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Article

# Olfactory Laterality Is Valence-Dependent in Mice

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**Abstract:** (1) Background: Although olfaction is the predominant sensory modality in rodents, studies focusing on lateralisation of olfactory processing remain scarce, and they are limited to the exploration of brain asymmetries. This study aimed to test whether outbred and inbred mice (NMRI and C57BL/6J mice strains) show nostril-use preference in processing olfactory *stimuli* differing in terms of emotional valence under unrestrained conditions. (2) Methods: Five odour *stimuli* were used in the study: vanilla, female urine, garlic, rat, distilled water. We measured the number of times mice used their left or right nostril for each testing session. (3) Results: We here showed that mice preferentially used their right nostril when sniffing attractive *stimuli* (female urine, vanilla), and their left nostril when sniffing aversive *stimuli* (rat odour). Results were consistent for both strains. (4) Conclusions: Surprisingly, the results obtained seem opposite to the valence theory assessing that the left and the right hemispheres are dominant in processing *stimuli* with a positive and a negative valence, respectively. It remains to be determined whether this valence-dependent pattern is specific or not to olfaction in mice. These new findings will be important to better understand how both hemispheres contribute to odour processing in rodents.

**Keywords:** olfactory laterality; *Mus musculus*; valence theory; emotional processing

## 1. Introduction

Although olfaction is the predominant sensory modality in rodents, influencing a large array of behaviours (e.g., mating, intra-specific communication, predation, predator detection) [1], research into olfactory laterality remains scarce. The basic circuit of the olfactory system begins with the sensory olfactory neurons located in the nose epithelium. The axons of these neurons project ipsilaterally to the olfactory bulb (OB) [2]. Then, these neurons send their axons ipsilaterally to the olfactory cortical areas: the anterior olfactory nucleus, the olfactory tubercle (OT), the piriform cortex (PC), the amygdala, and the lateral entorhinal cortex [3]. Bilateral convergence occurs at most central olfactory structures via the anterior commissure [4] and via commissural fibres originating in layer II of the PC [5]. In mice, a zone of the main OB is dedicated to aversive odours processing (e.g., predators, spoiled food [6]). Indeed, mutant mice devoid of glomerular structures in this zone are still able to detect and learn these odours but not to behave adaptively. The firing rate of OT neurons in mice during an odour discrimination task is sensitive to the associative outcome of conditioned odours [7]. In anaesthetised rats, an electrophysiological study suggests that the recruitment of OT neurons might be directly dependent on the biological significance of the odour [8]. Bilateral comparisons can be used by rats to accurately report the direction of odour gradients [9]. Anterior PC responds to odours delivered to either the ipsilateral or contralateral naris [10]; and following unilaterally training, memory can be accessible via either nostril [11,12].

The limited air exchange between the two nasal pathways associated with the strongly ipsilateral primary pathway may allow asymmetry in olfactory processing (Table 1). In rats, Litaudon and

colleagues [13] have recently shown a positive correlation between behavioural responsiveness to an odour and the activation of olfactory cortical areas (i.e., anterior PC and lateral amygdala) located in the left hemisphere. The odours used in this study were chosen to be neutral: i.e., inducing neither spontaneous aversion nor preference at the group level. Dantzer and colleagues [14] have shown that left-bulbectomised rats do not behave adaptively in response to the odour of a stressed conspecific, while being still able to discriminate between stressed and unstressed conspecifics (i.e., increase in plasma corticosterone in the first case, not in the second). These authors have suggested that the left hemisphere takes charge of behaviour in response to emotionally negative social odours. When considering olfactory learning, a study in rats has shown a highly significant lateralisation of translocation of enzyme protein kinase C from the cytosol to the membrane in the left PC [15]. More recently, Cohen and colleagues [16] showed a transient interhemispheric asymmetry in the anterior PC oscillatory activity at the initial stages of an odour discrimination task. This asymmetry was mainly the result of an enhanced event-related oscillatory activity in the left than in the right anterior PC. Interhemispheric coherence was recovered when rats started to succeed in the task. More recently, Cohen and Wilson [17] showed that this left bias in the anterior PC was associated with a right bias in the orbitofrontal cortex. The authors suggested that this functional lateralisation may enhance circuit specialisation for increased efficiency in tasks requiring behavioural flexibility.

