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Social Recognition in Male Rats: Age Differences and Modulation by MIF-I and Alaptide

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Summary

Social investigatory behaviour was used as a measure of olfactory recognition in two experiments to assess social memory in adult male rats. In Experiment 1, time spent in social investigation of juvenile males by 3-month-old adults was significantly higher than time spent by 7- and 11-month-old animals. Furthermore, a reexposure to the same juvenile male 30 min after the initial exposure elicited significantly less social investigation in adult males aged 7 and 11 months but not in those aged 3 months. If the reexposure occurs 2 h later, the same juvenile is thoroughly investigated by adult males irrespective of the age. The age-related differences in social recognition are discussed in terms of the internal readiness of adult males. While the social recognition was confirmed in older adult males, it is suggested that an ability to recognize the same juvenile may be masked in young animals by a high sexual arousal. Behavioural phenomenon of the social recognition was used in Experiment 2. An administration of hypothalamic MIF-I or its synthetic derivative Alaptide to adult males 7 or 11 months old immediately after their 1st exposure to a juvenile male resulted in decreasing the time spent in social investigation of the same juvenile during a reexposure performed 120 min later. Both drugs were ineffective if adult males were reexposed to a novel juvenile. The results suggest that both MIF-I and Alaptide improved an animal's capacity to store information received through olfactory cues .

Key words:
Social memory – Age differences – MIF-I – Alaptide – Male rat

Introduction

Most research on odour communication has been done using rodents. A behavioural preference for oestrous over dioestrous female odour of gonadally-intact and sexually-experienced male rats is well known (Carr *et al.* 1965, Lydell and Doty 1972, Stern 1970). However, rodents are able to distinguish between each other. According to Bronson (1971) individual recognition in rats and mice may be contingent upon a remarkable sensitivity to the melange of odours that apparently defines each individual. Sexually experienced male rats can easily discriminate between the odour of two different cagemates (Carr *et al.* 1976).

Thor and Holloway (1982) reported that mature rats confronted with a sexually immature rat display a dramatic reduction of investigatory behaviour upon

reexposure to the same juvenile when this exposure takes place 10 or 30 min after the first exposure, but not when it is delayed by an interval of two hours. Furthermore, since novel animals are investigated longer than familiar ones, the difference between social investigation times of the same stimulus, animal presented at different intervals, provides a convenient index of short-term memory for this particular individual. This memory subsequently was shown to be based mainly upon chemosensory cues (Sawyer *et al.* 1984).

More recently, Dantzer *et al.* (1987) proved that neurohypophyseal peptides may have a prepotent role in modulating the mnemonic processing of chemosensory information associated with social interaction: vasopressin facilitated while oxytocin disrupted social recognition of juveniles in adult male rats. Furthermore, vasopressin injected directly into the lateral septum of adult males facilitated this form of memory, whereas local injection of a specific vasopressin antagonist impaired it (Dantzer *et al.* 1988). These findings suggest that vasopressin may be involved in the physiological regulation of social recognition in male rats.

Another such peptide, hypothalamic MIF-I as well as its synthetic analogs and fragments have been shown to improve the performance of rats in several classic experimental paradigms used for evaluation of drugs influencing learning and memory (Krejčí *et al.* 1984, 1986). The question arises if and to what extent hypothalamic peptides could be involved in the social recognition mediated through chemoolfactory stimuli.

The first aim of the present study was to examine the age factor which may influence social recognition of adult male rats. While the difference in the time spent by immature and mature rats investigating the odour of strangers and cagemates was suggested but not statistically proved (Carr *et al.* 1976), differences in social investigatory behaviour were not studied during adulthood. Since this was found to be the case, the second objective, to determine whether social recognition is modulated by hypothalamic MIF-I and its derivative Alaptide (VUFB 15954) was investigated in older males.

Material and Methods

Experiment 1

Adult albino male rats (Wistar–Hannover, Konárovice Farm) 3, 7 and 11 months old (N = 24 animals per each age group) were used. They were housed in a temperature controlled room (20–22 °C) in standard plastic cages with three animals in each. The colony room was naturally illuminated. Food and water were freely available. Juvenile 24-day-old male rats were used as social partners.

