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Object preference and nicotine consumption in rats with high vs. low rearing activity in a novel open field

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Abstract

Our previous work has shown that normal male Wistar rats can differ systematically with respect to rearing activity in a novel open field: animals with high rearing activity (HRA rats) differed from those with low rearing activity (LRA rats) in dopaminergic and cholinergic brain activity, as well as in their behavioral responsiveness to a cholinergic antagonist, but not in measures of anxiety in the elevated plus-maze. Here, we tested (a) whether HRA vs. LRA reflects responsiveness to novelty, (b) whether such rats voluntarily consume different amounts of the cholinergic agonist nicotine and (c) whether these measures are related to those of anxiety in the plus-maze. Using a novel object test, we found that HRA showed a trend for more object exploration than LRA rats when confronted with two identical novel objects in a familiar open field. When subsequently confronted with a familiar vs. a new object, HRA rats showed substantially more exploration of the new but not of the familiar object than LRA rats. In a subsequent test, HRA vs. LRA rats did not differ in voluntary or forced consumption of oral nicotine, or water. In contrast to rearing activity in a novel open field, measures of anxiety in the plus-maze were neither related to behavior in the novel object test nor to voluntary oral consumption of nicotine, or water. Among others, these data are discussed with respect to dopaminergic and cholinergic forebrain mechanisms, which have previously been found to differ between HRA and LRA rats. Since forebrain dopamine and acetylcholine functions are critical for novelty processing, we suggest that they are also important for the differential behavioral patterns of HRA and LRA rats in the open field, and in the novel object test.

Keywords: Individuality; Novelty; Nicotine; Habituation; Acetylcholine; Dopamine

1. Introduction

An increasing body of literature has shown that rats, although identical in strain, sex and age, can differ systematically in their behavioral response to a novel environment. Here, high and low responding rats can be characterized based on their levels of exploratory activity, which is often measured in terms of horizontal locomotor activity (Dellu et al., 1996). Such individual behavioral profiles of locomotor activity were found to be related to differences in the reactivity to stressors, novelty and drugs of addiction, in particular psychostimulants (e.g., amphetamine; Bardo et al., 1996; Piazza et al., 1989, 1990).

Next to locomotion, rearing behavior can also serve as a powerful tool to differentiate systematically between rats. Thus, we previously found that rats displaying higher rearing activity (here termed HRA) in an open field differed from those with lower rearing activity (LRA) in ventral and dorsal striatal dopamine activity (Thiel et al., 1999), and in cholinergic activity in the hippocampus (Thiel et al., 1998). Further evidence for a role of acetylcholine was obtained in a psychopharmacological study (Thiel et al., 1999). There, we found that blockade of muscarinic cholinergic receptors re-induced the behavioral differences of rearing activity in HRA and LRA rats, which had been evened out before by repeated habituation to the open field (Thiel et al., 1999). Finally, we found that the individual differences in open field rearing between HRA and LRA rats are not related to anxiety profiles in the plus-maze (Schwarting et al., 1998; Thiel et al., 1999). Thus, anxiety, as a component of emotionality, which can affect rearing in the open field (Gironi Carnevale et al., 1990; von Hörsten et al., 1998), is probably not the major factor that determines the differential open field profiles of HRA and LRA rats.

Behavioral differences between HRA and LRA become especially prominent in novel testing environments (Thiel et

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al., 1998, 1999). Therefore, they may be determined by differential reactivity to, or processing of novel stimuli in such animals. This hypothesis was previously supported experimentally in rats with high vs. low locomotor activity (Dellu et al., 1996). However, the two measures, locomotor activity and rearing behavior, are not consistently correlated with each other and do not necessarily reflect the same physiological mechanisms (Gironi Carnevale et al., 1990; Thiel et al., 1998, 1999; von Hörsten et al., 1998). Thus, one part of the present study (Experiment 1) was designed to test whether our HRA and LRA rats (based on the measure of rearing) differ in a test which has repeatedly been used to analyze reactivity to novelty (Ennaceur and Delacour, 1988; Ennaceur and Meliani, 1992). In this test, we expected that HRA rats show more exploration of novel objects than LRA rats.