**Table 1.** Review of the studies on lateralisation of olfactory processing in rodents. OB: olfactory bulb; PC: piriform cortex.

Species/Strain/Sex	Brain Structure	Side	Measure	Reference
Rats/Long Evans males	anterior PC	LEFT	Enhanced beta frequency band oscillations during the first stages of odour discrimination learning Performance-and context-dependent asymmetry	[16]
Rats/Long Evans males	anterior PC orbitofrontal cortex	LEFT RIGHT	Enhanced odour-evoked activity during initial stages of odour discrimination learning	[17]
Rats/Sprague Dawley females	PC	LEFT	Olfactory-learning specific lateralisation of translocation of enzyme protein kinase C from cytosol to membrane	[15]
Rats/Wistar males	OB	LEFT	Absence of adaptive response to the odour of a stressed conspecific when lesioned	[14]
Rats/Wistar males	anterior PC lateral amygdala	LEFT	Activation correlated with behavioural responsiveness	[13]

Most studies have focused on brain correlates of olfactory processing in rodents, but none has focused on nostril-use preference when exposed to different olfactory *stimuli* under unrestrained conditions. These behavioural asymmetries have mainly been studied in horses and dogs. In both species, right nostril is preferentially used when first exposed to novel olfactory stimuli whatever their emotional valence (horses: stallion faeces in a plastic bag [18]; dogs: vaginal secretion, lemon, veterinary sweat odour and adrenaline [19]). While the results are non-significant (possibly because of a visual inspection conducted first), de Boyer des Roches and colleagues [20] showed a slight tendency (67% of individuals) to use the right nostril rather than the left one to sniff a novel object for the first time. Taken as a whole, these studies suggest that novelty associated with arousal is processed by the right hemisphere. This is also consistent with the decrease in the right bias when mares were exposed 24 h later to the same stallion faeces [18]. In horses, a right nostril bias was observed after repeated exposure to adrenaline and urine of oestrous mares [21]. The higher the increase of cardiac activity, the higher the

preference to use the right nostril at last. The authors suggested that the right hemisphere is involved in the analysis of intense emotion and sexual behaviour. In dogs, a shift towards the use of the left nostril was observed with repeated exposure to food odour, while the right nostril was still preferentially used for aversive *stimuli* (veterinary sweat odorants and adrenaline [19]). More recently, Siniscalchi and colleagues [22] also showed a right nostril use bias when exposed to the conspecific odour of a dog placed in a stressful situation (i.e., left alone for 5 min in an unfamiliar environment). The right nostril use preference for aversive *stimuli* (adrenaline in horses: [21]; veterinary sweat odorants, adrenaline, and odour of an isolated congener in dogs [19,22]) appears consistent with the hypothesis of the involvement of the right hemisphere in processing intense emotional *stimuli* [23]. Concerning the shift towards the use of the left nostril for food, the results are consistent with a left hemisphere involvement when performing routine behaviours in non-stressful conditions [23]. However, it should be noticed that Siniscalchi and colleagues [21] did not observe such a left bias in horses exposed to food odour.

Considering the lack of data concerning olfactory laterality in rodents in literature, the aim of this study was to test: (1) whether mice show nostril asymmetries in processing olfactory *stimuli* differing in terms of emotional valence under unrestrained conditions; (2) whether the strain of mice tested (NMRI strain and C57BL/6) affects either the direction or the strength of asymmetries in odour processing. Two different mice strains were chosen for this study: the NMRI (Naval Medical Research Institute) outbred strain and the C57BL/6J inbred strain. The NMRI strain is frequently used in many fields of general biology as well as in pharmacological studies, and C57BL/6J is the most widely used inbred strain.