The procedure was similar to that initially described by Thor and Holloway (1982). However, some modifications were introduced. Each adult male rat was tested in an unfamiliar box (80x40x35 cm) lighted with a 25 W fluorescent tube. Tests were conducted between 8.00–12.00 h. After placing a male into the box, a 3 min adaptation period followed. Each test consisted of a 5 min exposure to a juvenile rats followed by reexposure to the same juvenile 30 or 120 min later. In this experiment, a reexposure to a novel juvenile was not performed because the above mentioned authors (see Introduction) and our unpublished findings proved the same level of social investigation as during the 1st exposure. Between two successive exposures, the juveniles as well as adults were kept individually in small cages, the bottom of which was covered with pieces of a wet filter-paper.

Social investigatory behaviour defined as being oriented toward the juvenile and consisted of body sniffing, anogenital exploration, grooming and nosing was measured in adult males. Touching the flank, manipulation with the forepaws, climbing over, close pursuing and copulatory attempts were also recorded. For a detailed description of individual behavioural elements as well as their functional significance see the paper of Hliňák (1990).

One-way analysis of variance followed by the t-test with Bonferoni modification was used to express the age differences in social investigation. To express behavioural changes between the 1st and

2nd exposure Student's t-test (paired) was used for each testing condition. Statistical significance was accepted when $P < 0.05$.

Experiment 2

The retroactive enhancement procedure originally described by Dantzer *et al.* (1987) was used. The initial exposure of an adult male to a juvenile lasted 5 min. Immediately after finishing the 1st interaction adult animals received subcutaneously 1 mg/kg MIF-I or 1 mg/kg Alaptide (both drugs were dissolved in 0.9 % saline) or saline. Injection volume was 1 ml/kg. Two hours later, at a time when the same juvenile should be no longer recognized, the 2nd exposure was carried out: either the same or a novel juvenile was presented for 5 min to adult animals. The other experimental conditions were the same as described in Experiment 1.

Adult males ($N=24$) treated with MIF-I (or saline) were 7 months old, animals ($N=23$) injected with Alaptide (or saline) were 11 months old.

To express the effect of MIF-I as well as of Alaptide the ratio of social investigation times measured in the 2nd and 1st exposures was calculated for each individual. Then, the ratios obtained under MIF-I or Alaptide treatment and saline treatment were processed by the Kruskal-Wallis analysis of variance. If the overall H-value was proved to be statistically significant, comparisons among the groups were made according to Conover. Statistical significance was accepted when $P < 0.05$.

Results

Experiment 1

Age-related difference in social investigation time was found in the males exposed to the juveniles ($F_{2,69}$:16.1, $P<0.001$) for the first time. Three-month-old males had a significantly higher social investigation score than 7- and 11-month-old males. As to the 2nd exposure, the similar age-related differences in social investigation of males exposed to the same juvenile were found 30 min ($F_{2,33}=14.9$, $P<0.001$) and 120 min ($F_{2,33}=7.29$, $P=0.002$) later. A significant increase in social investigation time can be seen (Fig.1) in 3-month-old males during the 2nd exposure as compared with the 1st one performed 30 min before. However, no change was

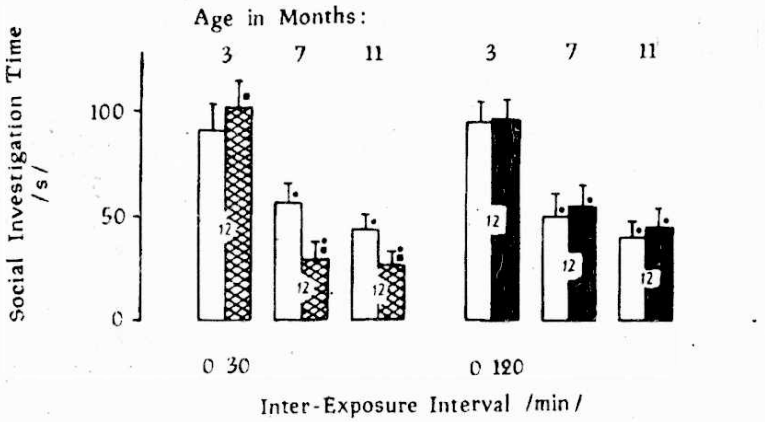

Fig. 1.
Social investigation time in adult male rats aged 3, 7, and 11 months. The same juvenile was presented twice: at time 0 (white columns) and after an inter-exposure interval of 30 min (hatched columns) or 120 min (black columns). Values are means ± S.E.M. Numerals in columns are numbers of animals. Abbreviations in detail see Methods. $P<0.05$: squares – compared to time 0. full dots – compared with 3-month-old animals.

found when 3-month-old males were reexposed to the same juvenile 120 min after the 1st exposure. Social investigation time of 7- and 11-month-old males was significantly reduced during the 2nd exposure in comparison with the time during the 1st exposure 30 min before. There were no differences in social investigation times of 3, 7, and 11-month-old males when 120 min inter-exposure interval was maintained.

