Auris Nasus Larynx xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Auris Nasus Larynx

journal homepage: www.elsevier.com/locate/anl

Newly developed method for mouse olfactory behavior tests using an automatic video tracking system

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ARTICLE INFO

Article history:
Received 4 November 2016
Accepted 8 March 2017
Available online xxx

Keywords:
Olfactory test
Behavior
Preference
Avoidance
Mouse
Anosmia

ABSTRACT

Objective: The mouse is the most popular animal model in olfactory research. Behavior tests with odorants are essential for determining olfactory phenotype. To the best of our knowledge, the mouse olfactory behavior test has not been standardized, making the results vulnerable to inter-observer variation. We sought to develop a new mouse olfactory behavior test assessed by an automatic video tracking system with minimal inter-observer variation.
Methods: A video-tracking system was used to automatically track mouse behavior in standard breeding cages with C57BL/6N mice. We tested two odorants (peanut butter for the preference test, 2MB acid for the avoidance test) and distilled water (for a control). Mouse behavior was recorded for 3 min and analyzed. For the preference test, investigation time was measured. For the avoidance test, time spent in sectors away from the odorant zone was measured. To confirm our experimental settings, we also evaluated an anosmia mouse model prepared with intranasal administration of $ZnSO_4$.
Results: All strains of mice showed reproducible behavior patterns of preference or avoidance for the odorants. The anosmia mouse model, as expected, failed to show an olfactory ability for preference or avoidance, and this was well-matched by histologic changes caused by the $ZnSO_4$ treatment. The automatic video tracking system successfully tracked and automatically calculated mouse behavior with good reproducibility.
Conclusion: Our olfactory behavior test offers a simple and accurate method to evaluate olfactory function in mice. This test can be utilized as a possible standard method to search for features of olfactory phenotypes in mice.

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http://dx.doi.org/10.1016/j.anl.2017.03.007
0385-8146/© 2017 Published by Elsevier Ireland Ltd.

1. Introduction

Olfaction is an essential sense for most creatures. Olfactory sense in humans is particularly important, and its disturbance causes mistaken results in social tasks and interferes with daily performance. Olfactory disturbance can develop at varying steps along the olfactory pathway, from the odorant receptors on olfactory neuronal cells to the olfactory cortex in the brain. Mouse models have been widely utilized for olfactory research, offering an accurate assessment of olfactory function that is crucial but tricky. Among various experimental procedures, the olfactory behavior test provides very basic but critical information about in vivo olfactory phenotypes of genetically or environmentally modified mice [1]. This information suggests that certain genes or environmental conditions affect olfactory function in mice.

To date, various methods to assess olfaction in mice have been utilized for basic research. The elementary concepts of olfactory behavior tests include preference and avoidance or discrimination with various odorants [1–4]. To analyze the mouse response to an odorant, many researchers have manually measured time parameters [1,2]. This approach may be affected by subjective points of view of observers and requires intensive labor, resulting in a lack of objectivity and risk of inter-observer variation.

In this study, we sought to establish a new behavior test for assessing mouse olfactory function using an automatic video tracking system without inter-observer variation. In addition, we applied our method to an anosmia mouse model to determine whether the method was objective and well correlated with histologic findings of the olfactory epithelium.

2. Materials and methods

2.1. Experimental setting

C57BL/6N male mice (12 weeks old) were used in this study, which was approved by the Institutional Animal Care and Use Committee of our institution (Protocol No. 2015-0043). Each mouse was placed in a standard polypropylene rodent breeding cage during the experiments. The breeding cage contained standard depth wood chip bedding and was not covered with its lid during experiments in order to prevent internal saturation with odorant particles. The experiments were performed in an isolated animal experiment room in the SPF zone, and the room was ventilated by the laminar flow of filtered air. Use of this set up prevented the room from being disturbed by environmental odorants. Before the measurement of behavior, habituation experiments were carried out; the methods are detailed below in the preference and avoidance test sections, respectively (Fig. 1). We tested two odorants (peanut better for the preference test, 2MB acid for the avoidance test) and distilled water (for a control).

The video camera (HD lens 720P 30FPS Auto Widescreen) was fixed vertically about 1 m above the mouse cage and was connected to a computer with a Smart video-tracking system (Smart 3.0, Harvard apparatus©, Holliston, MA, USA). Mouse behavior in the standard breeding cages was recorded and automatically tracked (Fig. 2). The targeting zone for preference tests included the odorant area (green box in the right panel of Fig. 3A). The targeting zone for the avoidance test was allotted to the sector behind the curtain from the odorant zone (blue box in right panel of Fig. 3B). The path of mouse

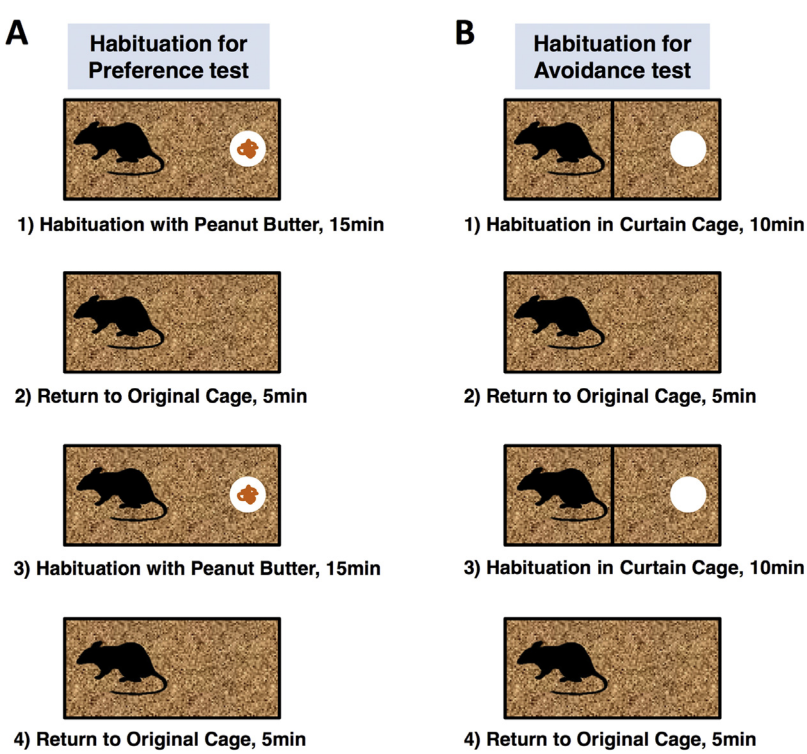

Fig. 1. Habituation protocol for olfactory behavior tests. (A) Habituation for the preference test. Two cycles of odor exposure and rest were performed before the preference test. (B) Habituation for the avoidance test. Two cycles of odorless exposure and rest were performed before the avoidance test.

Please cite this article in press as: Cho H-J, et al. Newly developed method for mouse olfactory behavior tests using an automatic video tracking system. Auris Nasus Larynx (2017), http://dx.doi.org/10.1016/j.anl.2017.03.007