} = 0.469$ ,  $P = 0.010$ ), indicating that dogs with higher scores for predatory behaviour used the left nostril more when sniffing at the "human-running" odour (see Fig. 5B). No other statistically significant correlations were found ( $P > 0.05$  in all comparisons between  $LI_{(\text{nostril use})}$ , cardiac activity (HV, LV, AUC and AAC), behavioural and questionnaire scores).

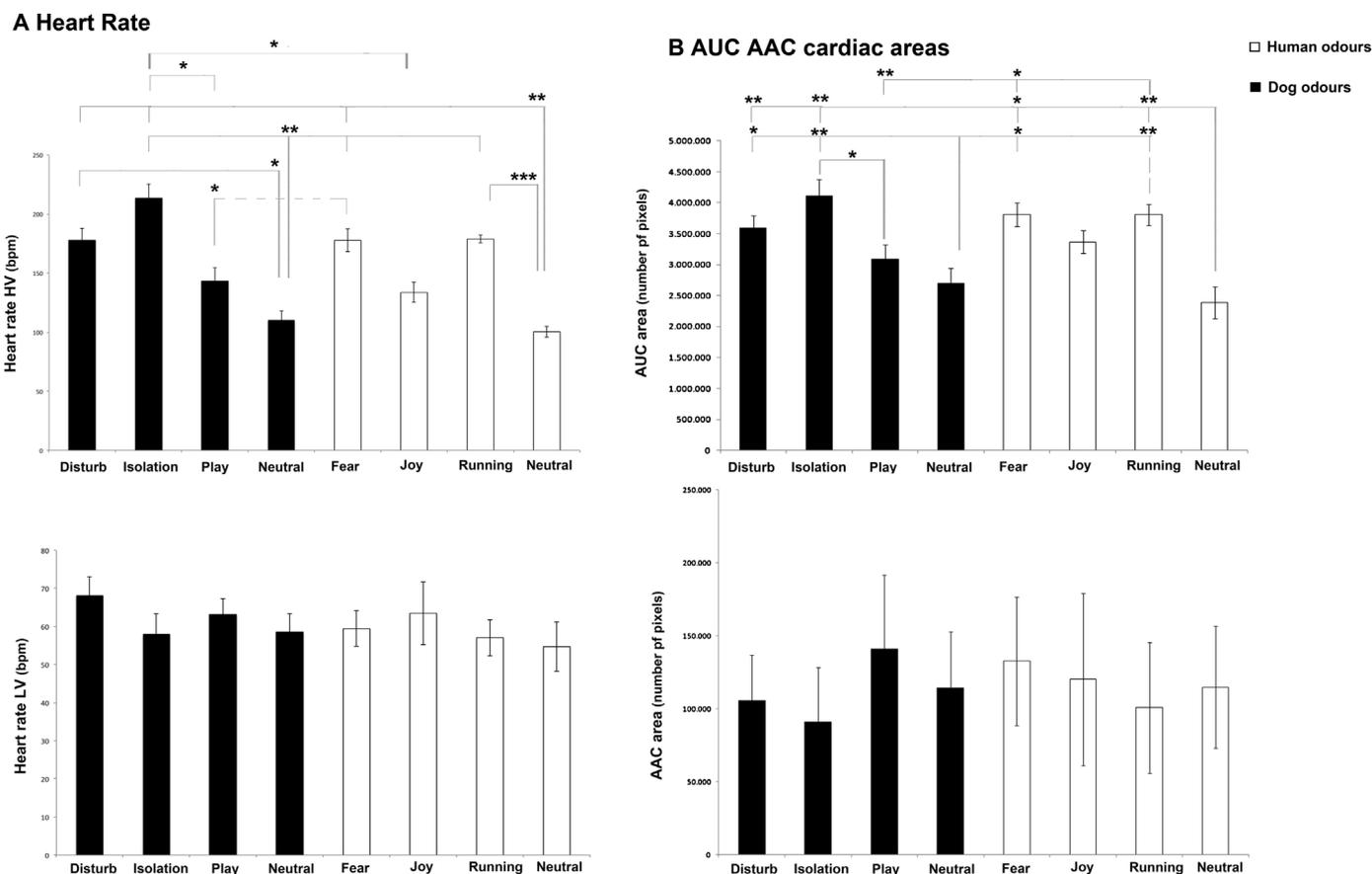
#### 3.6. Sex ratio

Although a significant main effect of sex was revealed on the AUC (male:  $F_{1,8} = 8.949$ ,  $P < 0.05$ ), no statistically significant interaction was revealed between AUC and sex during odour presentations (sex x AUC:  $F_{7,56} = 1.051$ ,  $P = 0.407$ ).