Regardless of the male's age, changes in social investigation during the 1st exposure to the juveniles were evaluated. Statistical analysis (Friedman two-way analysis of variance) revealed a significant difference in the total frequency (T_2/df 4,284/=20.5, $P<0.001$) as well as in the total duration (T_2/df 4,284/=19.6, $P<0.001$) of social investigatory episodes measured in 1-min intervals. *Post hoc* analysis performed with Conover's test (significance set at 95% confidence level) showed that both the frequency of investigation and the time spent in investigation measured during the first minute (4.52 ±0.18 and 20.38 ±1.28 s, mean ± SEM) was significantly higher as compared with the frequency and the time measured later (2nd minute: 2.60 ±0.21 and 10.04 ±0.95 s; 3rd minute: 2.61 ±0.18 and 9.78 ±1.17 s; 4th minute: 2.51 ±0.19 and 11.61 ±1.21 s; 5th minute of the exposure: 2.46 ±0.18 and 12.04 ±1.23 s).

Table 1
Age-related differences in individual behavioural elements exhibited by male rats during exposure to a juvenile conspecific

Behavioural elements	Age of adult males		
	3 months	7 months	11 months
Body–sniffing	24	24	24
Anogenital exploration	24	21	16
Touching the flank	20	10	1
Climbing over	12	3	0
Manipulation with the forepaws	4	1	0
Close pursuing	24	17	18
Copulatory attempt	5	1	0

The numerals express the number of animals exhibiting the given behavioural elements.

In addition, Table 1 demonstrates the number of males exhibiting the individual behavioural elements toward the juveniles during the 1st exposure. Although the data were not statistically processed, an age-related difference can be derived. The number of males exhibiting anogenital exploration, touching the flank, climbing over, close pursuing and manipulation with the forepaws decreases with age. However, body-sniffing was observed in all males irrespective of age. A certain change in copulatory attempts is also suggested.

Experiment 2

The overall analysis revealed a significant difference in times spent by adults in social investigation for both MIF-I treatment ($H=15.1$, df 3, $P<0.001$) and Alaptide treatment ($H=10.4$, df 3, $P<0.001$). Significant reduction in social investigation times during the 2nd exposure to the same juvenile 120 min after the 1st exposure was found in males treated with both MIF-I and Alaptide as compared with corresponding saline-treated males. The same holds for comparisons with those males reexposed to a novel juvenile irrespective of the MIF-I or Alaptide and saline treatment. No differences in social investigation times were found between saline- and drug-treated animals if they were exposed to a novel juvenile for the second time.

