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Post-trial treatment with the nicotinic agonist metanicotine: Differential effects in Wistar rats with high versus low rearing activity

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Abstract

Laboratory rats, although identical in strain, sex, age and housing conditions, can differ considerably in behavior and physiology. When screened in an open-field, for example, Wistar rats can be assigned to subgroups, based on the measure of rearing activity (high, low rearing activity; HRA/LRA). Such rats have previously been found to differ in dopaminergic and cholinergic brain mechanisms, reactivity to cholinergic drugs, and in tests of learning and memory. Here, we asked whether HRA and LRA rats might respond differently to nicotinic treatment, when given during the consolidation of an aversive experience. Therefore, we tested them for performance in an inhibitory avoidance task where they received post-trial injections of either saline, or the nicotinic agonist metanicotine (RJR-2403, 0.017–1.7 mg/kg, i.p.). In support of previous findings, saline-treated LRA rats showed a trend for higher step-in latencies than HRA rats after shock experience. Furthermore, metanicotine was effective only in LRA rats: Compared to their respective saline-treated controls, the retention scores of LRA rats were decreased after post-trial treatment with the highest dose (1.7 mg/kg). Thus, the nicotinic agonist had an amnestic-like effect dependent on dose and subject-dependent factors (HRA/LRA). These findings are discussed with respect to possible drug actions on mnestic and non-mnestic mechanisms, and the importance of taking subject-dependent variability into account when analysing drug effects.

Keywords: Rearing; Locomotion; Open-field; Step-in; Post-trial; Acetylcholine; Nicotine; Metanicotine

1. Introduction

Although identical in strain, breeder, age, sex, and housing conditions, laboratory rats can differ consistently, both at the behavioural and physiological level (Cools and Gingras, 1998; Schwarting and Pawlak, 2004; Mittleman, 2005). Various standardised test procedures can be used to screen rats according to certain quantifiable behavioural criteria, and to then assign them to subgroups with either high or low expression of a given criterion. Several research groups have used measures like locomotion, or rearing to define so-called high or low responder rats (Cools and Gingras, 1998; Dellu et al., 1996; Thiel et al., 1999). With such methods, larger samples of animals are usually tested under identical conditions and ranked individually according to the measure of interest, e.g. rearing activity. Using a median split, animals above versus below the median are then assigned to subgroups, which are tested with respect to other behavioural, physiological, or pharmacological measures (Piazza et al., 1989; Hooks and Kalivas, 1994; Thiel et al., 1998, 1999). Such approaches are thought to gauge a trait in the rat, which may be comparable to the sensation seeking trait in humans (Dellu et al., 1996), and which has helped to study mechanisms of stress and addictive liability in animal models (Mittleman, 2005).

With respect to individual differences, our work has shown that rearing in a novel open-field can be used to distinguish between male Wistar rats with high or low rearing activity (HRA/LRA). The stronger behavioral

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response of HRA rats probably reflects reactivity to novelty, since such rats also show more exploratory activity in a novel object test (Pawlak and Schwarting, 2002). Furthermore, differences between HRA and LRA rats typically disappear with repeated exposure to the same environment due to stronger habituation of HRA rats (Thiel et al., 1998, 1999). Nevertheless, the HRA/LRA designations probably reflect a stable trait, since they can still be observed when such rats are again tested in other novel open-fields (Pawlak and Schwarting, in press). Furthermore, the measurability of this trait depends on the kind of testing environment, since differences between HRA and LRA rats need not become apparent when tested in an elevated plus-maze, that is, a test of anxiety-related behavior (Pawlak and Schwarting, 2002). Finally, the trait hypothesis is supported by neurochemical and pharmacological data showing that HRA and LRA rats differ in their behavioral reactivity to cholinergic drugs (Thiel et al., 1999; Pawlak and Schwarting, in press), and with respect to dopamine and acetylcholine activity in the brain (Thiel et al., 1998, 1999).

