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THE INDIVIDUAL RECOGNITION AMONG C57BL MICE IN THE *Y*-MAZE

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Individual recognition, but not strain recognition, was observed among 3-week old male C57BL mice using the *Y*-maze apparatus. The results were interpreted in terms of familiarity of the subject mouse to the stimulus mice and the rearing effects which might have led to labelling of stimulus mice as novel strain mice. Furthermore, the occurrence of individual recognition was achieved through the presentation of olfactory cues together with other preferential cues (e.g. visual and auditory cues).

The present study investigated the occurrence of strain and individual recognition in male C57BL mice using the *Y*-maze. Strain recognition is defined as the choice of a same strain stimulus regardless of being a sibling or a non-sibling over a different strain of stimulus. Individual recognition on the other hand, is the complete attachment of preference for a single mouse regardless of strain or kin relatedness.

Several specific cues attached to the processes contributing to recognition have been identified such as visual, chemical and sound cues (Blaustein, 1982). However, the most potent factor affecting recognition in rodents are the chemical cues (Blaustein, 1982; Davis, 1982; Myrberg, et al., 1985; Quinn, et. al., 1985: Lampredht, 1985). It is regarded that these chemical cues are sensed by olfaction or taste (Blaustein, 1982). Furthermore, these chemical cues, which give rise to odors, are important in several behavioral and physiological contexts related to recognition (Cowley, 1972). As pointed out, many species of mammals, the mouse included, produce odorous secretions that serve as social communicative functions. These social odors can be used to identify the species, colony or family, age, sex, reproductive state, and social status of the animal that produces them (Brown, 1982). Im most animal studies on recognition, the approach paradigm of preference tests has been used (Doty, 1975). This paradigm suggests that a preference exists when an animal spends more time investigating one odor than another odor with which it is paired.

In individual recognition experiments, most researchers employed time spent (Hepper, 1987), mate choice (Hepper, 1986), or social behavior between opposite sex (Holmes, 1984) as indices of such recognition. Differences in these indices are mixtures of discriminative effect and reinforcing effect. However, the use of conditioning in individual recognition experiments has shown the occurrence of preferential behavior more clearly because functions of discriminative stimulus and reinforcing stimulus are analyzed separately (Husted et. al., 1966).

In mammals, life-time reproductive success is often taken as a measure for fitness. A high reproductive success does not only involve the production of many young but also requires that they survive to reproduce (Konig, 1989). It is within this context, the avoidance of extreme inbreeding and/or outbreeding, why most recognition studies are done in a sexually-related setting. Although researchers used the words social interactions, the concentration of their study was preferential sexual behaviors as index of recognition. Thus, additional information is needed to verify these same recognition types under a non-sexual or socially-related situation. Not disregarding the fact that the degree of inbreeding and outbreeding affects the fitness consequences of mating, social interactions during the period when the individual is not yet capable of reproduction could be another factor that could also influence its later mate choice behavior for reproduction. These social interactions, prior to the appearance of mating capability, therefore could not be neglected as part of the individual's life history having components such as survivorship and competitive ability which would ultimately relate to mating success. It is within this line of experimentation, on the ocurrence of individual and strain recognition under a socially-related context, that the researchers engaged in this study.

METHOD

Two strains of C57BL6/J and BALB/c of three weeks of age male mice were obtained from Charles River Breeding Company of Japan. Five out of the seven C57BL6/J mice were randomly assigned as subject mice and the other two mice were used as stimulus mice. The BALB/c mice were only used as stimulus mice. Upon arrival, each mouse was housed individually in cages made of thick transparent plastic that measured 10×16×28 inches. The floors of the cages were covered with sawdust. Replacement of the sawdust and cleaning of all the cages were done at the same time once a week. The subject mice together with the stimulus mice were maintained in a single room with temperature at 15+/−3 degrees celsius.