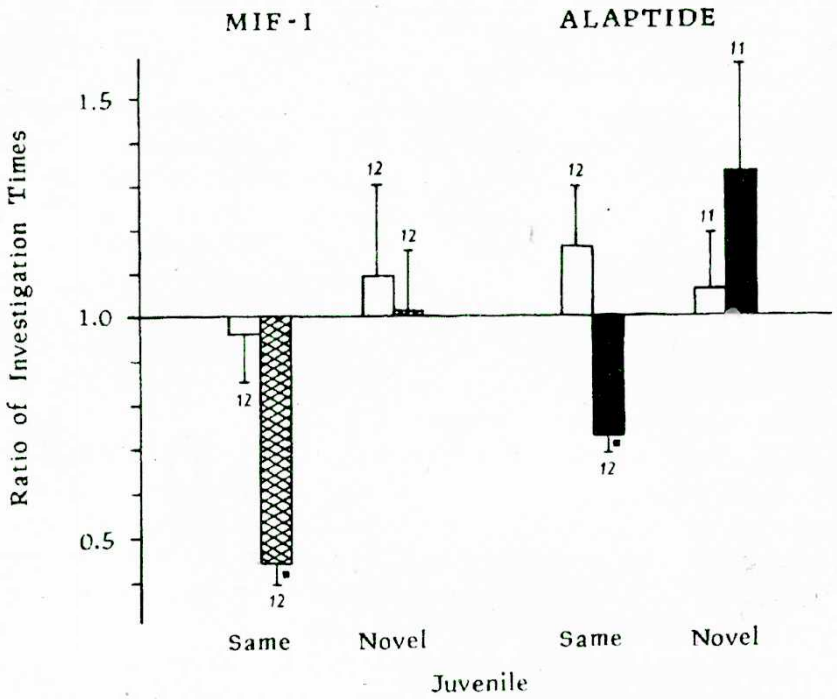

Fig. 2.
Effect of MIF-I and Alaptide on social recognition in adult male rats aged 7 and 11 months, respectively. Data are expressed as the ratio of social investigation times measured in the 2nd and the 1st exposure (inter-exposure interval being 120 min). White columns = saline-treated animals, hatched columns = MIF-I-treated animals, black columns = Alaptide-treated animals. Values are means ± SEM. Small numerals are numbers of animals. Full squares indicate significant differences ($P<0.05$) compared with the other three groups of the treatment.

Discussion

The present experiments confirm previous findings (Thor and Holloway 1982, Dantzer *et al.* 1987) that adult male rats are able to discriminate between juvenile conspecifics and to recognize the same individual through olfactory cues within a certain period of time. The more time that has passed after the first interaction, the more probable it is to eradicate what has been acquired. This is in accord with a general view on short-term memory mechanisms.

However, the age of adult males seems to be an important determinant in social recognition. Namely, a significantly longer time spent in social investigation was found in young adults as compared with times measured in older animals. Moreover, comparable, if not greater interest of young adults in familiar juvenile during the 2nd exposure compared with an exposure 30 min before has been demonstrated. Therefore, two points of interest call for a closer explanation. Firstly, why are young adults more responsive to the stimuli coming from the juveniles ? Secondly, what may be the cause of the increased social investigation of young adults during reexposure to the same juveniles?

We suggest that age-related differences in social investigation as well as the inability of young adults to recognize the same juvenile may be explained in terms of a different behavioural responsiveness determined by changes in the internal and/or hormonal readiness of animals. Evidently, the internal readiness of male rats is highest at the age of about 90 days including several subsequent weeks. This statement is supported by the findings of very high sexual-precopulatory as well as copulatory-readiness (Larsson 1956, Madlafousek and Hliňák 1983, Sachs and Meisel 1988) accompanied by maximum level of testosterone in plasma (Mock *et al.* 1978, Smith *et al.* 1977, Sodersten *et al.* 1980). At later ages, a decrease of sexual readiness appears in male rats which apparently is due to both a long-term sexual abstinence and a natural decrease of testosterone level (Chan *et al.* 1977, Mock and Frankel 1978, Lupo Di Prisco and Dessi-Fulgheri 1980). Present data show that both the high occurrence of precopulatory elements including copulatory attempts and long times of social investigation are characteristic for young adult males. To copulate, the males lacked the appropriate stimuli coming from juvenile partners. On the other hand, both behavioural parameters were lower in older males without any differences between 7- and 11-months old animals.

Therefore, we can anticipate that changes in hormonal status of adult males are accompanied by changes in sexual readiness as well as in social recognition. The higher the internal readiness of an animal, the higher is the behavioural responsiveness, and *vice versa*. In general, there are direct and relatively close relations between the internal readiness and the behavioural responsiveness in male rats. However, the relation is needed to be verified in a very old or aged animals. Moreover, present data suggest that a high internal readiness of young adults can be reinforced during the 1st exposure to a juvenile animal. Consequently, a maximum behavioural response is achieved. Then, the social recognition in young adults may be masked by high sexual interest in the same juvenile. Such an effect is apparently suppressed in older males. It can be stated that a high internal readiness of young adult males is a severe obstacle in testing the memory abilities of young adult males based on olfactory information. Therefore, older adult males are desirable to be used.