No other statistically significant effects of sex were apparent: LI of nostril use (sex:  $F_{1,7} = 0.358$ ,  $P = 0.569$ ; sex x LI of nostril use:  $F_{7,49} = 0.694$ ,  $P = 0.677$ ); HV (sex:  $F_{1,8} = 0.257$ ,  $P = 0.626$ ; sex x HV:  $F_{7,56} = 0.105$ ,  $P = 0.998$ ); LV ( $F_{1,8} = 0.639$ ,  $P = 0.447$ ) (sex:  $F_{1,34} = 0.006$ ,  $P = 0.939$ ; sex x LV:  $F_{7,56} = 0.964$ ,  $P = 0.466$ ); AAC (sex:  $F_{1,8} = 3.044$ ,  $P = 0.119$ ; sex x AAC:  $F_{7,56} = 1.844$ ,  $P = 0.097$ ); behavioural score (sex:  $F_{1,7} = 0.067$ ,  $P = 0.803$ ; sex x neutral/relaxed category:  $F_{7,49} = 1364$ ,  $P = 0.242$ ); sex x stress/anxiety:  $F_{7,49} = 0.522$ ,  $P = 0.813$ ); sex x alerting/targeting:  $F_{7,49} = 0.711$ ,  $P = 0.663$ ).

#### 3.7. Age

A significant main effect of age on the AAC was revealed ( $F_{3,6} = 136.708$ ,  $P < 0.001$ ); post-hoc analysis revealed that this effect was due to AAC mean values of 5–9 year-old dogs being significantly higher than for younger dogs (2–3 years old) (i.e. as expected,



**Fig. 3.** Cardiac activity. A: Highest value (HV) and lowest value (LV) of the dogs' heart rate (HR) in response to presentation of different odours (means with S.E.M. are shown). B: The Areas Under Curve (AUC; A) and Above Curve (AAC; B) in response to presentation of different odours (means with S.E.M. are shown).

during testing adult dogs spent longer with a lower heart rate than the basal average).

Interactions between AAC and age during odour presentations (age  $\times$  AUC:  $F_{21,42} = 15.533$ ,  $P < 0.001$ ) revealed that this difference was more evident for "D-disturb" and "D-isolation" stimuli ( $P < 0.001$ ) and for "D-play", "H-fear", "H-joy" and "H-running" odours ( $P < 0.05$ ).

