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INDIVIDUAL DISCRIMINATION BY OLFACTORY CUES IN MICE (*MUS MUSCULUS*): A MULTIPLE CHOICE CONFIRMATION

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ABSTRACT: The ability to discriminate four individual conspecifics by olfactory cues in laboratory mice was investigated. Adult male mice of the CD-1 outbred strain were water deprived and placed in a Plexiglas four-arm maze, twice a day, five days a week. Subjects were assigned to two groups: Training and Control. Training mice were trained by water reinforcement (a drop of water) to choose the maze's arm containing the sawdust of one of four donors (adult males, same strain), while for Control mice the association between sawdust and reinforcement was continuously varied. Data collected during three weeks showed that Training mice made significantly fewer errors in finding the water than Control mice, thus confirming the results of previous experiments based on a two-way choice that proved mice to be able to discriminate conspecifics individually by olfactory cues.

RIASSUNTO: Si è esaminata la capacità di topi di laboratorio di discriminare individualmente quattro conspecifici mediante la percezione olfattiva. Topi maschi adulti del ceppo non consanguineo CD-1 sono stati deprivati di acqua e posti in un labirinto a 4 bracci di Plexiglas, due volte al giorno, per cinque giorni alla settimana. I soggetti sono stati divisi in due gruppi: Training (Addestrati) e Control (Controlli). I topi Training sono stati addestrati a trovare una ricompensa costituita da una goccia di acqua, tale ricompensa era sempre associata a una solo tipo di segatura tra le quattro presenti (i quattro diversi donatori erano dello stesso ceppo), mentre per i topi Control l'associazione tra segature e ricompensa era continuamente variata. I dati raccolti nel corso di tre settimane hanno mostrato che i topi Training hanno effettuato un numero significativamente minore di errori nel raggiungere l'acqua rispetto ai topi Control (Controllo), confermando così i risultati di precedenti esperimenti basati su una scelta binaria che evidenziavano come i topi fossero in grado di discriminare individualmente dei conspecifici sulla base di segnali olfattivi.

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INTRODUCTION

Olfactory cues play an important role in the social behaviour of mice (Bronson, 1979; Brown, 1979; Hurst, 1990a, b, c). Mice can use odours to discriminate among closely-related strains (Hahn & Simmel, 1968) and to recognize gender (Bowers & Alexander, 1967), reproductive condition (Hayashi & Kimura, 1974) and social status of individual conspecifics (Jones & Nowell, 1973; Bishop & Chevins, 1987). Moreover, among mice, both mating and aggressive behaviour seem to be greatly influenced by conspecific odour cues depending on the social group (own/alien) and/or degree of kinship (Bronson, 1979; Barnard, Hurst & Aldhous, 1991).

Urine is considered the most important source of socially meaningful odours (Archer, 1968; Jones & Nowell, 1974; Barnard & Fitzsimons, 1988; Gosling & McKay, 1990; Novotnoy, Harvey & Jemiolo, 1990; Krackow & Matuschak, 1991). Urine marks seem to possess individual characteristics that appear to be determined partially on a genetic basis (Beauchamp, Yamazaki & Boyse, 1985; Jemiolo et al., 1991). According to several authors, identity signals can be correlated with differences in the polymorphic loci of the major histocompatibility complex (MHC) genes, since mice can be trained to discriminate between urine of individual congenic conspecifics that differ only in the MHC (Yamazaki et al., 1979; Yamaguchi et al., 1981; Beauchamp, Yamazaki & Boyse, 1985). Environmental factors (i. e. diet, microbial fauna) have also been proved to participate in the formation of a distinctive urinary odour (Schellinck, West & Brown, 1992).

The methods currently employed to demonstrate individual discrimination involve a series of two-way choices between two individual conspecifics which do not differ in either age or sex (Halpin, 1986). Mice to be discriminated are both unfamiliar in the positive reinforcement paradigm, firstly used by Bowers & Alexander (1967), while differences in the familiarity with the stimulus subjects represent the critical factor in the habituation-discrimination paradigm (e. g., Kimelman & Lubow, 1974). The ability to discriminate individual conspecifics both in rodents and in birds has been proved to be better evaluated when animals are presented with a multiple choice in place of a two-way choice (Haynes & Purvis, 1972; Gilder & Slater, 1978; Bateson, 1982). The aim of the present experiment was to investigate the extent of individual discrimination when mice are given choices between four unfamiliar stimulus animals.

METHODS

Animals

Twenty-eight outbred albino Swiss (CD-1) male mice (mean age 70 days) purchased from Charles River (22050-Calco, Italy) were used. On arrival, twenty-four of them (Subjects) were marked with picric acid and housed in groups of five individuals in 42x27x15 cm Plexiglas cages with stainless metal tops (Tecniplast, 21020-Varese, Italy). The remaining four (Donors) were individually housed in similar cages differing only in dimensions (33x13x14 cm). Bedding consisted of sawdust obtained from white and red fir-wood, free from chemical solvents, and containing neither resin nor dust (FTS, 20136-Milano, Italy). The housing room was air-conditioned at 21± 1 C° and 70% relative humidity, with a 12-12 red light/white light cycle (red light on at 9:30 a.m.). Pellet food (Enriched standard diet, D. Piccioni, 25100-Brescia, Italy) and tap water were continuously available. The sawdust was removed twice a day, 30 min before use, and replaced with an equal amount of clean sawdust. The collected sawdust was maintained at room temperature in a light-proof plastic box to prevent olfactory changes from occurring in the course of the experiment.