Additionally, and in a final part (Experiment 2) of the present study, we asked whether HRA and LRA rats may differ in their self-administration of nicotine. This experiment was determined by the following empirical background: (A) Work obtained from animals with high vs. low locomotor activity has shown that they can differ in their self-administration of drugs, especially psychostimulants (Cools and Gingras, 1998; Piazza et al., 1989). However, we do not yet know whether HRA and LRA rats also differ with respect to drug self-administration. (B) HRA and LRA rats are known to differ in dopaminergic and cholinergic brain activity and in the behavioral responsiveness to a cholinergic antagonist (Thiel et al., 1998, 1999). Therefore, one can assume that HRA and LRA rats should differently respond to drugs, which combine a strong dopaminergic and a cholinergic component. (C) Nicotine is such a drug which acts substantially via cholinergic and dopaminergic mechanisms in the brain (Balfour et al., 1998; Corrigall et al., 1992; Kameda et al., 2000; Picciotto et al., 1998; Pidoplichko et al., 1997). Furthermore, rats are known to self-administer nicotine intravenously and orally (Mosner et al., 1997), and pronounced individual differences in such self-administration rates were observed (Donny et al., 1999; Glick et al., 1996), which may be due to systematic mechanisms like those underlying the differences between HRA and LRA rats. Therefore, one might expect that HRA and LRA rats respond differently to nicotine when presented in a self-administration paradigm. Thus, our main question in Experiment 2 was to test whether rearing behavior in a novel open field is positively associated with oral consumption of the cholinergic agonist nicotine.

The study was performed in the following order: First, we screened a sample of male Wistar rats ($n = 35$) in a routine procedure used before (Schwarting et al., 1998; Thiel et al., 1999), that is, we tested them (A) twice in an open field and then (B) twice in an elevated plus-maze. None of the animals had been exposed to these tests before. Based on rearing behavior in the first open field test, we divided the animals into HRA and LRA rats. Then, we performed the test for novel object preference (Experiment 1). Finally, we tested for oral nicotine self-administration (Experiment 2). Here, we used only 24 animals; that is, those 12 rats with the highest and the lowest levels of rearing activity, respectively.

2. Experiment 1: novel object preference in HRA and LRA rats

2.1. Methods

2.1.1. Animals

Thirty-five male Wistar rats (Harlan Winkelmann, Borchen, Germany), weighing between 280 and 330 g at the beginning of the experiments, were used. They were housed in groups of five per cage (cage size: $57 \times 35 \times 24$ cm) under standard laboratory conditions and had free access to food and water. Throughout Experiment 1, HRA and LRA rats were kept in the same group to which they randomly had been assigned upon arrival in the laboratory. The colony room was maintained on a 12-h light–dark cycle (lights on: 7:00–19:00 h). Ambient temperature was 23 ± 1 °C. All animals were handled daily for 3 days (5 min each) prior to behavioral testing. All experiments were conducted in accordance with the ethical regulations for animal experimentation at the University of Marburg, Germany.

2.1.2. Open field testing

The open field consisted of a grey plastic chamber ($60 \times 60 \times 40$ cm) with a grey floor. Behavior was recorded under red light (28 lx) with a video camera suspended 150 cm above the centre of the testing device. The floor of the open field was subdivided into 16 virtual squares (15×15 cm each), and the following behavioral variables were analyzed from videotape: rearing, locomotor activity (total number of squares entered), thigmotactic scanning (number of squares entered along the walls of the open field), center moves (number of central squares entered) and latency to enter the centre. Additionally, the number of faecal boli was counted. Behavior was tested twice on 2 consecutive days (10 min each). On the first day, rats were screened for their behavioral response to the novel open field and divided into HRA and LRA rats based on the number of rearings (including on- and off-wall rearing).

2.1.3. Plus-maze testing

Four days after the second open field exposure, animals were tested in the elevated plus-maze. This maze was made of plastic and consisted of two opposite open arms (50×10 cm) and two closed arms (with 40 cm high walls). The maze was elevated 50 cm above the floor. Behavior was tested under red light and recorded with a video camera. The following behaviors were scored from videotape: the number of entries into and the time spent on open or closed arms, the latency to the first open arm entry, and the numbers of rearings and faecal boli. Finally,

risk assessment was measured as follows: the rat's body is positioned in a closed arm (at least one hindpaw still in a closed arm), but its head is poking into an open arm. Each rat was tested twice; that is, 5 min each on 2 consecutive days.

2.1.4. Novel object test

Twelve days after the last plus-maze test, the animals were exposed to a modified novel object test (see details in Ennaceur and Delacour, 1988) in the open field used before. Behavior was tested under the same general conditions as in the open field testing and was again recorded with a video camera. Each rat was submitted to one habituation session in the open field for 10 min to explore the apparatus without objects. Novel object testing was performed 24 h after this habituation session and consisted of two trials. In the first trial (T1), rats were exposed to two identical novel objects. In the second trial (T2), the rats were exposed again to two objects, one from the previous trial (familiar object) and a new object (novel object). In every trial, each object was placed in one of the back corners of the box, with the object's center point 15 cm away from both walls. We used two different objects: a red iron block ($5 \times 5 \times 8$ cm) and a solid glass pillar (6 cm in diameter, 8 cm high). Both objects had never been presented before. The kind of object presented during T1 (i.e., iron block vs. glass pillar) as well as its position during T2 (left or right) were counterbalanced and randomly permuted in HRA and LRA rats. The duration of each trial was 3 min with an intertrial interval of 15 min. During the interval between T1 and T2, the open field was not cleaned. The following behavioral measures were taken: the latency to approach the objects and the time of their exploration. Object exploration was defined as follows: directing the nose to the object within a distance of ≤ 2 cm and/or touching it with the nose.