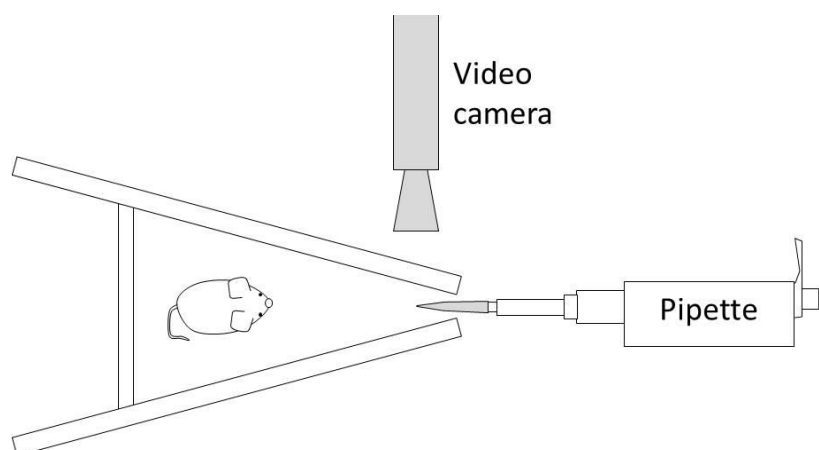
## 2. Materials and Methods

### 2.1. Animals

Experiments were performed on two-month-old male Naval Medical Research Institute mice (NMRI;  $n = 20$ ) and on two-month-old male C57BL/6J mice ( $n = 20$ ), purchased from Janvier Laboratories (Le Genest-Saint-Isle, France). Mice were housed per five in standard polycarbonate cages ( $42 \times 29 \times 15$  cm). They were maintained on a reversed 12 h L-D cycle (lights on at 7:00 pm) with food and water *ad libitum*, constant temperature ( $22 \pm 2$  °C) and humidity ( $55 \pm 10\%$ ). Behavioural tests were undertaken between 09:00 am and 04:00 pm. Animals were acclimatised to the experimental room 30 min before testing. All experiments were carried out in accordance with the Directive of the European Council (2010/63/EU), the French regulation regarding the protection of animals used for scientific purposes, and the regional ethics committee (Comité d’Ethique NOmandie en Matière d’Experimentation Animale, CENOMEXA; agreement number 54).

### 2.2. Apparatus and Odour Stimuli

The corner-shaped apparatus consisted of two vertical boards ( $30 \text{ cm} \times 20 \text{ cm} \times 1 \text{ cm}$ ) attached on one side (angle of  $30^\circ$ ), and a third board used to close the apparatus on the other side and reduce the length of the apparatus to 20 cm (Figure 1). A small opening (width 0.3 cm) between the boards allowed the experimenter to insert the tip of a  $10 \mu\text{L}$ -pipette containing an odour *stimulus* (length of the tip inserted in the apparatus was 1.7 cm long). According to preliminary experiments, the optimum height of the tip of the pipette, which allows a clear assessment of the nostril used during sniffing, was 10.5 cm and 8.5 cm for NMRI and C57BL/6J mice, respectively. A video camera was placed above the corner of the apparatus.

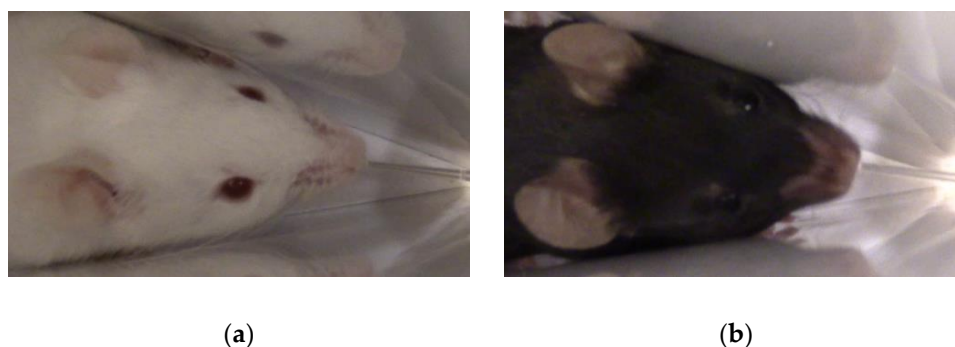


**Figure 1.** Apparatus used to test olfactory lateralisation in mice. The tip of the pipette filled with 10  $\mu$ L of one of the five odour *stimuli* is inserted through the slit before placing the mouse in the apparatus.

Five odour *stimuli* were used in the study: (1) vanilla; (2) odour of female NMRI mice or female C56BL/6J mice (according to the strain of the tested mouse); (3) garlic; (4) rat odour; (5) distilled water. The vanilla odour was prepared by mixing five drops of concentrated vanilla flavouring (Déco'Relief®, France) in 20 mL of distilled water. Garlic odour was prepared by mixing 2.4 g of mashed garlic in 60 mL of distilled water for 10 min. Female mice odour was prepared by mixing 35 g of female-soiled bedding with 300 mL of distilled water for 30 min. Rat odour was prepared by mixing 45 g of rat-soiled bedding with 300 mL of distilled water for 30 min. All odour *stimuli* (pure distilled water included) were aliquoted in Eppendorf tubes (0.5 mL), and stored at  $-20$  °C until testing.