Evidence about the hormonal determination of social investigation is further supported by the results obtained in castrated males. An androgen-related dependence of social recognition has been independently proved by Bluthe *et al.* (1990) and in our laboratory (unpublished observations). Castrated males exhibited less social investigation of juvenile conspecifics than intact males. In contrast, testosterone-treated castrated males investigated juveniles for as long as intact males (Thor *et al.* 1982). Furthermore, anogenital exploration decreases after castration and increases again with testosterone replacement (Singer 1972, Thor

1980, Hliňák and Madlafousek 1984). On the other hand, body-sniffing is less dependent on testosterone levels; it was observed in males several weeks after castration when anogenital exploration was no longer exhibited (Hliňák and Madlafousek 1984). In fact, anogenital exploration together with body-sniffing are the most important components of behavioural activity in adult males directed towards a partner during any social interaction. Both behavioural elements are usually included in social as well as the sexual behavioural systems. The functional significance of both behavioural elements consists apparently in the verification of the species and sex relevancy as well as the functional state of the partner (Hliňák 1990).

In addition, it is possible that locomotor activity of the juveniles can imitate, to a certain extent, stimuli emitted by a soliciting female and therefore, can provoke a male to pursue a juvenile and to exhibit various precopulatory elements. A similar phenomenon was recorded in adult males interacting with an ovariectomized female (Dvorská *et al.* 1986). Such behavioural responses can easily be induced in males more sexually aroused. This is apparently true of young adult males.

The data of Experiment 2 yielded a clear effect of MIF-I and Alaptide regarding social recognition. MIF-I- and Alaptide-treated males had a decreased social investigation response during reexposure to the same juvenile 120 min after an initial exposure as compared to saline-treated males. In contrast to this, no evidence of this effect was obtained when MIF-I- and Alaptide-treated males were exposed for the second time to a novel, unfamiliar juvenile. Changes in social investigation under the influence of both drugs may be interpreted as enhanced olfactory recognition of the same juvenile. Moreover, there are two conclusions resulting from the experiment. Firstly, MIF-I seems to be more effective than Alaptide in enhancing olfactory recognition (comparison not made). Secondly, the effect of drugs holds for olfactory recognition in 7- and 11-month-old males. However, it is still open question if and to what extent the effects of both drugs are comparable in animals of the same age. We add that Alaptide was also effective in ovariectomized adult female rats (Hliňák and Krejčí 1990).

We can exclude that some non-specific effects of both drugs intervene in social investigation time during the 2nd exposure and therefore, in social recognition. We note that although times spent by adult males in sniffing of scent traces left on the floor of the box during the 2nd exposure were significantly reduced in comparison with the 1st exposure, no differences were found between saline and drug-treated males (data not shown). Because the sniffing behaviour decreases with reexposure of adult males to the same environment (Hliňák and Madlafousek 1980, Hliňák *et al.* 1989), it can be considered as a characteristic which corresponds very well with decreasing locomotor and exploratory activity usually used in the evaluation of habituation phenomena of animals (Groves and Thompson 1970, Lát 1973). Thus, a simultaneous measurement of times spent by adult males in social investigation and in sniffing the floor can differentiate between a specific social recognition and a non-specific habituation to an experimental environment.

MIF-I and its derivative Alaptide have been shown to influence the behaviour of laboratory animals in tests used in evaluating drug effects on learning and memory processes (Walter *et al.* 1978, Krejčí *et al.* 1980, 1984, 1986). The present data suggest that it is the influence of both drugs on processing and short-term storage of information obtained through olfactory cues which are responsible

for easier recognition of the same juvenile in the delayed test. As already stated above, vasopressin facilitated short-term olfactory memory in male rats (Dantzer *et al.* 1987, 1988). More recently, Bluthe *et al.* (1990) reported that androgen-dependent vasopressinergic mechanisms are involved in the modulation of social recognition in rats. Present data do not allow to conclude whether MIF-I and Alaptide could be involved in similar physiological mechanisms. So the neurochemical brain picture of hypothalamic MIF-I and Alaptide is far from clear and will be the subject of additional investigation.

In conclusion, the present results have made it possible to extend the olfactory recognition test to 1-year-old or older males. Furthermore, using the experimental procedure described, an effect on short-term social memory of structurally different compounds could be detected. At present, facilitative effects of cholinomimetic drugs, nootropic drugs and benzodiazepine inverse agonists on social memory were reported (Perio *et al.* 1989, Hliňák and Krejčí 1990). Nevertheless, a number of theoretical problems relative to a social recognition deserves experimental attention.

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