2.1.5. Data analysis

According to Thiel et al. (1999), all 35 animals were ranked using the number of rearings in the novel open field. Those animals above the median were assigned to the HRA group and those below the median to the LRA group. The remaining intermediate animal was assigned to the LRA group by chance. Unpaired two-tailed *t* tests were used to compare open field or plus-maze behavior between HRA and LRA rats. Within-group comparisons of open field data were performed by paired two-tailed *t* tests.

2.2. Results

Assigning the 35 animals to subgroups with high and low levels of rearings in the novel open field yielded the following behavioral profiles.

2.2.1. Open field behavior

2.2.1.1. Day 1. In the novel open field, when rearing behavior was used to assign rats to the HRA and LRA subgroups, HRA rats showed a range of 55–80 rearings (for means, see Table 1) compared to LRA rats with a range of 31–53. In contrast to rearing, HRA showed only a trend for higher levels of locomotion than LRA rats (Table 1; P=.091), and no substantial indications for differences in thigmotactic scanning and centre moves (Table 1). Furthermore, the latencies to enter the centre (HRA: 51.6 ± 8.2 s; LRA: 64.7 ± 18.4 s) and the number of faecal boli (HRA: 3.8 ± 0.8; LRA: 3.8 ± 0.6) did not differ significantly between HRA and LRA rats ($P > .05$).

During this 10-min period in the novel open field, changes of behavioral activity, indicating within-session habituation (0–5 vs. 6–10 min), were observed in the measures of rearing, locomotor activity, thigmotactic scanning and centre moves in both, HRA and LRA animals (Table 1; $P < .01$).

Table 1
Open field behavior: Intra- and between-session habituation

	Rearings (frequency)		Locomotion (no. of squares)		Thigmotaxis (no. of squares)		Centre moves (no. of squares)	
	HRA (n=17)	LRA (n=18)	HRA (n=17)	LRA (n=18)	HRA (n=17)	LRA (n=18)	HRA (n=17)	LRA (n=18)
Day 1								
0–5 min	41.9 ± 1.2	27.9 ± 1.5	104.7 ± 4.1	87.1 ± 5.9	91.5 ± 3.7	77.4 ± 4.9	13.2 ± 1.5	9.7 ± 1.3
6–10 min	23.3 ± 1.6**	15.6 ± 1.0**	47.1 ± 3.6**	46.6 ± 2.9**	40.6 ± 3.2*	40.7 ± 2.8*	6.5 ± 1.1*	5.8 ± 0.7*
Total	65.2 ± 1.8‡	43.5 ± 1.9	151.8 ± 6.7	133.7 ± 7.8	132.1 ± 6.1	118.1 ± 6.7	19.6 ± 1.9	15.6 ± 1.6
Day 2								
0–5 min	32.6 ± 1.8	20.5 ± 2.4	100.5 ± 4.7	75.8 ± 6.5	86.2 ± 4.6	68.7 ± 5.7	14.3 ± 1.5	7.1 ± 1.1
6–10 min	15.1 ± 1.6**	10.3 ± 1.4**	44.6 ± 4.6**	40.1 ± 4.5**	37.9 ± 4.2*	37.4 ± 4.3*	6.7 ± 0.9**	2.7 ± 0.5**
Total	47.6 ± 2.8†	30.8 ± 3.4	145.1 ± 8.2†	115.8 ± 9.9	124.1 ± 8.0	106.1 ± 9.0	21.0 ± 1.9‡	9.8 ± 1.3

Data reflect means ± S.E.M.

* Intra-session habituation is indicated by $P < .01$.

** Intra-session habituation is indicated by $P < .001$.

† Differences between HRA and LRA rats are indicated by $P < .05$.

‡ Differences between HRA and LRA rats are indicated by $P < .001$.

Table 2
Plus-maze behavior

Days	Open arms				Closed arms			
	Absolute time (s)		No. of entries		Absolute time (s)		No. of entries	
	1	2	1	2	1	2	1	2
HRA ($n=17$)	119.3±12.0	83.4±13.2	6.6±0.6	5.8±0.7	141.7±11.9	182.7±14.4	8.5±0.6	8.8±0.6
LRA ($n=18$)	128.7±11.7	75.5±16.0	7.0±0.6	4.9±0.9	132.2±13.4	195.1±16.4	7.4±0.6	7.5±0.7

Days	Rearings		Risk assessment	
	1	2	1	2
HRA ($n=17$)	18.2±1.3	19.5±1.4	24.7±3.7	28.8±4.5
LRA ($n=18$)	14.2±1.3 *	15.9±1.2	18.0±2.6	20.3±4.5

Data reflect means±S.E.M. obtained in two 5-min test sessions.
* Between-groups differences are indicated by $P<.05$.

