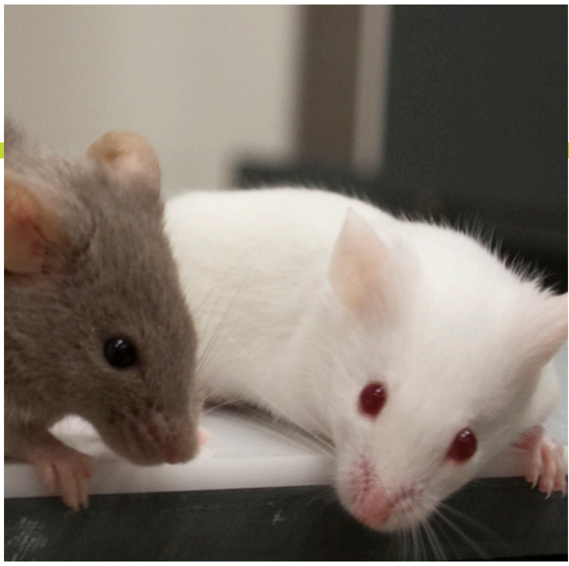

OLFACTORY Haxha

The role of peers and non-shared stressful experience

By J. Verhagen and G. Coronas_Samano - The olfactory habituation/cross-habituation test (HaXha) is a non-invasive spontaneous behavioral task that has been used to study the ability to smell and the capacity to discriminate between stimuli (odors). When any stimulus is repeatedly evoked, the behavioral response decreases (habituation). Meanwhile the presentation of a different stimulus leads to a change in the amplitude of the habituated response (cross-habituation) [1, 2].

GENERAL PROTOCOL

The general protocol for olfactory HaXha in rodents consists of presenting an odor (in a paper filter or cotton applicator) generally in the center of the experimental cage, and measuring the time in which an animal is oriented to and within 2 cm of the odorant (i.e. exploring the odorant).

To measure the habituation phase, the novel stimulus is presented several times (trials). The cross-habituation phase can be studied by changing the stimulus for a novel unfamiliar odor. The standard means to measure the exploration time is by a human experimenter using a stopwatch. This provides the potential for observer bias in cases where subjects cannot be tested without prior knowledge of status, as when testing anatomically different pheno-types (e.g. fur, body weight). Further, as individual exploratory bouts can be quite short (<1 sec) the use of a stopwatch limits accuracy.

Also, a human observer will have limited accuracy in scoring the behavior according to exact criteria such as proximity within 2 cm of the odor source and precise head angle towards it. Last, olfactory exploration inevitably is mediated by active sniffing, an increase of sniffing rate over the baseline rate.

Here, we propose a novel approach to accurately assess HaXha using the Noldus system to track the behavior of every animal combined with whole-body plethysmography to non-invasively evaluate their sniffing [3].

METHODS

The subjects in the study were 4-5 months old WT/129sv mice (n=7). The vivarium had a 12-h/12-h inverted light cycle with lights off at 10:30 am. All animals were housed individually in polycarbonate cages with controlled

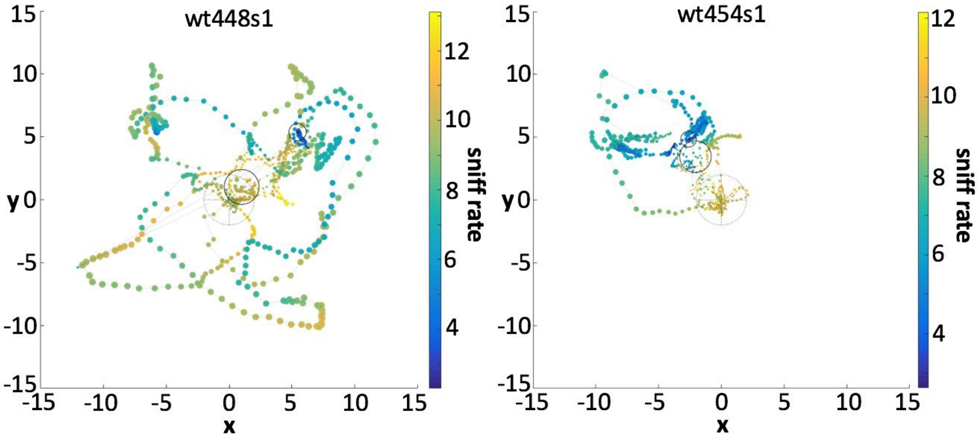

Figure 1. *Trajectory of two mice with their associated sniff rate (color) at each 100ms time bin during the first presentation of social odor on cotton tip 1 cm from the floor (cross-habituation trial). Smaller circles indicate smaller head angles to the odorant.*