Other work has shown that high and low responder rats can also differ in tests of learning and memory, especially when spatial tests are used (Cools et al., 1993; Tuinstra et al., 2000). In addition, we recently found that retention behavior, but not baseline performance, of HRA and LRA rats differed in a step-in inhibitory avoidance task (Borta and Schwarting, in press), since HRA rats showed shorter step-in latencies after shock experience than LRA rats. Here, we asked whether HRA and LRA rats might also differ with respect to pharmacological reactivity in such an inhibitory avoidance task. Specifically, we decided to use a nicotinic agonist based on the following reasons: For one, we had previously found that HRA and LRA rats differ in cholinergic brain activity, and in their behavioral reactivity to cholinergic drugs, including nicotine (Thiel et al., 1998, 1999; Pawlak and Schwarting, in press). Secondly, nicotine and nicotinic agonists are well established tools in research of learning and memory (Levin and Simon, 1998; Attaway et al., 1999; Rezvani and Levin, 2001; Schildein et al., 2002), since acute treatment with nicotine or nicotinic agonists, especially when administered before learning, can have pro-mnestic effects in several tasks, like radial-mazes (Levin et al., 1994), or inhibitory avoidance (Decker et al., 1993). Such effects can follow an inverted U pattern (Picciotto, 1994), depending on factors like site or time point of injection. These outcomes are often attributed to actions in the brain; however, nicotine has actions on the peripheral and vegetative nervous system, which also may play a critical role (Clarke and Kumar, 1983; Stolerman, 1990). In order to minimise such peripheral effects we decided to use metanicotine (RJR-2403), an agonist with high affinity, selectivity and potency for $a4\beta2$ nAChR receptors (Bencherif et al., 1996; Papke et al., 2000; Lippiello et al., 1996), which are frequently found in the mammalian brain (Dani, 2001), including areas involved in learning and memory, like hippocampus or nucleus accumbens (Levin et al., 2002; Schildein et al., 2002). The selected dosages were geared to the study of Lippiello et al. (1996) who observed pro-mnestic effects of metanicotine on scopolamine-induced amnesia in an inhibitory avoidance task. We also used an inhibitory avoidance task, but injected the drug after one-trial shock experience (i.e. post-trial) to investigate its effects during memory consolidation rather than acquisition, and asked whether such effects might differ between HRA and LRA rats.

2. Materials and methods

2.1. Animals

Male Wistar rats (N=122; Harlan Winkelmann, Borchen, Germany) were used with body weights that ranged between 250 and 265 g at the beginning of the experiment. They were housed in groups of five in acrylic cages (cage size: 56×34×35 cm) in an animal room with a 12 h light–dark cycle (lights on 07:00–19:00 h) and with food and water provided ad libitum. Each animal was handled on 5 consecutive days (5 min/day) prior to the experiments. The experiment started 3 days after the handling period.

2.2. General procedure

Initially, the animals underwent a routine testing procedure, which consisted of an open-field test followed by a test in the plus-maze 3 days later. After a further interval of 3 days, testing in the inhibitory avoidance task was begun. All behavioral tests were started between 09:00 h and 10:00 h. First, the animals were weighed in the animal room. Then, they were placed individually in a clean cage (cage size: 39×23×25 cm) and transported to a dim observation room. Defecation during transport and behavioral testing was scored. The test equipment was thoroughly cleaned with a 0.1% acetic acid solution followed by thorough drying before each rat was tested. The behavioral parameters were analysed by an automated computer program, or by scoring from videotapes. All experimental procedures used were approved by the local institutional review committee for the use of animal subjects.

2.3. Behavioral tests

2.3.1. Novel open-field

The open-field consisted of an acrylic box (size in cm 41×41×40) monitored by an automated activity monitoring system (Tru Scan™, Photobeam Sensor-E63-22; Coulbourn Instruments; USA). Activity was measured for 10 min under conditions of red light (28 lux). The

following measures were taken: number of rearings, total locomotion (in cm), margin locomotion (locomotion outside the 27×27 cm centre area of the box), centre time, and centre entries (number of entries into this area).

2.3.2. Elevated plus-maze

The apparatus was made of plastic and consisted of two opposed open arms (arm size: 50×10 cm), two opposed enclosed arms with no roof (arm size: 50×10 cm), and an open square (10×10 cm) in the centre. The maze was elevated 50 cm above the floor. The animals were placed into the centre of the plus-maze facing one of the open arms. Each rat was tested once for 5 min under conditions of red light (28 lux in the centre). The following parameters were analysed from videotapes: latency to the 1st entry into an open arm (an entry was defined if all four paws were placed on that arm), number of entries into open or enclosed arms, time spent on open or enclosed arms, rearing on the open or enclosed arms, and "risk assessment", that is, the animals centre of gravity is within an enclosed arm, but its head or forepaws are within the centre or an open arm.