2.2.1.2. Day 2. Similar to the novel open field, HRA rats again showed more rearings than LRA rats when reexposed to this environment on Day 2 (Table 1; $P<.001$). Furthermore, HRA animals now displayed higher levels of locomotion (Table 1; $P<.05$) and centre moves (Table 1; $P<.001$), as well as shorter latencies to enter the centre (HRA: 32.4±5.7 s; LRA: 73.8±17.1 s; $P<.05$) than LRA rats. Thigmotactic behavior (Table 1) and the number of faecal boli (HRA: 3.7±0.7; LRA: 3.6±0.6) did not differ significantly between groups ($P>.05$). Again, within-session habituation was observed in the measures of rearing, locomotion, thigmotactic scanning and centre moves in HRA and LRA animals (Table 1; $P<.01$).

When comparing behavior to that shown in the novel open field, we observed changes indicating between-session habituation. The levels of rearing were lower on Day 2 compared to Day 1 in both, HRA ($P<.001$) and LRA rats ($P<.01$). In contrast, centre moves decreased only in LRA animals from Days 1 to 2 ($P<.01$). Locomotor activity, thigmotactic scanning and faecal boli did not significantly differ between Day 1 and Day 2 ($P>.05$).

2.2.2. Plus-maze behavior

In the plus-maze (Table 2), HRA and LRA animals spent most of the time in the enclosed arms, and this effect was even larger on the second day of testing. Similar to the open field, HRA rats showed more rearing activity in the plus-maze than LRA rats (Day 1: $P<.05$; Day 2: $P=.068$). All other measures, including the number of faecal boli, latencies to first open arm entry, time spent and entry numbers in open or closed arms, and risk assessment (data not shown) did not show significant differences ($P>.05$).

Furthermore, the time in the open arms on Day 1 and rearing behavior in the novel open field were not substantially correlated ($n=35$; $r=.033$; $P>.05$; data not shown).

2.2.3. Novel object test

Since we presented as novel objects either only iron blocks or glass pillars first, we tested whether exploratory time might differ between the two kinds of objects. Therefore, we compared exploratory time during T1 when animals were either exposed to glass pillars or iron blocks. This analysis showed that rats with glass pillars tended to show more exploration than rats with iron blocks; however, there were no significant differences between glass pillar and iron block, neither when looking at all 35 animals (iron block: 26.3±2.7s vs. glass pillar: 31.0±2.0s; $P>.05$) nor when analyzing HRA (iron block: 29.3±4.2s vs. glass pillar: 33.9±2.3s; $P>.05$) or LRA rats (iron block: 24.2±3.6s vs. glass pillar: 27.4±3.2s; $P>.05$) separately. Thus, we considered these different objects as largely comparable and performed the subsequent analyses irrespective of this factor.

We observed that HRA rats showed a tendency for more object exploration than LRA rats when confronted with two novel identical objects (T1; $P=.057$; Fig. 1). In T2, when one of the now familiar and another completely new object were presented, exploratory activity towards the familiar object did not differ between HRA and LRA animals

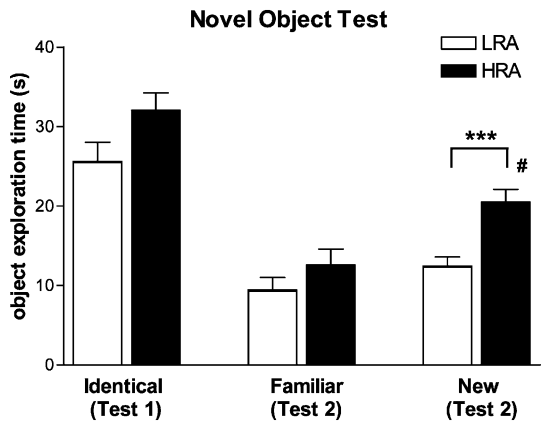

Fig. 1. Data reflect means±S.E.M. of exploration time (s) in a novel object test with two novel identical objects (Test 1), and one familiar and one novel object (Test 2). Between-groups differences are indicated between HRA and LRA rats (***$P<.001$), and within-session differences are indicated between familiar vs. new object ($^{\#}P<.05$).

($P>.05$; Fig. 1). In contrast, HRA rats showed significantly more exploratory behavior during T2 towards the novel object than LRA rats ($P<.001$; Fig. 1). Furthermore, within-group comparisons showed that HRA animals displayed significantly more exploratory behavior towards the new object than to the familiar object ($P=.015$; Fig. 1), whereas LRA animals showed only a trend for more exploration of the new vs. the familiar object ($P=.058$; Fig. 1). The latencies to approach the objects did not differ between groups, neither during T1 (first object, HRA: 9.7 ± 1.4 vs. LRA: 10.7 ± 1.7 s; second object, HRA: 27.0 ± 2.3 vs. LRA: 42.1 ± 10.2 s) nor during T2 (first object, HRA: 9.9 ± 5.0 vs. LRA: 6.8 ± 1.5 s; second object, HRA: 27.1 ± 6.9 vs. LRA: 21.6 ± 3.2 s; $P>.05$). When further differentiating into new and familiar object in T2, the object latency periods for the new (HRA: 19.2 ± 5.7 s; LRA: 12.3 ± 2.5 s) or the familiar object (HRA: 17.9 ± 7.0 s; LRA: 16.0 ± 3.5 s) showed no significant differences between HRA and LRA rats ($P>.05$).