SUBJECTS

Five male mice of C57BL6/J (hereafter referred to as C57) strain were used as subjects. Prior to the start of habituation phase, the mice were first deprived of food for 20 hours for two consecutive days and thereafter, a 23-hour food deprivation was monitored. Water was freely available in their home cages. The mean weight of the subject mice was 23.2 grams.

STIMULI

Stimulus mice for discrimination training were one male C57 and one male BALB/c (hereafter referred to as BALB) mice. Two more mice of both strains were used in the testing phase. Each stimulus mouse were also housed individually in cages similar to the subject mice. The positions of the stimuli mice in the room were not visible from the subject mice to prevent olfactory cues and other possible variables (e.g. visual and auditory cues) arising from the stimulus mice that could mask testing results. During discrimination training, C57 mouse was designated as the positive stimulus (S^+). Reinforcement through presentation of a food pellet followed the response of choosing the C57 mouse. On the other hand, the choice of BALB mouse, having been assigned as the negative stimulus (S^-), followed no reinforcement of food pellet. Throughout the discrimination training, only this pair of C57 and BALB mice were used. During the testing phase, this pair was labelled as the training stimulus mice whereas the other pair of mice was named as the new stimulus mice. All the stimulus mice gained free access to food pellet and water.

APPARATUS

The apparatus was a *Y*-maze made of black Plexiglass (Fig. 1). The height of the wall was 7.5 cms. and a ceiling of transparent Plexiglass covered every arm. The arms were 35 cms. long and 10 cms. in width.

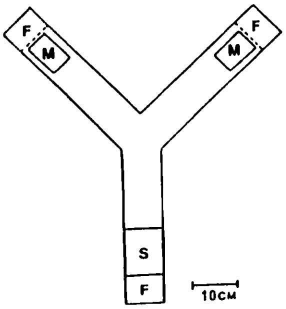

Figure 1: The *Y*-maze apparatus.

Angle of the choice arms was 100 degrees. At the end of each arm, there was a small fan (CD: 6 volts; 5700 rpm) which produced air flow from the choice arms to the start arm. Throughout the experiment, the stimulus mice were placed in a metal box (12×6×7 cms.) in front of the fan of each choice arm. There were six holes (0.7 cm. in diameter) on the rear wall of the box and a window of wire mesh (6×5 cms.) on the front wall. A small cup (3 cms. in diameter) in which a 20 mg. food pellet could be baited was fixed in front of each box. The start area was 10 cms. long from the end of the start arm and separated by a wire mesh door from the other area.

PROCEDURE

HABITUATION: Each stimulus mouse was placed into the small metal box and in turn the box was placed at the end of each choice arm. The subject mouse was then introduced to the start area. Ten seconds later, the wire mesh door of the start area was opened and the subject was allowed to run the maze. Both choice arms were baited with a 20 mg pellet. The choice response was defined as entering of the whole body of the subject mouse except for its tail in one of the choice arms. Time from opening of the mesh door to the response of choosing one arm was also measured. After the subject consumed the pellet, it was placed back to the start area and the next trial also began after 10 seconds.

One daily session consisted of 20 trials and on each trial, the position of the stimulus mice was randomly changed. At the end of each daily session for each subject mouse, the metal boxes were washed using an ultrasonic cleaner then wiped with ethanol. Also after the daily session, the animals gained access to food freely for one hour in their home cage. The habituation session was done for two consecutive days.

DISCRIMINATION TRAINING: The discrimination training was similar to habituation except that for every choice of the negative stimulus (BALB), no reinforcement was introduced. It was only during choice of the positive stimulus (C57) was reinforcement given to the subject mouse. The training continued until the subjects showed at least 80% correct response of choosing the positive stimulus on three successive sessions.