2.3.3. Inhibitory avoidance

A step-in paradigm was used to measure inhibitory avoidance behavior. The apparatus consisted of a brightly illuminated compartment made from transparent plastic (10×20×25 cm; 500–600 lux) and a dark compartment made from dark plastic (31.5×31.5×40 cm; <1 lux). The entrance into the dark compartment, which could be closed by a guillotine door, was 10 cm wide and 12 cm high. The floor of the dark compartment was made of 2 mm diameter stainless steel rods spaced 1.5 cm apart. This compartment could be electrified through a shock scrambler (521/C, Campden Instruments). The floor of the bright compartment was made of transparent plastic and was positioned 90 cm above the floor.

Each rat was tested on 5 consecutive days. On each day, the rat was placed in the illuminated part, facing away from the dark compartment. On the first day (termed baseline 1, B1), the rat could move freely into the dark compartment. If the rat entered the dark compartment with all 4 paws, the guillotine door was closed carefully. Immediately thereafter, the rat was removed from the dark compartment. On the 2nd day (termed baseline 2, B2), the rat was treated in the same way as on the preceding day but in addition it received a foot shock (0.15 mA, duration 1 s, 50 Hz) after the door had been closed. Immediately after the shock, the rat was removed from the dark compartment. The first retention test (T1) was performed 24 h thereafter and the latency to enter the dark compartment was measured. If an animal entered the dark compartment, it was removed and returned to its home cage. If the rat did not enter the dark compartment within 300 s, the test was terminated, a ceiling score of 300 s was assigned, and the animal was returned to its home cage. This test procedure was repeated on two further days (T2, T3). No shocks were applied during these three retention tests.

2.3.4. Drug treatment

Metanicotine [(*E*)-*N*-Methyl-4-(3-pyridinyl)-3buten-1amine fumarate; RJR 2403 fumarate, TOCRIS], dissolved in saline, was injected intraperitoneally in doses of 0.017, 0.17 and 1.7 mg/kg (calculated from the base). These injections were given immediately after shock experience on day 2. The group sizes were as follows: LRA: saline n=15, 0.017 mg/kg n=16, 0.17 mg/kg n=15, 1.7 mg/kg n=15; HRA: saline n=17, 0.017 mg/kg n=14, 0.17 mg/kg n=15, 1.7 mg/kg n=15.

2.4. Data analysis

Identically to previous experiments (Thiel et al., 1999; Pawlak and Schwarting, 2002), the animals were ranked based on the criterion of rearing behavior in the novel open-field. Animals above the median were assigned to the HRA groups, whereas those below the median were assigned to the LRA group. Behavioral data obtained in the open-field and plus-maze were analysed with two-tailed t-tests for unpaired data. The latency measures obtained in the inhibitory avoidance tests were analysed with ANOVAs for repeated measures using group (HRA/LRA) and trial (B1,B2 or T1, T2, T3) as factors.

3. Results

3.1. High/low responder differentiation (HRA/LRA)

Based on the number of rearing activity in the open-field test, the animals were divided into HRA versus LRA rats. HRA rats had a mean of 58.4 rearings in this 10 min test, compared to 37.1 rearings in LRA rats (Table 1). In addition, HRA rats showed more locomotor activity, when determined as total (p<.001; two-tailed t-tests), peripheral (p=.017), or centre locomotion (p=.001). Also, they showed more centre entries (p<.001), but did not differ in centre time from LRA rats (p=.988).

Table 1
Open-field activity in rats with low (LRA) or high (HRA) rearing activity

	LRA	HRA	p-values
Rearings (number)	37.07±1.05	58.41±1.65	<.001
Locomotion (total, cm)	2771.42±60.36	3217.81±43.94	<.001
Locomotion (margin, cm)	936.18±37.04	1054.95±32.33	.017
Locomotion (centre, cm)	1321.50±45.39	1544.03±45.78	.001
Centre entries (number)	88.93±2.43	102.00±2.46	<.001
Centre time (s)	304.58±10.56	304.37±9.39	.988

Given are means±S.E.M. (LRAn=61; HRA: n=61). p-values are based on two-tailed t-tests.

In the subsequent plus-maze test (Table 2), HRA did not differ from LRA rats in open arm percentage, the latency to the 1st open arm entry, numbers of arm entries, total rearing, or open arm rearing (p-values between .115 and .918; two-tailed t-tests). The mean values of rearing in the closed arms were higher in HRA than LRA rats; however, this difference was not statistically significant (p=.089).