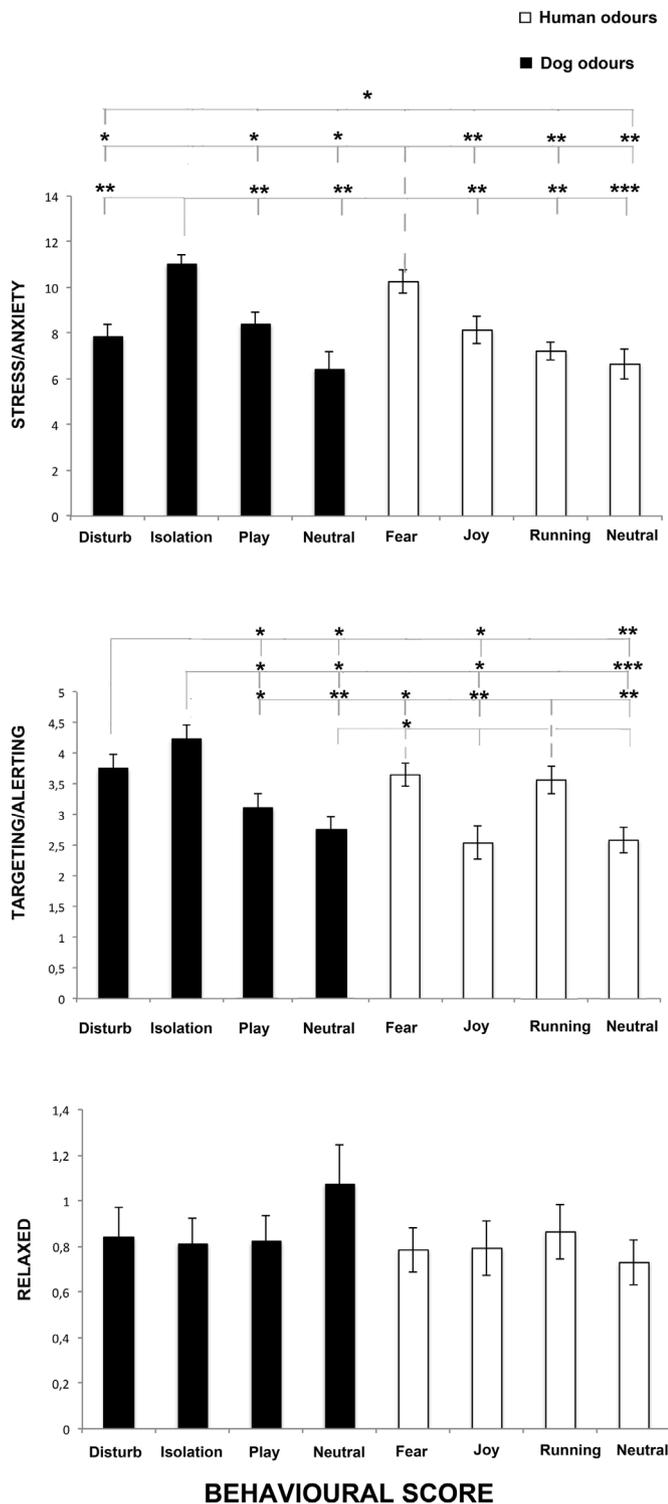
No other statistically significant effects of age were apparent: LI of nostril use (age:  $F_{3,5} = 1.420$ ,  $P = 0.341$ ; age  $\times$  LI of nostril use:  $F_{21,35} = 1.090$ ,  $P = 0.400$ ); HV (age:  $F_{3,6} = 0.232$ ,  $P = 0.871$ ; age  $\times$  HV:  $F_{21,42} = 0.757$ ,  $P = 0.750$ ); LV (age:  $F_{3,6} = 0.259$ ,  $P = 0.852$ ; age  $\times$  LV:  $F_{21,42} = 0.760$ ,  $P = 0.748$ ); AUC (age:  $F_{3,6} = 3.650$ ,  $P = 0.083$ ; age  $\times$  AUC:  $F_{21,42} = 0.815$ ,  $P = 0.687$ ); behavioural score (neutral/relaxed-age:  $F_{3,5} = 0.348$ ,  $P = 0.793$ ; age  $\times$  neutral/relaxed category:  $F_{21,35} = 0.650$ ,  $P = 0.850$ ); stress/anxiety-age:  $F_{3,5} = 1.294$ ,  $P = 0.373$ ; age  $\times$  stress/anxiety:  $F_{21,35} = 0.794$ ,  $P = 0.708$ ); alerting/targeting-age:  $F_{3,5} = 0.100$ ,  $P = 0.957$ ; age  $\times$  alerting/targeting:  $F_{21,35} = 0.633$ ,  $P = 0.865$ ).

#### 4. Discussion

Previous studies have reported striking asymmetries in dogs' nostril use during sniffing at different emotive stimuli [4]. Here we report for the first time that this asymmetry is also manifested during sniffing of both human and conspecific odours collected during different emotional events, suggesting that dogs could detect the emotional states of a member of its social group through olfaction. The results specifically showed that during sniffing of "D-isolation" odour, dogs consistently used their right nostril. Given that the mammalian olfactory system ascends mainly ipsilaterally

to the brain [22], the pattern of right nostril use during sniffing of "D-isolation" odour suggests involvement of the right hemisphere. In dogs, right nostril use (right hemisphere activity) has been observed during sniffing at clear arousal stimuli such as adrenaline and the veterinarian's sweat [4]. More in general, in the animal kingdom, right hemisphere use has been associated with the expression and control of intense emotions, such as aggression, escape behaviour and fear (see for extensive review: [5,23]).