Furthermore, exploratory behavior of the familiar or novel object was compared to open arm time in the plus-maze (Day 1); however, there were no substantial correlations ($n=35$; $r \leq .166$; $P>.05$; data not shown).

2.3. Summary

Differentiating animals into those with higher vs. lower levels of rearing activity in a novel open field showed that behavioral activity in HRA animals is not enhanced in general: For one, HRA rats showed only a trend for more overall locomotion activity. Secondly, thigmotactic scanning, which serves as a measure of exploratory behavior and which also requires locomotor activity (Thiel et al., 1999), did not differ between HRA and LRA animals. Also, centre moves, latencies to enter the centre and the number of faecal boli did not differ between the two subgroups. Habituation towards the open field on Day 2 was less expressed in HRA rats (locomotion, centre moves), since HRA animals showed significantly more activity in these measures than LRA animals, whereas thigmotactic scanning remained similar between the two subgroups. Finally, rearing behavior, locomotor activity, thigmotaxis and centre moves were indicative of within-session habituation, since they decreased significantly within each testing day in HRA and LRA rats.

The novel object test revealed further differences between the two groups. Thus, HRA rats showed a trend for more object exploration than LRA rats when confronted with two identical novel objects in a familiar open field. When subsequently confronted with a familiar vs. a new object, the HRA animals showed substantially more exploration of the new but not the familiar object, whereas LRA animals only showed a tendency for increased exploration of the new object. These results indicate that HRA and LRA rats differ in their responsiveness towards novel stimuli; however, since these differences were detectable mainly in T2, they may depend on additional factors, like habituation to the environment.

Finally, measures of anxiety in the plus-maze were neither related to rearing activity in the novel open field, nor to exploration of novel objects. This lack of relationship indicates that anxiety, as measured in the plus-maze, does not contribute substantially to the differential behavioral expressions of HRA and LRA rats, which supports and extends our previous findings (Schwarting et al., 1998; Thiel et al., 1999).

3. Experiment 2: voluntary nicotine consumption

3.1. Methods

3.1.1. Animals

In the test for voluntary nicotine consumption, only 24 of the previous 35 rats were used, since we selected only those 12 animals with the highest and those 12 animals with the lowest rearing scores, as measured before in the novel open field. The remaining 11 rats were excluded from the nicotine experiment. The 24 experimental animals were now housed individually (cage size: $42 \times 26 \times 23$ cm) under standard laboratory conditions. The selection procedure was chosen in order to maximize possible effects and due to limited capacity for individual housing in our laboratory.

3.1.2. Drugs

(−)Nicotine hydrogen tartrate (Sigma-Aldrich, Steinheim, Germany) was dissolved in water. The nicotine concentrations used were 0.06 and 0.12 mg/ml.

3.1.3. Nicotine testing

Seven days after the preceding novel object test, the animals were housed individually in order to assess the amount of individual liquid consumption. They were exposed to free or forced access to oral nicotine solutions using a three-bottle free-choice method with either tap water, 6 mg nicotine/100 ml water or 12 mg nicotine/100 ml water, and with food available ad libitum. Initially, the animals were given only tap water for 3 days (baseline). Then, they had access to a 6 mg nicotine solution, a 12 mg nicotine solution and tap water for 6 consecutive days (voluntary 1). Thereafter, a period of forced nicotine consumption was performed where only 6 mg nicotine solutions were available for 2 days (forced). The final condition was identical with voluntary 1, except that it lasted for 4 days (voluntary 2). The order of the bottles was identical for all animals and was alternated daily from left to right. When one bottle of any given content needed a refill, the contents of all bottles (nicotine and water) were completely exchanged. All bottles were weighed daily, and the difference between the previous and the present day was taken as the index of fluid consumption (measured in gram). Every bottle was checked carefully for possible spillage.

3.1.4. Data analysis

Data were analyzed by two-way ANOVA with repeated measures using group (two levels) and days of application (two, three, four or six levels, respectively) as factors.

3.2. Results

During baseline when the animals received water only (data not shown in detail), HRA (33.0 ± 1.3 ml) and LRA (33.9 ± 1.2 ml) rats drank comparable amounts of liquid.