### 2.3. Procedure

At the beginning of a test session, the tip of the pipette was filled with 10  $\mu$ L of one of the five odour *stimuli* and inserted in the apparatus. The tested mouse was then placed at the opposite side of the apparatus, facing the tip of the pipette. The test session lasted 10 min. The behaviour of the mouse was recorded continuously during olfactory inspection for subsequent analysis. Each individual was tested with one odour per day, over five consecutive days. The five odour *stimuli* were presented in random order. The tip of the pipette was changed between each tested individual. All video recordings were analysed by two blind experimenters (unaware of the identity of the tested mouse, and of the odour *stimulus*). The first nostril used at the beginning of a testing session and the number of times a mouse used its left or right nostril (L and R, respectively) during odour sniffing was noted for each testing session (Figure 2). When the use of one or the other nostril could not be clearly stated by the experimenters, the data point was discarded.



**Figure 2.** (a) Image of an NMRI mouse using its right nostril to sniff the odour presented; (b) Image of a C57BL/6J mouse using its left nostril to sniff the odour presented.

#### 2.4. Data Analysis

A repeated-measures two-way ANOVA (with odour *stimuli* as a repeated factor, and strain as an independent factor) was conducted to compare the mean number of sniffs during testing sessions, and effect size was computed (ges: generalised Eta-squared measure of effect size). Benjamini–Hochberg post-hoc tests were used to make subsequent pairwise comparisons.

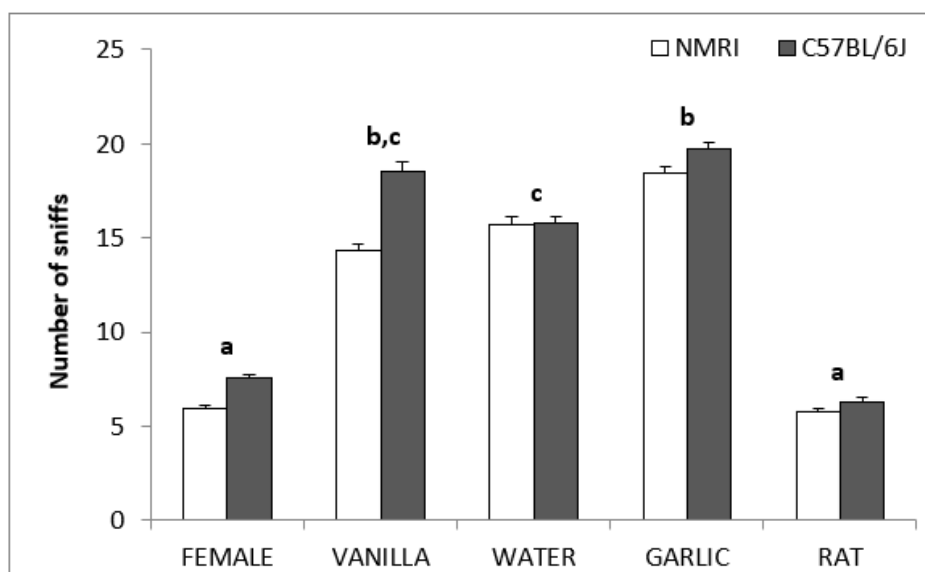
Binomial tests were used to determine whether a nostril (i.e., left or right) was used more frequently for the first sniff at the beginning of a testing session for each odour, and each strain. Cochran tests were run in each strain to determine whether the proportion of mice using their right or left nostril first were different according to the odour tested. Confidence intervals and odds ratio are available in Tables S1–S3.