Apparatus

The apparatus was a grey Plexiglas maze (radial, four-arm) raised 50 cm above the floor. Each of the arms was 32 cm long and 4 cm wide. The maze walls were 4 cm high. A box (4 x 4 x 0.5 cm) was placed in each arm, 4 cm away from its opening onto the central platform (circular, diameter 13 cm). The maze was covered with a removable, transparent Plexiglas top with a central round opening (6.2 cm wide), supplied with a cover. The maze was placed in a quiet experimental room under temperature, relative humidity, and lighting conditions very similar to those of the housing room.

Procedure

After one week each subject was given a 10-min familiarization trial in the maze and then placed on a 23-h water-deprivation schedule. Twenty-four h later, each subject was given two 10-min familiarization trials with a between-trial time lapse of 3 h. One drop of water (50 µl) was then placed in a small hole (1 cm deep) at the end of a randomly-chosen arm. Following the testing of each animal, the maze was

thoroughly cleaned with a solution of alcohol and water (50%) and dried. During familiarization trials the boxes were empty.

The experiment started 24 h after familiarization, and took place during the dark period (between 12:00 and 20:00 hours). All mice were given two trials a day (between-trial interval: 3-4 h), 5 consecutive days a week. The boxes were filled with soiled sawdust from the donor animals (1-2 g). Each box contained sawdust from a different donor. The mice were randomly assigned to one of two groups: Training (16 subjects) or Control (8 subjects). Training mice always found the reinforcement (water) associated with the same sawdust, while for the Control mice water was randomly associated with one of the two types of sawdust. A greater number of subjects was used for the Training group as a more heterogeneous response was expected in this group than in the Control group. Indeed, we did not know if one of the sawdust types was easier or more difficult to discriminate than the others.

The order of testing was random. The relative positions of the boxes were varied between trials, and the boxes also moved with respect to extramaze cues. Before each trial, the maze and the boxes were wiped with an alcohol/water solution. Individual mice were placed on the central platform and the trial began when the animal touched the platform with its four paws. An error was counted when the subject passed beyond a box in an arm without reinforcement. During each trial, the number of errors was scored and the time to reach the reinforcement was recorded by a silent hand-held stopwatch. Each test was ended either when water was reached or after 3 min (whichever was first). This cut-off time was seldom exceeded.

During the water-deprivation phase, we continuously checked the mice for signs of distress. None were found.

RESULTS

On the first experimental day, Control and Training mice made the same number of errors before reaching the water (i. e. 1.7 errors). However, in the following days, data show that Training subjects made fewer errors than Control mice before reaching the water reinforcement. An ANOVA (2 x 3) with a between-groups factor (Control and Training) and a within-groups factor (three repeated 10-trial blocks, each block grouping one week) proved this difference to be significant [$F(1,22) = 5,24$, $p = 0.03$, see Fig. 1]. Analysis did not reveal an effect due to the interaction between group and block number.

The data of the Training group could be analyzed as a whole since

repeated ANOVA (4 x 3) measures found no significant differences between the performances of the four subgroups of the Training group [$F(1,22) = 1.79$, $p = 0.17$].

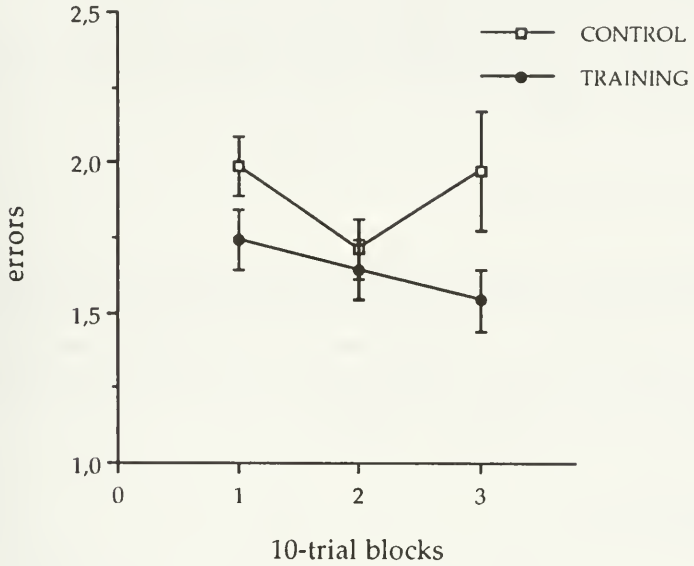

Figure 1. Mean (± SE) level of errors before reaching reinforcement scored for C (Control) and T (Training) subjects during three 10-trial blocks.

DISCUSSION

A recent study in a semi-natural setting (Hurst, 1993) has stressed the role of individual discrimination by olfactory cues in social communication among male mice living in the same area. In laboratory studies, this ability has been demonstrated by experiments involving two-way choice (Bowers & Alexander, 1967; Hahn & Simmel, 1968; Kimelman & Lubow, 1974; Yamaguchi et al., 1981; Beauchamp, Yamazaki & Boyse, 1985; Gosling & McKay, 1991).

Giving animals multiple choices is more likely to reveal the existence of a discrimination process (Gilder & Slater, 1978; Bateson, 1982). Our experiment verified that the ability to discriminate individuals by olfactory cues is also expressed in mice presented with multiple choices. The discrimination curve was not clear however, possibly due to the limited number of trials (see Bowers & Alexander, 1967; Yamazaki et al., 1979) which was agreed upon with our intramural

Ethical Committee before the beginning of the study.

We considered urine odour to be the basis of the discrimination reported. But since the odour source was represented by sawdust we cannot exclude an effect due to other organic components present (secretion of specialized exocrine glands, feces, hair), that have been shown to affect behaviour (Apps, Rasa & Viljoenet, 1988; Goodrich, Gambale, Pennycuik & Redheadet, 1990a, b; Marchlewska-Koj, Pochron & Sliwowska, 1990).

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