TESTING: Immediately after the subject mouse reached the criterion of discrimination, it was introduced twice for each of the six tests consisting of twenty extinction trials. After each test, the subject was retrained to maintain its 80% discriminative behavior of choosing the positive stimulus. This 80% choice after each test was a prerequisite before proceeding to the next test. Testing was similar to habituation and discrimination training procedures but the stimulus mice presentations were manipulated. For tests 1 to 5, the combinations of training and new pairs of stimulus mice were manipulated while in tests 6 and 7, only the nesting material and air flow were manipulated, respectively. Tests 1 to 5 measured the presence of strain and individual recognitions while tests 6 and 7 measured the possible influence of olfactory cues for the occurrence of the recognition process(es) in the previous tests. The different setups are summarized below: TEST 1: No reinforcement. The training pair of stimulus mice used during habituation and discrimination training were presented to the subject but no food pellet was placed in both choice arms.

TEST 2: $S^{+\prime}$ and $S^{-\prime}$. The [′] denotes that a new pair of C57 (positive) and BALB (negative) stimulus mice were used.

TEST 3: S^{+} and $S^{-\prime}$. Training positive stimulus (C57) and new negative stimulus (BALB) mice were presented.

TEST 4: S^{+} and $S^{+\prime}$. Training and new positive stimulus mice were presented.

TEST 5: S^{-} and $S^{+\prime}$. Training negative stimulus mouse and new positive stimulus mouse were presented.

TEST 6: Nesting Material. No stimulus mouse was presented to the subject mice. Only the floor material (sawdust or flakes) of the cages which had been soiled by the stimulus mice for a day was collected just prior to testing and placed in the metal box.

The flakes contained feces and traces of urine labelled by the stimulus mice. The weight of the sawdust from both stimulus mice was maintained at +/−15 grams so as to somehow control similarity of olfactory cues arising from these materials of two different strain stimulus mice.
TEST 7: Contrary Wind. The training stimulus mice were presented but the direction of the fans was reversed so that air flowed to the direction of the choice arms.

RESULTS

The total reaction time (TRT) for the subject mice showed a downward trend from phase 1 up to test 1 (Fig. 2). This would imply that prolonging the exposure of the subjects to the stimulus mice regardless of strain relations, led to the nonhesitant approach towards both of the stimulus mice. However, separate analyses of the reaction times (SRT; Fig. 3) for C57 and BALB stimulus mice showed that there was a significant difference (two-tailed t-test with $df=4$ and $p>.05$, $t=5.5886$). The reaction time for the choice of C57 was significantly lower than the reaction time for BALB across phases up to test 1. This would suggest that in choosing a non-strain BALB stimulus mouse, the approach was always accompanied with hesitance thus a markedly slow movement was observed and a high reaction time was recorded. In this sense, the downward trend of the total reaction time was due to a very low reaction time for the choice of C57 that managed to pull down the high reaction time of BALB.

The total reaction time recorded in test 2 showed a pattern similar to habituation. This finding would suggest that there was no transfer of the non-hesitant approach from the training stimules mouse to the new stimulus mouse. Interestingly, this finding reflected the same results obtained from the data of number of choices for the two new stimuli. Both results would suggest that no stimulus generalization was observed in Test 2. Analysis of separate reaction times for C57 and BALB stimulus mice ($t=1.91$) was insignificant, pointing to the indiscriminate approach of the subject mouse towards the two stimuli.

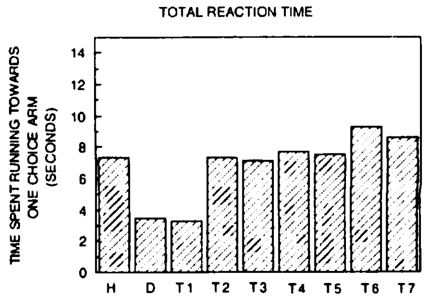

Figure 2: Total Reaction Time (TRT) as recorded during the havituation phase (H), discrimination phase (D), and testing phases 1 to 7 (T1–T7).