A Laterality Index (LI) was calculated for each test session for each mouse, according to the formula:  $LI = (R - L)/(R + L)$ . LI is a continuous variable ranging from +1 to −1. Positive values would thus indicate a preference to use the right nostril, and negative values a preference to use the left nostril. Absolute values of laterality indexes (absLI) were calculated for each test session for each mouse to assess laterality strength. absLI is a continuous variable ranging from 0 to 1. A value of 1 would indicate that the mouse was always using the same nostril during the test session, a value of 0 would indicate that the number of times a mouse used its left or right nostril was the same during the test session (i.e., no preference). To analyse LI and absLI results, only animals that had sniffed the tip of the pipette at least 6 times during a training session were considered. This criterion was used as it is the lowest value for which it is possible to obtain a significant result using a binomial test to test nostril preference (i.e., if an individual is using the same nostril six times out of six sniffs, it is preferentially using this nostril: binomial test:  $p = 0.03$ ). Significant departures from chance level (0) were estimated by running two-tailed one-sample  $t$ -tests on LI for each odour in each strain.  $p$ -values obtained were corrected according to the Benjamini–Hochberg procedure in each strain. As the number of mice was different according to the odour presented (see above criterion of inclusion), it was impossible to run a repeated-measures two-way ANOVA (with odour *stimuli* as the repeated factor, and strain as the independent factor) to compare the mean LI or absLI during testing sessions. Then, Welch two-sample  $t$ -tests were used to compare LI or absLI between the two strains for each tested odour. Confidence intervals and effect size (Cohen's  $d$ ) for mean comparisons are available in Tables S4–S7.

Data analyses were conducted using R software (Free Software Foundation, Vienna, Austria; R Development Core team 2009). The statistically significant threshold was  $\alpha < 0.05$ .

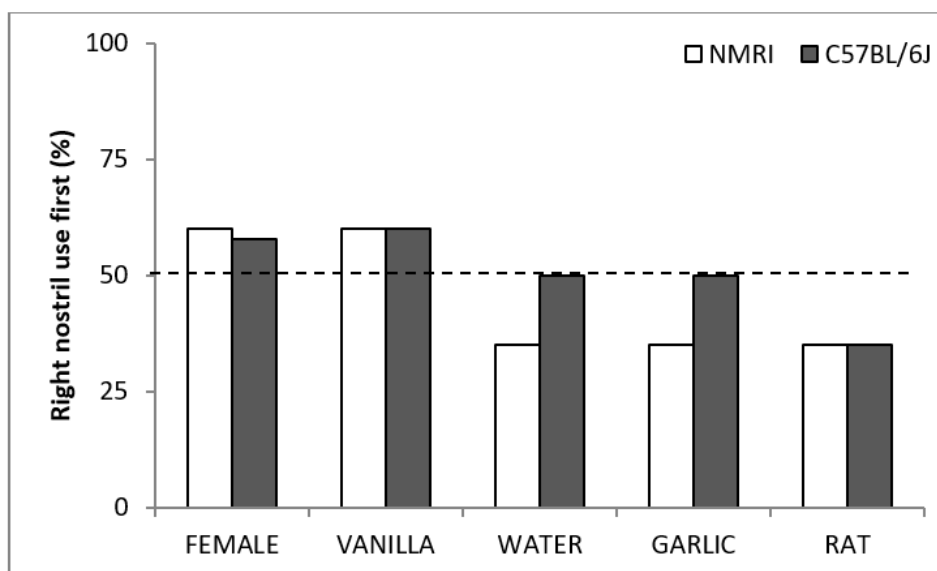
### 3. Results

The mean number of sniffs during a session was significantly different between odours, but not between strains, and no interaction was observed (two-ways ANOVA: odours:  $F = 40.36, p < 0.001$ , ges = 0.40); strains:  $F = 1.43, p = 0.24$ , ges = 0.01; odours/strains:  $F = 0.73, p = 0.57$ , ges = 0.01; (Figure 3). Pairwise comparisons showed that the number of sniffs was lower during female and rat odour sessions compared to the other odour sessions (female or rat, compared to vanilla, water, or garlic: all pairwise comparisons:  $p < 0.001$ ), but not different between female and rat odour sessions (female/rat:  $p = 0.27$ ). The mean number of sniffs during water sessions was not different in comparison with vanilla odour sessions ( $p = 0.68$ ) but was lower compared to garlic odour sessions ( $p = 0.02$ ). The number of sniffs during vanilla and garlic odour sessions was not different ( $p = 0.13$ ).



**Figure 3.** Number of sniffs (mean ± s.e.m) directed to the tip of the pipette during a session. Different letters indicate statistical differences between odours (Benjamini–Hochberg pairwise comparisons).