3.2. Inhibitory avoidance

3.2.1. Baseline

During the two baseline trials (Table 3; B1, B2), the animals rapidly entered the dark compartment. These latencies decreased from the 1st to the 2nd trial ($F_{1,120}$=12.743, p<.001), but did not differ between HRA and LRA rats ($F_{1,120}$=.990, p=.322).

3.2.2. Behavior after treatment

When tested on the 3 days after shock experience, latencies to step into the previously shock-paired dark compartment were increased in all groups, irrespective of post-trial treatment or HRA/LRA assignment (Fig. 1).

3.2.3. Comparisons between HRA and LRA rats

In saline treated rats, the mean step-in latencies of HRA rats were lower than those of LRA rats; however, this difference was not significant ($F_{1,30}$=3.678, p=.065). In drug treated animals, there were no indications for differences between HRA and LRA rats (low dose: $F_{1,28}$=.164, p=.689; medium dose: $F_{1,28}$=.015, p=.904; high dose: $F_{1,28}$=.213, p=.648).

3.2.4. Comparisons between control and drug treatments

3.2.4.1. LRA rats. The step-in latencies of saline-treated LRA rats did not differ from those treated with the low ($F_{1,29}$=2.450, p=.128), or medium dose of RJR ($F_{1,28}$= 2.105, p=.158), but were higher than those treated with the high dose ($F_{1,28}$=6.0, p=.021).

3.2.4.2. HRA rats. The step-in latencies of saline-treated HRA rats did not differ from those of HRA rats treated with the low ($F_{1,29}$=.115, p=.736), medium ($F_{1,30}$=.017, p=.896), or high dose of RJR ($F_{1,30}$=.069, p=.794).

Table 2
Plus-maze activity in rats with low (LRA) or high (HRA) rearing activity

	LRA	HRA	p-values
Percentage of open arm time	44.09±1.93	45.38±2.18	.660
Open arm latency (s)	19.21±5.09	14.92±5.03	.549
Open arm entries (number)	7.16±.34	7.61±.31	.341
Closed arm entries (number)	7.75±.26	8.26±.35	.246
Open arm rearing (number)	1.83±.32	1.79±.28	.918
Closed arm rearing (number)	11.84±.48	13.26±.68	.089
Total rearing (number)	13.23±.51	14.61±.70	.115

Given are means±S.E.M. (LRA: n=61; HRA: n=61). p-values are based on two-tailed t-tests.

Table 3
Baseline step-latencies of rats with low (LRA) or high (HRA) rearing activity in the inhibitory avoidance task

	LRA	HRA
B1	9.01 (±.51)	8.18 (±.52)
B2	6.73 (±1.06)	6.01 (±.57)

Given are means±S.E.M. (LRA: n=61; HRA: n=61).

4. Discussion

Based on rearing behavior in an open-field test, we assigned male Wistar rats to HRA and LRA subgroups. These sub-groups were then tested with respect to performance in an inhibitory avoidance task, and the effects of post-trial treatments with saline or a CNS selective nicotinic agonist.

In the open-field, HRA and LRA rats did not only differ with respect to rearing activity (which was used to assign animals to these subgroups), but also in total, peripheral and centre locomotion, which is largely in line with our previous work (Thiel et al., 1999; Pawlak and Schwarting, 2002). These differences were not paralleled by differences in conventional measures of anxiety-related behavior, like centre time in the open-field, or open arm time in the plus-maze. Thus, previous findings have been supported which showed that our measure of open-field rearing is not substantially determined by mechanisms of anxiety, but rather by individual levels of psychomotor reactivity, and responsiveness to novelty (Pawlak and Schwarting, 2002). Furthermore, rearing activity of HRA and LRA rats did only weakly differ in the plus-maze (closed arms). This as well as prior evidence shows that the measurability of the HRA/LRA trait depends on the demands of testing, like the type of test environment used (Thiel et al., 1999; Pawlak and Schwarting, 2002).