One possible explanation for the right nostril use observed during the "D-isolation" stimulus is that this odour was collected during a stressful situation (in which a dog was separated from its owner in an unfamiliar environment) and, as a consequence, the donor dog's odour chemosignals could have enhanced the arousal state in the receiver subject, eliciting its escape behavioural response (right hemisphere activation). In other words, it could be useful for a dog to receive information through its sense of smell about a place where another dog experienced a stressful/possibly dangerous situation. In addition, the higher cardiac activity shown by dogs when presenting "D-isolation" odour is consistent with the idea that the hypothalamic-pituitary-adrenal (HPA) axis, which enhances cardiac activity in response to an emotional stressor, is mainly under the control of the right hemisphere [8]. Behavioural measures also supported the hypothesis that higher heart rate during presentation of the "D-isolation" stimulus reflected a state of increased arousal since subjects showed significantly higher stress and alerting behaviours compared to other stimuli. Other evidence comes from studies on marmosets, showing that visual scanning (indicator of vigilance) during exposure to a predator was correlated to serotonergic neurotransmission in the right hypothalamus, which activates an HPA-axis stress response [24].



**Fig. 4.** Behavioural score. Data for the score of the three behavioural categories determined from the behavioural score for each dog during inspection of different odours (means with S.E.M. are shown).

In addition, different studies exploring the functional implications of emotional chemosignals in humans and rats reported that smelling the sweat of stressed individuals enhances attention and vigilance [25,26], improves anger and fear discrimination [27] and augments the startle reflex [28,29].

More recently in humans, consistently with theories of possible “emotional contagion” between dogs, de Groot et al. [30] tested the ability of emotional chemosignals to recruit joint processes

between sender and receiver and found that inhaling chemosignals emitted during emotional states induced the same state (fear, disgust) in the receiver.

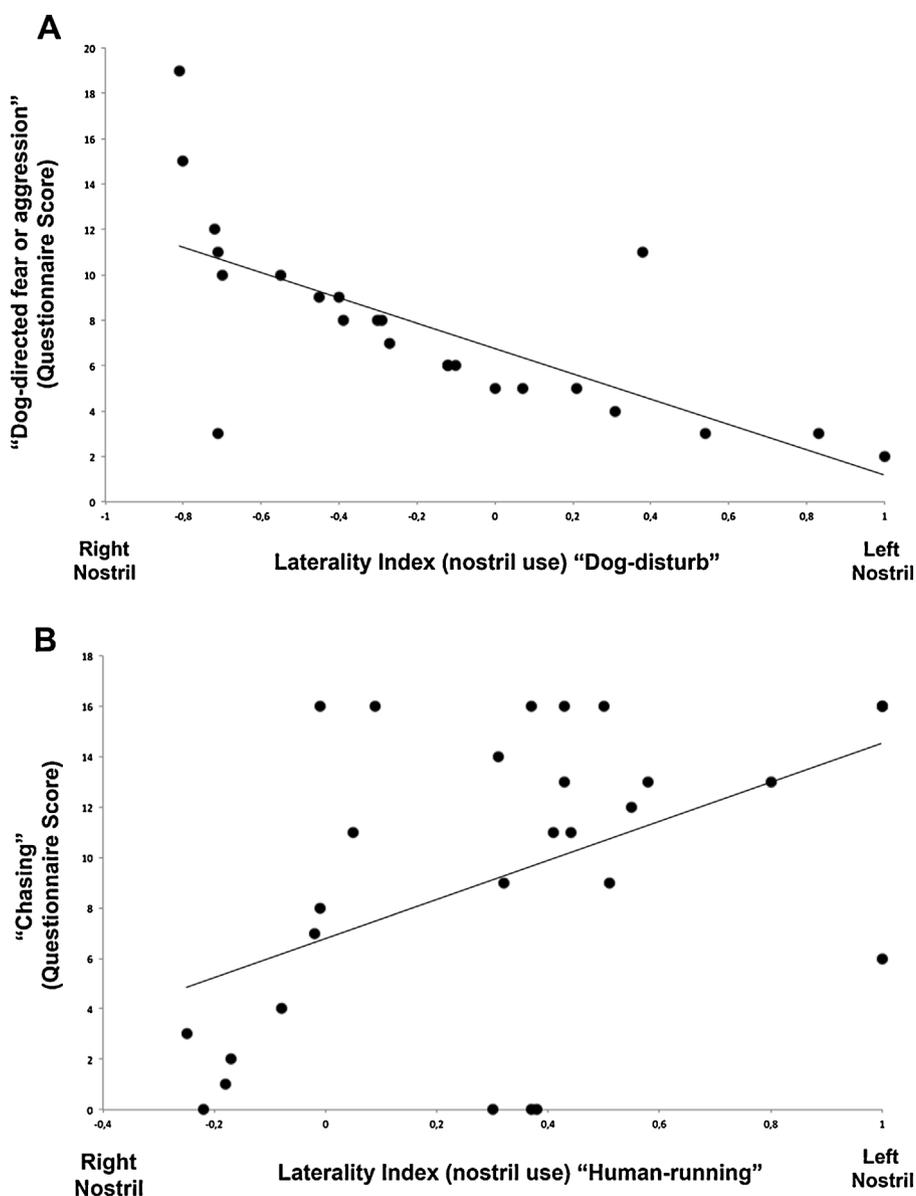
Although we failed to find any statistically significant bias in nostril use during sniffing at “D-disturb”, it is interesting to note that the stronger the aggressiveness/fear towards other dogs, the more likely the right nostril was to sniff at this odour, suggesting that temperament could influence behavioural response to emotional odours.

