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Short report

Induction of habits in rats by a forced-choice procedure in T-maze and the effect of pre-test free exploration

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ABSTRACT

A forced-choice procedure in T-maze designed for the induction of habits was used to induce strong habits in rats. The response choices of rats in 20 free-choice trials were compared after the rats had been subjected to 1 or 200 forced-choice trials to one side of the T-maze. After 200 forced-choice trials the rats showed a significant ($p < .001$) propensity for the habitual arm of the maze in the subsequent free-choice trials. The habit was at least as pronounced when analysed over the last 10 free-choice trials as when it was analysed over the first 10 free-choice trials. When the rats were given the opportunity to explore the entire maze immediately before the free-choice challenge after 200 forced-choice trials, this resulted in a large variation in the choice pattern of the individual rats, and a subgroup of rats choose the newly opened maze arm in 95–100% of the 20 free-choice trials.

1. Introduction

Behavioural procedures for creating habits in laboratory animals are potentially useful for modelling human habit formation and aspects of pathological habit formation, occurring for instance in obsessive compulsive disorder or compulsive drug-seeking (Korff and Harvey, 2006; Everitt et al., 2008; Graybiel, 2008). Methods for development of habits in animals include asymmetric reinforcement training paradigms, e.g. overtraining paradigms (Sutherland and Mackintosh, 1971; Mackintosh, 1974; Graybiel, 2008), and animals exhibiting spontaneous rituals or stereotypies have been studied (Graybiel, 2008). The approach used in the present study was forced-choice training of rats in a T-maze—a method to our knowledge used here for the first time with the purpose of creating habits in rats.

The term "habit" is used here for cemented responses of the kind traditionally regarded to be learned incrementally in accordance with stimulus–response theory (Mishkin and Petri, 1984; Mishkin et al., 1984; Dickinson, 1985; Graybiel, 2008). Various theories have been published describing how initially flexible responses as a result of learning develop into "automatic" and inflexible habits (Dickinson, 1985; Norman and Shallice, 1986; Tiffany, 1990). Based on the theory by Dickinson and collaborators (Adams and Dickinson, 1981; Dickinson, 1985), some authors use resistance to reward devaluation as an indicator of habits (e.g. Miles et al., 2003). Recently, there has been an increasing interest in studying the neural substrate of habits (Everitt and Robbins, 2005; Graybiel, 2005; Everitt et al., 2008; Graybiel, 2008). Although the exact neural substrate suggested in these studies varies somewhat with the underlying definition of "habit" – and thereby the behavioural paradigm chosen – the general picture is that habits are mediated by neural circuits involving the basal ganglia and the dopaminergic transmitter system (Graybiel, 2008; Everitt et al., 2008). One interesting finding is an apparent shift from ventral to dorsal striatal control over instrumental behaviour as it develops into stimulus–response habits resistant to reward devaluation (Everitt et al., 2008; Graybiel, 2008).

In the present T-maze paradigm, spontaneous alternation – the tendency of experimentally naïve rats to spontaneously shift between choosing the right and the left arm of the maze when allowed to choose freely, especially during the initial trials (Montgomery, 1952; Lalonde, 2002) – must be expected to influence behaviour. Strong habits can only be displayed after the forced-choice training if spontaneous alternation behaviour is significantly decreased by the procedure.

The aims of the present study were (1) to examine if "automatic" habits – i.e. habits without visits to the non-habitual side of the maze in a subsequent free-choice challenge – could be created by training rats for 200 forced-choice trials in a T-maze. (2) To test if potential habits created by the method would last for 20 free-choice challenge trials. (3) To test if brief free exploration of the entire maze immediately before the free-choice challenge would have an effect on the distribution of responses in the free-choice challenge. The rationale behind testing the effect of a pre-test free exploration period was the following: based on preliminary results

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we found it likely that rats – even though they had been trained for 200 forced-choice trials to a specific arm of the maze – would not totally cease visiting the other, non-habitual arm of the T-maze in the free-choice challenge. As we mainly ascribed visits to the non-habitual maze side after extensive training to exploration tendencies, we hypothesized that the newly opened maze arm would loose its novelty if the rats were allowed to visit it immediately before the free-choice challenge, and that the rats would therefore exhibit less tendency to visit the non-habitual arm in the challenge after free exploration.

2. Materials and methods

2.1. Subjects

A total of 16 male experimentally naïve Wistar rats (Charles River Labs, Sulzfeld, Germany), weighing 250 g at the start of the experiment, were used. The rats were housed singly in Macrolone Type III cages (Scanbur, Koge, Denmark) with aspen bedding (Tapvei Estonia, Harjumaa, Estonia) in a temperature ($21 \pm 1\,^{\circ}C$) and humidity (45–65%)-controlled environment and maintained at a 12 h/12 h light/dark cycle. Testing was conducted during the light phase. Daily food intake of Altromin 1314 (Altromin GmbH, Lage, Germany) was restricted and rats were maintained on 90% of their free-feeding weight, adjusted for growth. Acidified (citric acid) tap water was available *ad libitum*.

2.2. Maze

The T-maze was a white Plexiglas maze (width: 10 cm; height: 20.5 cm; length of start alley: 38 cm; length of response arms: 30.5 cm) without start box.