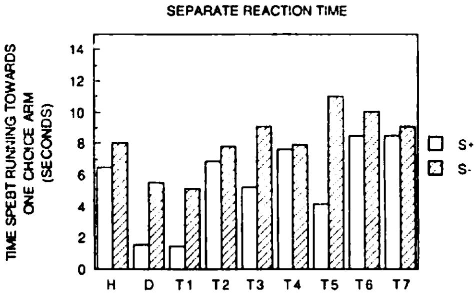

Figure 3: Separate Reaction Time (SRT) as recorded during similar phases of TRT. The time spent running towards S^+ was separately measured from the time spent running towards S^-.

In tests 3 to 5, also habituation-like total reaction times were recorded. Again such recordings would indicate that combinations of training and pair stimuli led to recurrence of hesitant approach to both stimuli. Analyses of separate reaction times of C57 and BALB mice however reveal that there was a significant difference between the two stimulus mice in tests 3 and 5 ($t=4.11$ & 5.34) wherein training C57 was paired with either one of the new stimulus mice. In test 4 wherein training BALB mouse was used as one of the stimulus mice, no significant difference in separate reactions times was recorded ($t=1.72$). Thus, it should be pointed out that the observation of total reaction times with patterns similar to habituation in these latter tests was due mainly to the use of new stimulus mice and the training BALB stimulus mouse. It would appear then that regardless of strain, a new mouse was treated in a manner similar to how the subject mouse treated a non-strain mouse. As such, the hesitant approach was exhibited by the subject mouse towards new and non-strain stimulus mice.

With regards to tests 6 and 7, again the patterns reflected habituation-like total reaction times and, no significant difference was seen in the analyses of separate reaction times for either of the two stimulus conditions. In test 6 (t=.0152), the presentation of olfactory cues produced by two different strains did not yield preferential behavior to either one of the stimuli. However, in test 7 (t=.942) wherein control for elimination of olfactory cues from the stimulus mice was in effect, still no prefence was observed. This would imply that limited presentation of olfactory cues or other cues aside from odor cues, e.g. visual or auditory, led to non-occurrence of preference.

The data on the number of choices for C57 and BALB during habituation did not differ significantly (t=1.15). Therefore, during this phase, no preference was exhibited by the subject mouse (Fig. 4). In the discrimination phase however, an increasing pattern of preferential responding towards C57 stimulus mouse was recorded (Fig. 5). During the 20th session of discrimination phase, all subjects exhibited at least 80% preference towards C57 for 3 consecutive sessions. This 80% choice of C57 was clearly significant from the level of responding towards BALB.

The first test showed that the subject mouse exhibited preference towards C57 at a level significantly similar to the preference level obtained from the last three consecutive trials of discrimination training phase (Fig. 6). Comparing test one results with the population mean of 80% (attained by subjects during discrimination training) no significant difference was seen using the t-test (t=1.1937). The C57 stimulus mouse therefore was seen as reinforcing and as such the preference was clearly manifested by the subject mice in choosing this stimulus. Therefore the suggestion based on the

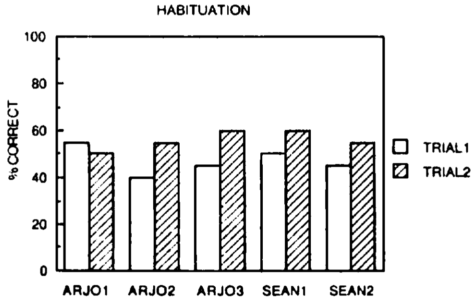

Figure 4: The percent of choosing S^+ was at chance level (50%+/−) during the two trials of habituation phase.

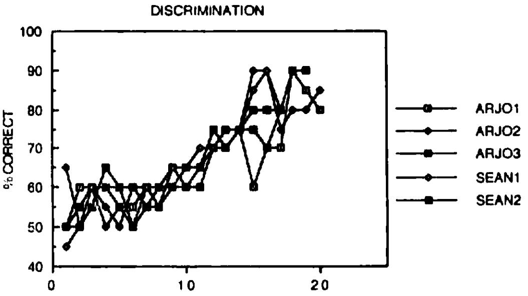

Figure 5: The percent of choosing S^+ had an upward trend during the discrimination phase with all the subject mice reaching the 80% level of preference for S^+ after the 20th session.