On the other hand, dogs consistently used the left nostril to sniff “H-fear” and “H-running” stimuli, suggesting prevalent activation of the left hemisphere. One possible logical explanation for the involvement of the left hemisphere in the analysis of these odours is that a greater discrimination accuracy between stimuli that display varying levels of arousal occurred in the left amygdala [31,32]. Furthermore, neuroimaging studies in humans [33,34] have shown that while the right amygdala rapidly and non-selectively detects stimuli that represent a potential threat (i.e. general detector of arousal), the left amygdala provides a fine-tuned and detailed mechanism which helps provide a more accurate determination of whether the potential threat is real. In other words, it is not said that what represents a potential threat for a human (e.g. the owner) represents a threat for the dog. Neural structures on the left side of the brain are also involved in the control of predatory behaviour in different animal models [23], including dogs [35]: as a consequence, human chemosensory arousal signals (“H-fear” and “H-running” odours) could elicit approaching behavioural tendencies to the stimulus (dogs’ prey drive, i.e. the instinctive inclination of a carnivore to pursue and capture prey) through the selective use of the left nostril/hemisphere. This hypothesis is supported by the positive correlation between targeting behaviour and item 9 in the questionnaire (predatory behaviour) during sniffing at “H-running”, indicating that the stronger the dog’s targeting behaviour, the more likely it would be to use the left nostril to sniff the “H-running” stimulus. In addition, the prey-catching behaviour elicited by left nostril use during sniffing at human chemosensory arousal signals could be one explanation for canine aggression towards people who are afraid of dogs. In a more evolutionary perspective, during prey-catching behaviour, it could be useful for a dog to elicit an approaching behavioural response towards heterospecific arousal chemosignals produced during prey escape behaviour: the prey that is escaping produces arousal chemosignals in the environment that could inform conspecifics about the presence of the predator (eliciting a withdrawal behavioural response in conspecific subjects) and at the same time the same odour could reinforce the tracking behaviour of the predator. Furthermore, behavioural/neurochemical correlations in marmosets suggest that general motor activity (e.g. locomotory, exploratory behaviour) is related to dopamine levels in the left hemisphere [24]. Taken together, these hypotheses could support the long evolutionary role of the left side of the brain in sustained pursuit of prey using odours [5].

## 5. Conclusion

Overall, our data showed that dogs displayed higher behavioural and physiological reactivity to canine (conspecific) and humans (heterospecific) odours collected during different emotional conditions.

In addition, a clear bias in nostril use was observed during sniffing at clear arousal stimuli, but using opposite sides for canine and human odours (showing right nostril-use bias for inspecting conspecific anxiety odours and a prevalent use of the left nostril during sniffing at heterospecific arousal odours), suggesting that



**Fig. 5.** Correlations between  $LI_{(nostril\ use)}$  and questionnaire. Data for the significant correlation discussed in the text between A)  $LI$  "D-disturb" and "dog fear/aggressiveness to other dogs" (item 5 of the questionnaire); B)  $LI$  "H-running" and "chasing" (item 9 of the questionnaire); Data presented are means calculated for each dog over the three trials.

chemosignals communicate conspecific and heterospecific emotions using different sensory pathways.

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### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.bbr.2016.02.011>.

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