In the inhibitory avoidance task, the baseline step-in latencies did not differ between HRA and LRA rats, which is in line with our previous results (Borta and Schwarting, in press). When analysing behavior after experience of shock, we found a moderate difference between saline-treated HRA and LRA rats. This pattern was similar to that of our previous work (Borta and Schwarting, in press), where non-injected HRA rats showed shorter step-in latencies than LRA rats after shock experience, especially when a higher shock intensity was used (0.5 mA). Here, we used a comparably low shock intensity (0.15 mA). This intensity was chosen, since unpublished pilot work had shown that combining a given shock intensity with a post-trial injection of saline leads to higher step-in latencies as compared to the same shock intensity without injection. In order to avoid possible ceiling effects, we therefore

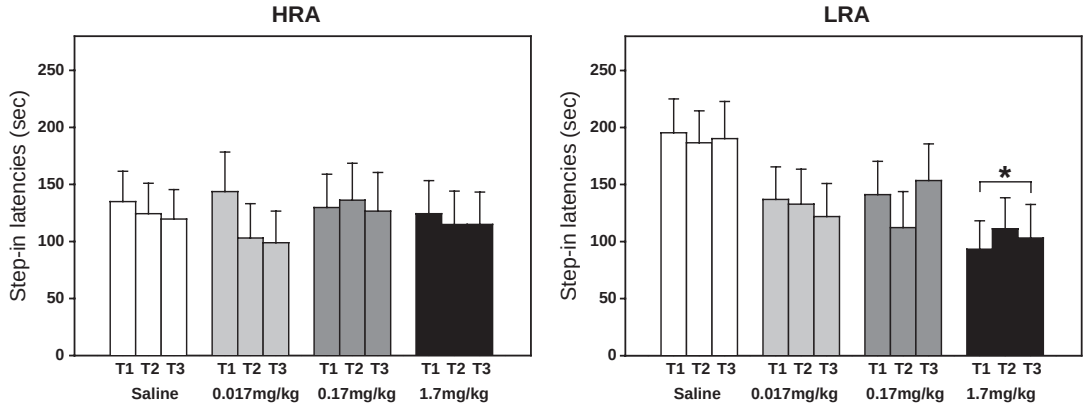

Fig. 1. Inhibitory avoidance behavior of rats classified as animals with high (HRA, left) or low rearing activity (LRA, right). Retention scores, that is, step-in latencies (in seconds; mean+S.E.M.) are given on the 1st, 2nd and 3rd day (T1–3) after post-trial treatment with saline, or a dose of metanicotine (0.017, 0.17, or 1.7 mg/kg; i.p.). The asterisk (*) denotes a difference ($p<.05$) between drug and the corresponding saline control group (ANOVA for repeated measures).

decided to lower the shock intensity, which apparently led to a shock level where differences between HRA and LRA rats become marginal.

In accordance with our previous work (Borta and Schwarting, in press), baseline performance of HRA and LRA animals did not differ. Therefore, the subsequent shock-dependent effects were probably not due to a general difference in responsiveness to the step-in apparatus as such. Secondly, tests of pain reactivity (hot-plate, tail-flick) did not yield indications that acute pain processing might differ between HRA and LRA rats (Borta and Schwarting, in press). Therefore, the differential retention behavior of HRA and LRA rats in the inhibitory avoidance task may be determined by other factors. Thus, one could assume that HRA rats generally differ from LRA rats with respect to mnestic mechanism (see also Tuinstra et al., 2000), namely that acquisition, consolidation, or retrieval may be superior in LRA rats, since they had the higher retention scores. This assumption, however, is not in line with our previous work. There we found superior habituation learning and higher experience-dependent activation of acetylcholine in the hippocampus of HRA, but not LRA rats (Thiel et al., 1998, 1999). Therefore, other, perhaps motivational mechanisms have to be taken into account, like an experience-dependent conflict between avoiding the brightly lit start compartment versus approaching the shock-associated dark compartment. Here, LRA rats seem to be more likely to persist in avoiding the aversive dark compartment, whereas HRA rats are more likely to re-approach it. The behavior of HRA rats is often interpreted in terms of novelty-seeking and sensation-seeking (Bardo et al., 1996), which might include not only novel and appetitive, but also aversive stimuli (see also Dellu et al., 1996).

Differences between HRA and LRA rats also determined the outcome of post-trial treatment with the nicotinic agonist metanicotine, which was effective only in LRA rats, where decreased retention latencies were found with the high dose. Furthermore, these effects were only observable when comparing LRA rats to their respective saline controls, but not when comparing between HRA and LRA rats.