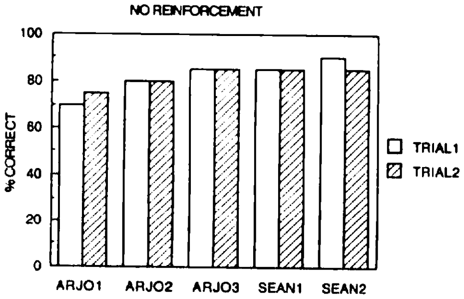

Figure 6: In Test 1, both stimulus mice were presented but no reinforcement followed S^+. Preference for S^+ was still exhibited by the subject mice.

total reaction time that both stimulus mice possessed reinforcing property was too general and, as have been done in separate analysis of reaction time for each stimulus mouse, must be clarified. During the last trials of discrimination training, there was an increasing choice of C57 and especially in the last three consecutive trials, BALB was only chosen at the most 3 times out of the 20 trials. In this case, the low number of choice would indicate that even in these choices of BALB, a low reaction can recorded due to chance error. Thus, to based the reinforcing property of BALB on the low reaction time taken from just three trials was not a good measure of reliability.

In test 2, the new stimulus mice did not elicit preferential behavior from the subject mice (Fig. 7). The result of test 2 was shown to be significantly different from test 1 (t=5.205). This would mean, therefore, that the reinforcing property of the training C57 stimulus mouse was not transferred to the new C57 mouse. Furthermore, in accordance with the findings on reaction time, the subject mouse did not generalize the reinforcing property of the training C57BL stimulus mouse.

In test 3, there was no significant difference (t=2.110) between the preference level of this test and that of test 1 (Fig. 8). This would mean that the training C57 stimulus mouse retained its reinforcing effects even with pairing it to a new BALB stimulus mouse.

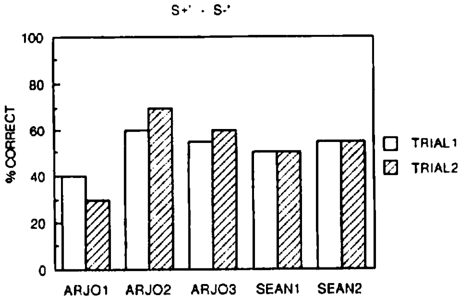

Figure 7: In Test 2, when two new stimulus mice were presented, the subject mice showed no preference.

In tests 4 and 5, comparing the preference level of each test to the population mean of 80%, the results revealed a significant difference between them (t=3.420 & 3.216). This would imply that no preference was exhibited in either of the two stimulus mouse (Figs. 9 & 10). In test 4 specifically, the transfer of reinforcing property of the training C57 to the new C57 stimulus mouse was

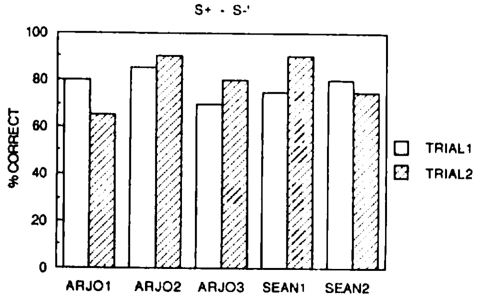

Figure 8: In Test 3, preference was observed when the training stimulus S^- was paired with a new stimulus S^-.

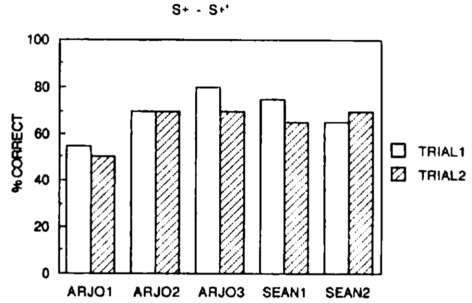

Figure 9: In Test 4, when training and new S^+ were presented, no preference was observed.

