Social Transmission of Food Preference in C57BL/6 Mice

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Abstract

Motivation/Aims

Our understanding of memory processes has greatly benefited from animal research. The assessment of learning and memory in rodents has so far intensely relied on simple tests with easily translatable experimental methods, and thus has concentrated largely on spatial tasks. Socially communicated food bias as a semantic knowledge task has been developed in our laboratory as a modified version of the Galef model of food bias induced through the social interaction with a conspecific [1,2]. This constitutes a natural odour-odour association and fulfills criteria of semantic knowledge including the acquisition during an episodic-like learning event, but the information then becomes unrelated to this mouse-mouse interaction and conceptually independent from the learning episode; it is finally declared in a different environmental setting. Such ‘learning from others’ can be employed in rats and mice, is not induced through a simple presentation of the odour cue, is transmitted by an anaesthetised animal and thus not reliant on the social encounter as such. Furthermore, memory for the novel odour is long-lasting (at least 24 hours) and dependent on elements of the temporal lobe as confirmed through hippocampal lesion studies (for example [3]) and more recently through measurement of activated immediate early genes [4]. Olfactory deficits and memory impairments have been linked to several neurological disorders (such as Alzheimer’s disease, Parkinson’s disease, autism, schizophrenia) with respect to detection, discrimination, recognition and identification [5], hence the need for identifying an early marker for disease prior to clinical manifestations.

In the present study, we carefully examined and constructed new methodologies of investigating olfactory memory in mice using the STFP paradigm. Principle manipulations introduced are the automation of the test which allows the recording of a large number of subjects simultaneously during all phases of the test (habituation, social interaction, test of food preference) and improved methods of measuring activity of the subjects by analysing time spent in particular zones of the arena.

Materials and Methods

Behavioural apparatus

Olfactory discrimination and STFP were assessed in the PhenoTyper cages (30cm x 30 cm x 35cm). The PhenoTyper system (Noldus, Wageningen, Netherlands) is a video-based observation system used for long-term continuous monitoring of behavioural activity in mice. The top unit of the cage contains a built-in digital infrared sensitive video camera and infrared lighting sources for video tracking. The infrared sources provide a constant and even illumination of the floor of the cage that enables continuous recording of the subject’s activity during light and dark periods. Video tracking was performed at a rate of 12.5 samples/second and recorded by the computer-based tracking software EthoVision 3.0 (Noldus Information Technology, Wageningen, the Netherlands).

Animals

Male (N=24; 12 Observers, 12 Demonstrators) and female (N=24, 12 Observers, 12 Demonstrators) C57BL/6 mice at the age of 6-8 weeks were used for this study (purchased from Harlan, UK). Animals were housed in groups up to 10 subjects per cage prior to testing. Animals were allowed food and water ad libitum and were kept under standard laboratory conditions (temperature 20-21°C, 60-65% relative humidity) on a 12h light/dark cycle (light on at 07:00). The testing took place during the light period.
Procedure (see Figure 1)

The Observer (N=24) animals were placed in the Phenotypers and given 3 days of habituation (96-h recording) to the novel environment, with free access to food pellets and water. Once mice were fully habituated to the Phenotypyer, the STFP task was employed. At the start of STFP test food hoppers were emptied and replaced with food jars containing ground mouse chow. Mice were exposed to the ground mouse chow for a period of 24 hours in order to allow for habituation and determination of subjects spatial preference for food jar location. Prior to testing animals were food deprived overnight (maximum 16 hours). The following morning the observer animals were exposed to a stranger mouse (demonstrator previously presented with a particular food scent) placed in a social interaction cylinder positioned in the corner of the Phenotypser cage. The social interaction (SI) phase was recorded for 30 minutes (simultaneous recording of 2 animals in individual cages). The measurement of observer x demonstrator interaction was based on time spent and distance moved of the observer in the ‘interactive zone’ drawn in the vicinity of the cylinder. After SI, the demonstrator mice were removed from the cages and following the determined delay (3 conditions: a) 15 min delay; b) 4-h delay; c) 24-h) the observer mice were exposed to two food jars, each containing a different scented food. It is worth to note that the 24-delay-test was performed on the subjects from condition a) and b) with no food deprivation prior to test. The jar containing cued food was placed in the least preferred corner for each subject, in order to avoid experimental bias. The activity of the subjects during food preference test was recorded for 30 minutes (or over night in the 24hr delay). Time spent in the food jars zones (squares 6cm x 6cm) recorded by EthoVision as well as the amount of food eaten from each jar comprise multiple measures of food preference. A longitudinal cross-over design was employed with the experiment performed over a period of 2 weeks using different delays between demonstrator interaction and food exposure and food scents (cocoa, cinnamon, nutmeg and coffee).
**Statistical analyses**

Group mean comparisons were performed with Prism software (GraphPad Software Inc., San Diego, USA) using parametric or non-parametric analysis of variance (one way or two way ANOVAs as appropriate) followed by suitable post-tests for comparison of selected data pairs. Normalized (%) amount of correct food eaten (in grams, with precision to 2 decimal places) served as a main index of food preference. Further behavioural activity data recorded and extracted by EthoVision software, in 1 hourly bins (for 96-h habituation recording) or 5 minute bins (SI and test) and the following parameters were analysed: time spent, distance moved and frequency of occurrence in social interaction zone, jar vicinity zones (square), water zone; overall distance moved, velocity and immobility. P’s< 0.05 were considered reliable.

**Results**

C57BL/6 mice displayed a strong preference for the cued food under all conditions (15-min delay 70.5% preference; 4h delay 65.1% preference; and 24-h delay 69.7% preference; *p’s<0.05 above the level of chance), with no effect of gender (p= ns). Further analysis revealed significantly more time spent and increased frequency in correct compared with incorrect food jar zones (15-min and 4-h delay groups: ***p<0.001; 24-h delay group **p<0.01). Latency to correct food jar reached significance in the 15-min delay group only (*p<0.05) and was much shorter for the correct scent. Moreover, food intake correlated significantly with time (%) (15-min delay-** r=.42; 4h delay- ***r=.72; and 24-h delay- *r=.37) and frequency in jar vicinity zones (15-min delay- ** r=.45; 4h delay- ***r=.95; and 24-h delay- *r=.40). Further correlation analysis of demonstrators and observers food intake was found positive for all conditions.

**Conclusions/Implications**

Our data confirm that C57BL/6 mice display social and contextual learning, as indicated by intact preference for the cued food, and concurrently prove the ability of these animals to maintain the olfactory memory for a period of 24 hours. Additionally, the introduction of time delays between the acquisition (SI) and retrieval (test) seems to broaden the scope of STFP and allows for assessment of memory at different time intervals. More importantly however, the additional behavioural measures designed in the system (PhenoTyper and the tracking software) employed in this study facilitated the analysis and quantification of STFP-related behaviours, which until now have been restricted to manual recording in other laboratories. The combination of STFP with automated video-observation might be a step forward in measuring behaviours related to olfaction and memory in mice.

**References**