2.3. Habituation

Prior to testing, each rat was given one 30 min habituation session in the T-maze on each of two consecutive days. During habituation mashed food was present by the end of each response arm, and the rats were allowed to explore and eat freely. No blocks restricting access to the arms were inserted into the maze. The experimenter was located by the start alley of the maze. After each habituation session all rats received a 3 min handling session.

2.4. Forced-choice procedure

During the forced-choice procedure mashed food was present by the end of both response arms of the maze. On all trials the rats were released in the start alley and allowed to run to the food and eat. On most trials one of the response arms were blocked by a block inserted into the maze. The block was a cardboard box of the same colour as the maze. The box had the same height as the maze and nearly the same width as the response alleys. Smell from the food could pass at both sides. For each individual rat the blocked arm was always the same (right or left), but the position of the blocked arm was randomised between rats. On the first session, the rats received one trial with one of the maze arms blocked. Thereafter, the block was removed and a randomised half of the rats ($n = 8$) received a 3 min period of free exploration of the entire maze without food being present (exploration group) and the other half of the rats ($n = 8$) did not (standard group). All rats were then given 20 trials in which they could choose freely between arms (1-trial challenge). The eating period was 8 s after all responses. The rats were then subjected to 195 more forced-choice trials spread over eight sessions with the block inserted into the usual maze arm. On the last session, five more trials were given with the block inserted followed by 20 block-free trials (200-trial challenge). The eating period was 6 s after all responses. Immediately before the 200-trial challenge rats in the exploration group only, were subjected to a 3 min free exploration period of the entire maze with no block or food in the maze.

2.5. Data and data analysis

Data consisted of the percentage of visits of the rats to the newly opened side of the T-maze, the percentage position bias, and the percentage spatial alternation in the 1-trial as well as the 200-trial free-choice challenge for the two groups of rats: (1) the group that were allowed to explore the entire maze freely immediately before the free-choice challenges (exploration group), and (2) the group that received no exploration period before challenges (standard group). The percentage spatial bias was defined as the percentage choices to the side of the maze preferred by that particular rat for that particular session. Non-parametric statistics were used as some groups could not be considered normally distributed (e.g. see Fig. 2, group 200E). In order to test the effect of the forced-choice training, results of the 1-trial and the 200-trial challenges were compared using Mann–Whitney *U*-test ($p < .05$ significance level, two-tailed) for the following trial blocks: A, trials 1–5; B, trials 6–10; C, trials 11–15; D, trials 16–20; and all 20 trials. In order to test the effect of the exploration period on response choices, we – for the same trial blocks – compared the results of the standard group and the exploration group using Mann–Whitney *U*-test ($p < .05$ significance level, two-tailed).

3. Results

Fig. 1 shows the results for the standard (non-habituation) group. For all trial blocks of the free-trial challenges there was a significantly decreased percentage of visits to the newly opened arm of the T-maze ($p < .001$ for all 20 trials; $p < .01$ for trials 11–15 and trials 16–20; $p < .05$ for trials 1–5 and trials 6–10, Mann–Whitney *U*-test, two-tailed) in the 200-trial challenge compared to the 1-trial challenge, while position bias was significantly increased in the 200-trial challenge compared to the 1-trial challenge only for all 20 trials and trials 6–10 ($p < .01$, Mann-Whitney U-test, two-tailed). The percentage spatial alternation was significantly decreased in

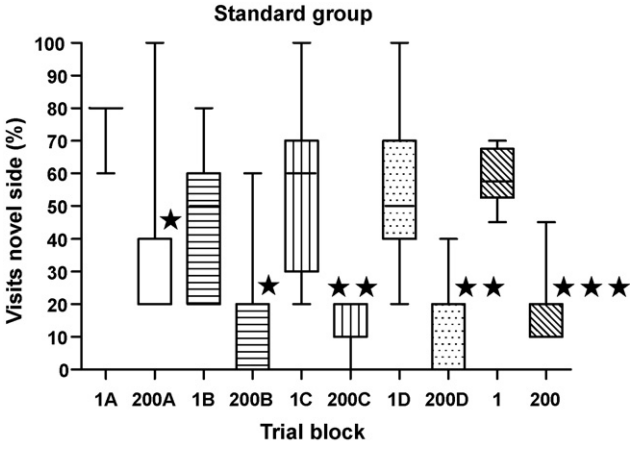

Fig. 1. The percentage of visits to the newly opened maze arm in the forced-choice paradigm. Results of the standard (non-exploration) group ($n = 8$) after 1 or 200 forced trials (1-trial or 200-trial challenge). Trial blocks: 1A, 1B, 1C, 1D and 1: trials 1–5, trials 6–10, trials 11–15, trials 16–20, and all 20 trials, respectively, of the 1-trial challenge. 200A, 200B, 200C, 200D, and 200: trials 1–5, trials 6–10, trials 11–15, trials 16–20, and all 20 trials, respectively, of the 200-trial challenge. Data represent medians with 25% and 75% percentiles and range. ***Significantly different from the 1-trial challenge ($p < .001$). **Significantly different from the 1-trial challenge ($p < .01$). *Significantly different from the 1-trial challenge ($p < .05$).