When subsequently confronted with the choice of water or nicotine solutions (Day 1), the animals drank from all bottles; however, they preferred water from nicotine/water (Fig. 2). On the subsequent 2 days, nicotine consumption dropped to < 5 ml/day (Fig. 2a,b), whereas water consumption increased to about 25–30 ml/day ($P < .05$; Fig. 2c). Water consumption remained rather stable on Days 2–6 of phase voluntary 1, whereas consumption of fluid form nicotine-containing bottles increased on Day 4, when the bottles were refilled with fresh solutions and again dropped thereafter (Fig. 2a–c). Although there was a moderate trend for more water consumption in LRA rats, there were no statistical differences between HRA and LRA rats. HRA rats drank more of the 6 mg than the 12 mg nicotine solution ($P < .05$), and LRA rats showed a trend for a similar effect ($P = .077$).

In the subsequent forced condition (nicotine 6 mg only), the animals consumed comparable amounts of fluid as during the preceding voluntary 1 phase (i.e., around 35 ml). There were no substantial differences between HRA and LRA rats (Fig. 2a).

In the final phase (voluntary 2), the animals again had the choice between water or nicotine. Similar to voluntary 1, they clearly preferred water from nicotine, with no differences of fluid consumption between groups ($P > .05$). The intake of water during voluntary 2 was rather variable, that is, highest on the day subsequent to forced nicotine (Day 11), and on Day 13, when fresh solutions were presented again. HRA rats drank more of the 6 mg than the 12 mg nicotine solution ($P < .05$), and LRA rats had a trend for a similar effect ($P = .062$). During this final phase, total liquid consumption was higher in both groups (42.5 ± 1.3 ml) than during the preceding phases where consumption was rather stable (voluntary 1: 33.4 ± 0.9 ml; forced: 34.9 ± 0.8 ml; Fig. 2a–c).

Furthermore, it was observed that some animals drank unusually high amounts of nicotine solution, that is, the amount consumed exceeded the overall mean by more than two standard deviations. These two extreme rats had a five-fold higher consumption of nicotine (6 mg: 20.2 ± 2.1 vs. 4.3 ± 0.4 ml), and more than twice the amount of 12 mg nicotine intake (8.2 ± 0.9) than the other animals (3.0 ± 0.1 ml). Again, no substantial relation to open field rearing was observed, since one of the two animals belonged to the HRA and the other to the LRA group.