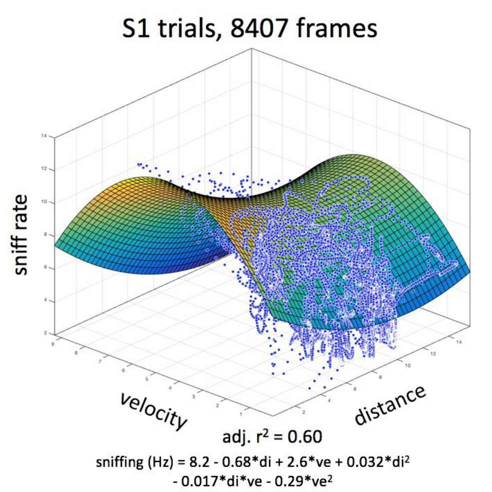

Figure 2. *Sniff rate increases with closer proximity to odor and increased mouse velocity (first social odor trial, 120x100ms bins per trial, n=7 mice).*

humidity (40%) and temperature (22 °C) and were provided with nestlets.

A total of 12 trials were performed per mouse, where each odorant was presented three times in succession per daily session to yield the following order: MO1-3, AA1-3, PE1-3, S1-3. The total duration of a single session was 35 min per mouse, each trial consisting of 2 min per odorant exposure and an inter-trial interval of 1 min between stimuli.

In order to reduce timing errors and experimenter bias in measuring the habituation/cross-habituation task, and to know if these behavioral responses were specific to the olfactory abilities of the animals, we combined a system to automatically record behavioral responses using the Noldus behavioral tracking system (EthoVision XT, version 10.1, Noldus Information Technology b.v., Wageningen, The Netherlands) with sniffing analysis obtained by whole-body plethysmography. We used an air-sealed experimental semi-transparent white acrylic box (26x38x16cm) with a USB camera (Logitech HD Pro C920,1920x1080 pixels) mounted at the ceiling of the box, aimed downward.

RESULTS

EthoVision XT allowed us to carefully analyze the behavior of the mice in relation to sniffing. Mice strongly modulated their sniff rate, but not sniff amplitude, as they walked inside the box (Fig. 1). The sniff rate was generally higher in close proximity to the odor stimulus (at the center of the box).

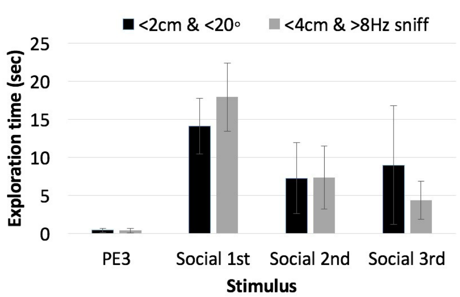

Figure 3. *Exploration time of phenylethanol and 3 presentations of social odor. Classic distance and head angle to odor criterion provides a statistically weaker dataset than distance and elevated sniff rate (n=7 mice; mean±sem).*

We further found that the sniff rate could be well predicted (60%) by the combined nose distance to the odor and their movement velocity (Fig. 2). The head angle to the odor source was not a useful measure when added as criterion to nose proximity. Figure 3 shows that the classical criterion for odor exploration of nose proximity (<2 cm) and head orientation (<20°) to odor source performed poorer, yet remarkably well, compared to the more informed criterion of nose proximity (<4 cm) combined with elevated sniff rate (>8.2 Hz, i.e. >mean+2.5 s.d. of baseline sniff rate).

Mice increased their exploration time when the social odor was introduced (social 1st) and subsequently habituated their exploration. The sniffing based criterion showed more cross-habituation exploration time and stronger habituation, all with less variance.

CONCLUSIONS

We confirm that standard HaXha exploration criteria are fairly accurate at assessing sniff-modulated odorant exploration. We suggest that using velocity or sniffing itself rather than head orientation, combined with proximity, as criterion provides more accurate and more robust results. We further suggest that combined use of video tracking and sniff measurements are optimally suited to perform HaXha experiments. For further details, see [3].

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CONTACT INFORMATION

Justus V. Verhagen (jverhagen@jbpierce.org), The John B. Pierce Laboratory and Yale School of Medicine, Department of Neuroscience, New Haven, U.S.A.