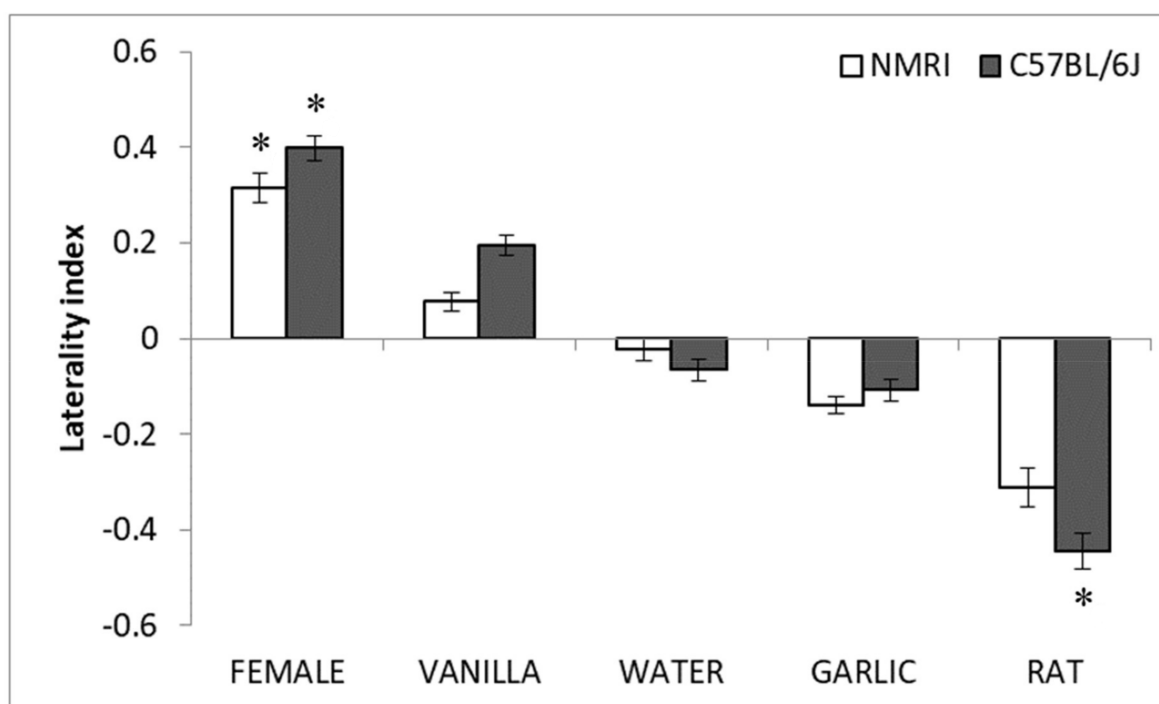
We found no nostril-use preference at the group level at the beginning of a session, whatever the strain or the odour (binomial tests: NMRI: female and vanilla:  $p = 0.50$ ; water, garlic, rat:  $p = 0.26$ ; C57BL/6J: female:  $p = 0.82$ ; vanilla:  $p = 0.50$ ; water, garlic:  $p = 1$ ; rat:  $p = 0.26$ ; Figure 4). The first nostril used was not significantly different according to the odour whatever the strain considered (Cochran test: NMRI:  $Q = 6.25$ ,  $df = 4$ ,  $p = 0.18$ ; CR7BL/6J:  $Q = 3.82$ ,  $df = 4$ ,  $p = 0.43$ ).



**Figure 4.** Percentage of mice using their right nostril to first smell the tip of the pipette filled with the odour stimulus at the beginning of a testing session. The dotted line indicates the theoretical expected distribution (50%).