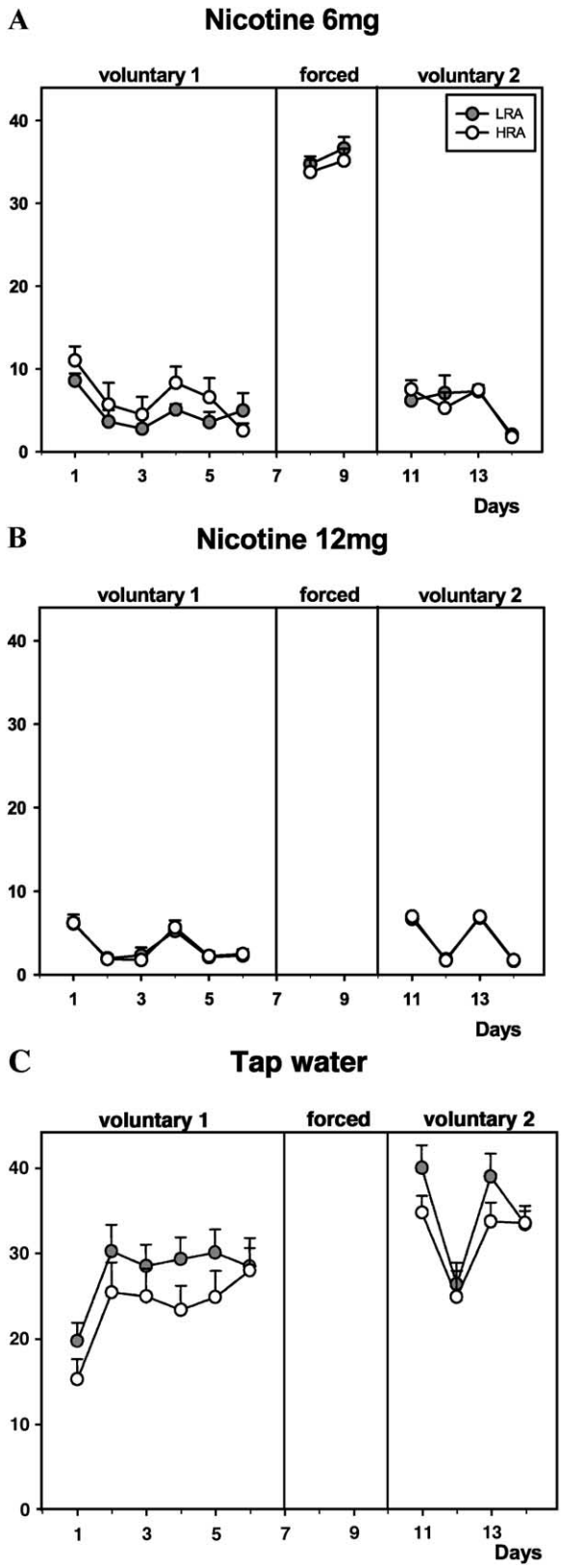

Fig. 2. Data reflect means ± S.E.M. of daily liquid consumption in HRA ($n = 12$) and LRA ($n = 12$) rats for (A) 6 mg/100 ml, (B) 12 mg/100 ml nicotine solutions and (C) tap water, respectively. Three consecutive test phases were performed: voluntary 1, forced and voluntary 2. Note: Only 6 mg nicotine solutions were available in the forced condition.

Apart from the HRA and LRA differentiation based on rearing in the open field, water or nicotine consumption was also analyzed in relation to other open field measures (locomotor activity, centre moves, centre latency) or behaviors in the plus-maze (rearing, arm entries, arm times, risk assessment, open arm latency). However, none of these analyses yielded evidence for substantial associations ($P>.05$; data not shown).

3.3. Summary

This experiment provided no evidence that HRA or LRA rats differ in their oral intake of nicotine, or water. Compared to water, most of the animals drank rather small amounts of nicotine, especially of the solution with the higher concentration of nicotine. Nicotine consumption was highest when the animals were confronted with nicotine for the first time, and dropped rapidly thereafter, indicating that the animals largely avoided the nicotine solutions, possible because of their bitter taste and/or because of their consequences. A 2-day period of forced nicotine consumption, which led to considerable nicotine intake, was not effective to increase subsequent voluntary intake of nicotine, but was followed by an overall increase of liquid intake. Two animals showed extremely high levels of nicotine intake; again, however, there was no relationship to the HRA and LRA differentiation.

3.4. General discussion

The present study consisted of several parts, including initial screening of rats in open field and plus-maze, and then testing (A) their reactivity to novel objects and (B) their consumption of the psychostimulant nicotine.

The open field analysis was used to differentiate rats into those with high (HRA) vs. low levels (LRA) of rearing. Apart from rearing, these HRA and LRA rats did not differ substantially in plus-maze behavior, which refutes anxiety to be important for the differential rearing responses in HRA and LRA rats. Employing a novel object test, HRA animals showed a trend to more object exploration compared to LRA animals when confronted with two identical novel objects in a familiar open field. When subsequently confronted with a familiar vs. a new object, the HRA rats showed significantly more exploration of the new object. In the subsequent nicotine test, HRA vs. LRA rats did not differ in voluntary or forced consumption of oral nicotine, or water.

3.4.1. Open field behavior

Based on our previous data, we employed rearing behavior in a novel open field, in contrast to locomotion, to differentiate between high and low responding rats. This behavioral measure was used, since we had previously found that cholinergic activity in the hippocampus (Thiel et al., 1998), and dopamine in the dorsal and ventral striatum (Thiel et al. 1999) were associated with rearing behavior in a novel open field.

In the present experiment, we observed that animals with higher rearing activity in a novel open field also showed a trend for more locomotor activity in this test. However, behavioral activity in HRA animals was not enhanced in general, since thigmotactic scanning, a measure of exploratory behavior, which also requires locomotor activity, did not differ between HRA and LRA animals. Furthermore, no significant differences were observed in other open field measures, that is centre moves, centre latency period and the number of faecal boli. These results are in accordance with our previous data (Thiel et al., 1999) showing that the differences between HRA and LRA rats in the novel open field were specific to rearing behavior, and to a much lesser extent to locomotor activity. Thus, it can be concluded that rearing behavior is partly different from locomotion which has also been used previously to differentiate between high and low responding rats, and to test their reactivity to voluntary drug consumption (Piazza et al., 1989) or novelty (Hooks and Kalivas, 1995).

When reexposing the animals to the open field, rearing activity, but also locomotion, and centre moves were higher in HRA as compared to LRA rats. Such differences in interindividual behavioral activity with repeated testing are in accordance with previous studies (Thiel et al., 1998, 1999; Zimmermann et al., 2001), indicating that the behavioral differences between HRA and LRA animals depend on the novelty of the open field, with rearing behavior declining between-sessions in both subgroups.

In addition, the analysis of within-session behavior (0–5 vs. 6–10 min of a given testing period) indicated habituation in both groups of animals. We observed a consistent behavioral pattern, with rearing behavior, locomotor activity, thigmotactic scanning and centre moves declining in HRA and LRA rats within each testing day, as has been shown before (Thiel et al., 1999).