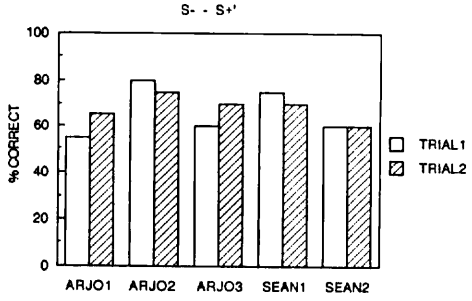

Figure 10: In Test 5, when new S^+ and training S^- were presented, no preference was observed.

again shown as not evident. In test 5 however, in contrast to test 3, the training C57 stimulus mouse did not retain its reinforcing property to produce preference for itself when paired with a new C57 stimulus mouse. The difference in results for tests 3 and 5 therefore could be interpreted as due to the strain of the other stimulus paired with the training C57BL stimulus mouse.

With regards to the mediation of olfactory cues for the occurrence of individual recognition among C57 mice, test 6 ($t=4.698$) response level when compared with the 80% mean preference level was way below and thus significantly different (Fig. 11). In the same manner, test 7 ($t=5.116$; Fig. 12) was also significantly different from the 80% mean preference level. These results support the prior data under the reaction times of the latter two tests. Both results would suggest that olfactory cues or other cues, aside from olfactory cues, when presented alone are not sufficient to evoke preferential behavior. Hence, this would imply that C57 mice needed more than olfactory cues, that is, relevant cues (e.g. auditory and visual) to exhibit individual recognition.

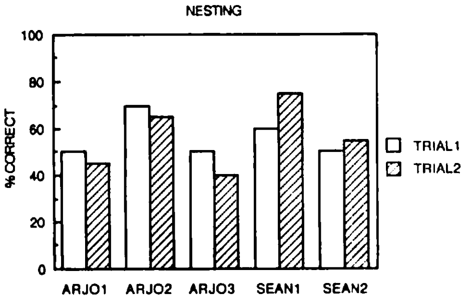

Figure 11: The presentation of the beddings (flakes) of S^- and S^- did not lead to preference for either of the two nesting materials.

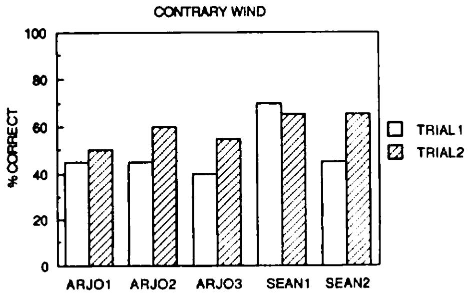

Figure 12: The presentation of cues (visual and auditory) aside from olfactory cues also did not lead to preference.

DISCUSSION

The present results demonstrated that 1) male C57BL mice introduced to a discrimination conditioning paradigm learned preference through individual recognition, and that 2) sole presentation of the olfactory stimulus was not a sufficient cue for individual recognition.

Familiarity of the same strain is perhaps the factor leading to assignments of which stimuli are similar and which are not (Alberts & Galef, 1973; Carr et. al., 1976; Cahoun, 1962; Barnett, 1967; Eibl-Eibesfedt, 1970). The early experience of three weeks during which all the same strain subject and stimulus mice were housed together was not sufficient enough to increase the probability of recognizing the similar cues among them. Such short time of exposure with the same strain stimulus mice would explain the absence of strain recognition. On the other hand, such situation could lead to the occurrence of individual recognition through the process of 'self-matching'. The subject mouse with very few environmental cues as a result of poor experience with his own strain would be using his own criterion in identification of which stimulus mice come close to his preference of company. What is similar or characteristic of himself or his strain whether or not a biological relationship exists between him and the individual being investigated depends upon his own criterion (Porter et. al., 1983; Poter & Wyrick, 1979; Huck & Banks, 1980, Borlongan & Watanabe, 1990).