During a testing session, the number of times mice used their right and left nostril choices were not significantly different from random when tested with distilled water (one-sample  $t$ -tests, Benjamini–Hochberg correction: NMRI:  $t = -0.23$ ,  $df = 17$ ,  $p = 0.82$ ; C57BL/6J:  $t = -0.68$ ,  $df = 19$ ,  $p = 0.51$ ; Figure 5) or garlic odour (NMRI:  $t = -1.8$ ,  $df = 19$ ,  $p = 0.15$ ; C57BL/6J:  $t = -1.13$ ,  $df = 19$ ,  $p = 0.34$ ). However, mice were more likely to use their right nostril when tested with female mice odour (NMRI:

$t = 3.45$ ,  $df = 8$ ,  $p = 0.04$ ; C57BL/6J:  $t = 3.68$ ,  $df = 15$ ,  $p = 0.01$ ). C57BL/6J mice were more likely to use their left nostril when tested with rat odour, and NMRI mice also tended to preferentially use their left nostril (NMRI:  $t = -2.54$ ,  $df = 8$ ,  $p = 0.07$ ; C57BL/6J:  $t = -3.52$ ,  $df = 10$ ,  $p = 0.01$ ). Although NMRI mice displayed no nostril preference when exposed to vanilla odour, C57BL/6J mice tended to preferentially use their right nostril (NMRI:  $t = 0.92$ ,  $df = 17$ ,  $p = 0.46$ ; C57BL/6J:  $t = 2.10$ ,  $df = 19$ ,  $p = 0.08$ ). Whatever the odour considered, no difference between the two strains were observed (Welch two-sample  $t$ -tests: female:  $t = 0.59$ ,  $df = 22.54$ ,  $p = 0.56$ ; vanilla:  $t = 0.95$ ,  $df = 35.90$ ,  $p = 0.35$ ; water:  $t = -0.30$ ,  $df = 35.61$ ,  $p = 0.77$ ; garlic:  $t = 0.26$ ,  $df = 36.41$ ,  $p = 0.80$ ; rat:  $t = -0.76$ ,  $df = 17.89$ ,  $p = 0.46$ ). Concerning absLI, whatever the odour considered, no difference in laterality strength was observed between the two strains (Welch two-sample  $t$ -tests: female:  $t = 0.93$ ,  $df = 22.71$ ,  $p = 0.36$ ; vanilla:  $t = 1.34$ ,  $df = 35.89$ ,  $p = 0.19$ ; water:  $t = 0.75$ ,  $df = 30.20$ ,  $p = 0.46$ ; garlic:  $t = 1.22$ ,  $df = 37.99$ ,  $p = 0.23$ ; rat:  $t = 0.99$ ,  $df = 17.70$ ,  $p = 0.33$ ).



**Figure 5.** Laterality indexes (mean  $\pm$  s.e.m) obtained for each odour in the two mice strains tested. One-sample  $t$ -tests: \*:  $p < 0.05$ .

#### 4. Discussion

In this study, we showed for the first time that mice display preferential nostril use according to the odour presented. During sniffing of female urine, mice preferentially used their right nostril. On the contrary, during sniffing of rat odour, mice preferentially used their left nostril. Finally, when tested with a neutral solution (i.e., distilled water) or garlic odour, no nostril-use preference was observed.

Here, mice have not shown any initial preference to use their right or left nostril independently of the valence of the odour presented, as it has been shown in other species. Indeed, in both horses and dogs, during the initial presentation of an odour, individuals preferentially use their right nostril whatever its emotional valence [18,19]. It has been suggested that, in mammals, the right hemisphere attends to novel *stimuli*. In our study, odour spreading inside the apparatus might explain the absence of stimulus-independent nostril-use preference when first approaching the tip of the pipette. Indeed, when placed in the apparatus, both visual and olfactory explorations are undertaken by tested individuals, and they might have already identified the type of odour they are exposed to, before starting to come close to the tip of the pipette. This is consistent with the fact that, even if not



significantly different from random, the percentage of mice using their right nostril is above 50% for female urine and vanilla for which they display a right-nostril use preference when considering the whole session, and under 50% for rat odour for which they display a left-nostril use preference when considering the whole session. To test whether mice consistently use one of their nostrils to initially explore a new odour *stimulus*, further studies may require an air extractor below the tip of the pipette.

Given that olfactory information ascends mainly ipsilaterally from each nostril to the primary olfactory cortex in the same hemisphere in mammals [24], the pattern of nostril use observed suggests an involvement of the right hemisphere for processing female urine. Although female urine has been shown to activate predominantly the posterodorsal medial amygdala in male mice [25], we do not know whether this activation is higher on the right side. Here, the right-nostril use preference for processing female odour is consistent with literature commonly pointing to a right hemisphere bias for processing social *stimuli*. In horses, a right-nostril use preference was observed when they were exposed to stallion faeces [18], or oestrous mares [21]. This right hemisphere bias for processing social *stimuli* has also been shown in the visual modality in a large array of non-human mammals (*Odobenidae*, *Equidae*, *Cervidae*, *Bovidae*, *Macropodidae*) [26]. In most species studied (both aquatic and terrestrial mammals), infants preferentially position themselves to keep their mother in their left visual field (i.e., right hemisphere involvement), than in their right visual field.