3.4.2. Plus-maze behavior

The open field procedure used here can be considered aversive, anxiogenic or stressful, since it is a procedure with forced exposure (Bardo et al., 1996; Hennessy et al., 1979; Thiel and Schwarting, 2001; Welker, 1957). Therefore, open field differences between HRA and LRA rats might reflect differences in anxiety or emotionality. However, the indices of anxiety in the plus-maze, like open-arm time, or risk assessment, were not related to behavior in the open field, whereas rearing in the open field and in the plus maze were related. These analyses bear substantial evidence for a dissociation between anxiety and open field rearing, since we repeated the same results as reported previously (Thiel et al., 1999). Importantly, the two similar patterns of data were obtained in different labs and with different experimenters, which underlines their consistency. Furthermore, behavior in the novel object test, or consumption of nicotine, or water were also not related to plus maze behavior, indicating that

interindividual differences in these two latter tests are not due to different anxiety levels.

3.4.3. Novel object test behavior

HRA rats showed a trend for more exploration than LRA rats when confronted with two identical novel objects. Most importantly, when subsequently confronted with a familiar vs. a new object, the HRA animals again showed more exploration of the new, but not of the familiar object, whereas LRA animals only showed a tendency for enhanced exploration of the novel object. Additionally, we observed more exploratory behavior towards the novel object in HRA compared to LRA rats. Thus, this novel object test shows that HRA and LRA rats can differ behaviorally in response to novel stimuli, which supports the assumption that their rearing differences in the open field reflect differential processing of novelty (but see also Aspide et al., 1998).

The behavioral similarities between the novel open field and the novel object test may depend on dopaminergic and cholinergic mechanisms in which HRA and LRA rats have been shown to differ (Thiel et al., 1998, 1999). Importantly, dopamine activity has been found to increase in response to novelty in areas such as the medial prefrontal cortex (Feenstra and Botterblom, 1996) and nucleus accumbens (Hooks et al., 1992; Hooks and Kalivas, 1995; Piazza et al., 1991; Rebec et al., 1997; Rougé-Pont et al., 1993). Next to dopamine, cholinergic differences were found between HRA and LRA rats, with a higher hippocampal reactivity of extracellular acetylcholine in HRA rats (Thiel et al., 1998). Since dopaminergic and cholinergic forebrain mechanisms are known to be related to novelty processing, we suggest that these two mechanisms may be important for the differential behavioral patterns of HRA and LRA rats in the novel open field and in the novel object test.

3.4.4. Nicotine consumption

Our test of oral nicotine consumption yielded similar intake patterns (amount of total oral voluntary intake per day) as in previous work where another strain of rats was used (Kameda et al., 2000; Maehler et al., 2000). However, the present experiment did not yield significant evidence, that HRA rats differ from LRA animals in a test of voluntary, or forced consumption of oral nicotine, or water. Both subgroups preferred water and avoided nicotine solutions. One explanation for the lack of effect could be that oral nicotine administration is not suitable, since the bitter taste may have prevented the animals from enhanced consumption. However, evidence against such a gustatory explanation comes from another study, which showed that oral self-administration of sucrose/nicotine did not differ from sucrose alone (Smith and Roberts, 1995).

In addition, we forced the animals to experience the possibly reinforcing effects of nicotine by exposing them to nicotine only, followed by another period of voluntary nicotine intake. However, forced exposure to nicotine did not increase subsequent consumption of this substance in both HRA and LRA rats, which is in agreement with previous findings showing that voluntary oral nicotine consumption could not be enhanced by a transient period of forced nicotine exposure (Kameda et al., 2000; Maehler et al., 2000).

Studies with other psychostimulants like amphetamine, or cocaine have revealed different reactivities of high and low locomotor responders to these drugs. The present lack of increased oral nicotine intake in our HRA rats may be due to one, or more of the following reasons. First, the present procedure of nicotine application may be not effective. Next, most of the previous drug studies employed locomotor activity, in contrast to rearing behavior, to differentiate animals behaviorally (Cools and Gingras, 1998). However, we also analyzed our nicotine data dependent on open field locomotion but found no evidence for relationships to nicotine consumption, which argues against the role of the screening variables, rearing vs. locomotion. Finally, self-administration of nicotine may not differ between HRA and LRA rats, even when using other routes of administration (Todte et al., 2001). Still, HRA and LRA rats may differ in behavioral measures, which are known to be affected by nicotine, like place-preference (Fudala and Iwamoto, 1986), acute psychomotor effects (Bevins and Besheer, 2001) or sensitization with repeated administrations (Miller et al., 2001), and these aspects have to be tested in the near future.

In conclusion, the present findings complement and extend the existing knowledge on differential behavioral responses in HRA and LRA rats. Animals with higher rearing activity also showed increased exploration of novel objects in a familiar open field compared to animals with lower rearing behavior. However, HRA and LRA rats did not differ in test of voluntary, or forced consumption of oral nicotine, or water. We suggest that dopaminergic and cholinergic forebrain mechanisms, which have previously been found to differ between HRA and LRA rats may be associated with their distinguishable behavioral patterns in the novel open field and in the novel object test.

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