The occurrence of individual recognition is also affected by the novelty of the strain stimulus (Gilder & Slater, 1978). The novelty of the strain stimulus was pointed out as a variable that could alter preference, at least, between parents and siblings (D'Udine & Partridge, 1981). The more novel the stimulus, the higher the possibility that preference for this stimulus is expected. However, the novelty of the stimulus is dependent also upon the familiarity of the subject to this stimulus especially during the early period of development. (Carr et. al., 1976; Lorenz, 1963). In-fostered mice (reared with same strain parents) would assess their parents as less novel than parents of different strain (cross-fostering). Therefore in-fostered mice would exhibit preference towards non-strain over same strain mice since in-fostered mice have not previously encountered the former mice and labelling of such mice as novel stimuli would occur (Dawkins, 1976; Holmens & Sherman, 1982; Bekoff, 1981; Blaustein, 1982; Myrberg et. al., 1985; Cowley, 1972). This assumption of strain recognition based on in-fostering which leads to classification of novel and familiar stimuli is not observed in the present study. The lack of extensive experience and thus familiarity of the subject mouse with his same strain stimulus mice led to a non-discriminant preference for either the C57 or BALB strain. That is, preference was not limited to a single strain.

The absence of strain recognition, therefore, is not in line with the view on the possible mediation of genetically encoded factors on recognition (Mainardi et. al., 1965; Yanai & McClearn, 1973; Yamazaki et. al., 1976, 1978). The assumption here is that, with the absence of the capability of the mice to incorporate their first three weeks of experience, recognition processes are based on naturally occurring factors namely the genes (Porter et. al., 1983; Bateson, 1983; Alberts, 1976; Barash, 1978; Greenberg, 1979; Bekoff, 1981; Waldman, 1981; Holmes & Sherman, 1982). However, as shown in the tests using new stimulus mice, the generalization process was not evident. It could be inferred then that similarity of genes of one of the stimulus mice with that of the subject mouse was not sufficient to label such stimulus as distinct from the other stimulus with different genetic composition. The present results, thus, favored familiarity or in general the experience of the subject mouse in relation to his outside environment over genetically encoded factors as factors for the occurrence of strain recognition. The success of strain recognition, regardless of biological relatedness, lies heavily on the nature of individual rearing (Porter et. al., 1983). The activities that take place between the organism and his environment during the early period of development is important (Porter et. al., 1981; Konig, 1989; Holmes & Sherman, 1982).

With regards to the olfactory cues as mediating individual recognition, it was shown that presentation of this cue alone would not lead to recognition. The observation of olfactory cues as not sufficient preferential cues is in contrasts with a similar experiment on recognition in mice (Mori & Watanabe, 1989). One major source of difference between these two studies is the experimental setting. The use of very young and only male subject and stimulus mice led to the classification of this study as having been conducted in a social setting and not in a sexually-related context as seen in Mori and Watanabe's research. At the age of 3 weeks, mice are not yet capable of exhibiting sexual behaviors (Barnard & Fitzsimons, 1988; Mainardi, 1965), but if ever they are, the fact that only male mice were used in this study would prevent any occurrence of sex-directed preferences. As mentioned in the literature, much of the studies on recognition have been done in a sexual setting, that is, the use of adult and opposite sex subject and stimulus mice. With this in mind, inconsistencies of results and such contradicting outcomes as a result of differences in the testing methods, sex and age of subject and stimulus mice used by different researchers are expected (D'Udine & Partridge, 1981; Yanai & McClearn, 1973; Hucks & Banks, 1980; Holmes & Sherman, 1983). Thus, in this study, the presence of other cues occurring together with the olfactory cue was shown as a prerequisite to produce individual recognition in young C57 male mice.

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