The left-nostril preference, when exposed to rat odour, suggests an involvement of the left hemisphere for negative emotional processing. Indeed, exposing C57BL/6J mice to a rat elicits anxiety responses (e.g., greater immobility) and increased plasma corticosterone levels [27]. In literature, rats are both described as predators and competitors of mice, their density negatively affecting mice abundance [28]. Conversely, rat eradication can greatly increase mice population [29]. As right hemisphere dominance is commonly described in mammals for negative emotional processing [30], we might have expected mice to display a right nostril preference when exposed to rat odour. However, asymmetric processing of stressful *stimuli* seems to depend on numerous factors (e.g., brain structure considered, sex of tested individuals [31], nature of the stressor, length of stress exposure, delay post-stress) in rodents. If we consider the olfactory bulb which allows mice to display adaptive behaviours when exposed to innate aversive odours (e.g., predator, spoiled food [6]), its integrity on the left side is critical to behave adaptively in response to the odour of a stressed conspecific (rats [14]). The amygdala also seems responsible for some innate olfactory behavioural responses, as rats with lesioned amygdala show reduced freezing compared to sham-lesioned congeners when facing an immobile predator [32]. At a brain level, a right hemisphere potentiation (between the central amygdala and periaqueductal gray) was observed at 11 to 12 days post-stress, but not at 1<sup>st</sup> day post-stress [33–35]. In rats exposed to a restraint stress, changes in dopamine activation shift from a greater left-sided (at 15 min) toward a greater right-sided effect (from 30 min) in the medial prefrontal cortex [36]. Taken as a whole, it seems that the two hemispheres might contribute differentially to the behavioural response to stressors, and the right hemisphere might be particularly important in long term response to stressors. That might be consistent with the fact that the left hemisphere is primarily involved during the initial stages of different olfactory tasks in rats (Table 1).

In our study, the odours of vanilla and garlic, both potential food items, were initially chosen for their expected emotional valence: respectively, attractive and aversive odours. Indeed, it has been shown that mice are attracted to vanilla flavour [37] and that conversely, garlic odour has been described as aversive for mice [38]. Detecting pleasant and unpleasant odours is particularly adaptive as they are important signs of potential resources in mice [1]. Both odours were actively explored during the test sessions, much more than female and rat odours. However, only a statistical trend for right nostril-use preference was observed for vanilla in C57BL/6J mice. Surprisingly, we were expecting a left-nostril use preference when exposed to a pleasant odour to be consistent with the hypothesis of left hemisphere dominance to process positive emotions [39]. This result associated to the left-nostril preference when exposed to rat odour might indicate an asymmetric pattern of olfactory processing that might be different in mice than in other mammals studied: with positive (female urine, vanilla)

and negative (rat) odours preferentially processed by the right and the left hemisphere, respectively. As it is the first time nostril-use preference has been tested in rodents, further studies will be helpful to better determine whether this pattern is common in rodents, or specific to *Mus musculus*.

This study describes for the first time nostril-use preference in rodents, and more specifically in mice. The absence of strain effect (excepting for vanilla) suggests that this pattern might be commonly shared by different mice strains. Further studies will be useful now to determine whether the specific pattern of olfactory lateralisation observed, is specific to the sensory modality studied (i.e., olfaction) or more generally the reflect of emotional lateralisation in mice. Better understanding asymmetric processing of emotional *stimuli* in mice (commonly used to model human pathologies) might provide important insights into environmental conditions and developmental mechanisms that may alter behavioural and brain asymmetries as observed in different neuropsychiatric disorders in humans.

**Supplementary Materials:** The following are available online at <http://www.mdpi.com/2073-8994/11/9/1129/s1>: Supplementary data (S1 S7).

**Author Contributions:** Conceptualisation, C.J.-A., V.B.; Data collection and analyses, S.P.; Original draft preparation, C.J.-A.; Review and editing, all three authors.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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