Decreased step-in latencies after post-trial treatments are usually interpreted in terms of amnesia (e.g. Schwarting, 2003, but see Carey, 1987). Accordingly, one can conclude that the high dose of metanicotine, administered after learning (post-trial), led to a dose-dependent amnestic effect. Importantly, this effect was observable only in the LRA sub-population, that is, it was apparently determined by subject-dependent factors. The amnesia-like outcome of the agonist may appear surprising since most previous studies with nicotine or other nicotinic agonists usually yielded pro-mnestic effects (e.g. Rezvani and Levin, 2001; Schildein et al., 2002). Also, metanicotine in similar doses as used here had yielded beneficial effects on learning and memory. However, prior work performed with metanicotine differs from the present in several important aspects, including type of learning task, pre-trial drug treatment, or analysis of animals with endogenous or experimentally induced deficits (Lippiello et al., 1996; Levin and Christopher, 2002; Ueno et al., 2002). Only one study published so far also reported an impairment with metanicotine, and there a repeated dosing regimen (1.4 mg/kg s.c.) retarded learning in a water maze task in rats (Abdulla et al., 1996). Interestingly, this dose is similar to the one, which was also effective in the present work (1.7 mg/kg, i.p.).

Our approach differs considerably from such previous work, since we used post- rather than pre-trial drug administration. This approach was chosen, since we wanted to test drug effects during the phase of consolidation, and not during the phase of acquisition. It is unlikely that metanicotine acted via a proactive way on performance during the retention test, since the available physiological data do not point at long-lasting effects of this drug (Bencherif et al., 1996; Lippiello et al., 1996; Summers et al., 1996). Also, the anti-nociceptive effects of metanicotine (Damaj et al., 1999) may not account for the

present effects, since the drug was administered after the aversive experience.

Therefore, one can assume that metanicotine affected processes relevant for consolidation. Given that the present doses enhanced cholinergic function in the brain in a physiological way (Levin and Christopher, 2002; Summers et al., 1996), and consistent with the well-grounded hypothesis that cholinergic brain function improves cognitive processes (Rezvani and Levin, 2001), one might expect metanicotine to promote rather than impair processes of memory. However, promotion of certain cognitive processes does not necessarily result in promotion of memory. For example, acetylcholine and its nicotinic receptors are critically involved in attentional functions (Rezvani and Levin, 2001; Sarter et al., 2003). Therefore, one might assume that metanicotine promoted attention after the learning task, and that such attention focused cognitive capacities on post-shock stimuli, and not on the previous shock experience and its cognitive consequences, thereby weakening consolidation of the aversive event. This hypothesis seems not to be supported by previous work with nicotine, which usually promoted memory when given post-trial (e.g. Faiman et al., 1991; Schildein et al., 2002); however, data with nicotine can perhaps not be generalised to metanicotine, since metanicotine is highly selective for central $a4\beta2$ receptors, whereas nicotine stimulates various types of nicotine receptors peripherally and centrally.

Interestingly, there is evidence that cholinergic agonists can have amnestic effects when given post-trial, at least in case of intraseptal infusion and a radial maze task (Bunce et al., 2004). This effect was attributed to the enhancement of hippocampal theta, which was suggested to be pro-mnestic in the phase of information acquisition, but amnestic in the subsequent phase of consolidation. It is possibly that the present higher dose of metanicotine also enhanced hippocampal theta, and that such a mechanism could impair memory in the inhibitory avoidance task. This interpretation, however, remains purely speculative, especially since previous evidence was based on other tests, and on unselective cholinergic or muscarinic agonists (Bunce et al., 2004). Therefore, it may not hold for nicotinic mechanisms and the test used here. It is known, however, that the hippocampus is critical for acquisition and retrieval of inhibitory avoidance (Izquierdo and Medina, 1997). Furthermore, it is rich in nicotinic receptors (Role and Berg, 1996; Wonnacott, 1997), including $a4\beta2$ receptors for which metanicotine is selective, and nicotinic receptors are furthermore known to modulate hippocampal theta (Cobb et al., 1999) and to affect learning and memory there (Levin and Simon, 1998; Levin et al., 2002). Interestingly, knockout mice lacking nicotinic receptors with $\beta2$ subunit showed enhanced inhibitory avoidance behavior (Picciotto et al., 2002), which points at a critical role of this receptor in the current context.

Importantly, the present amnestic effects were obtained in a sub-population of rats, namely the so-called LRA rats. It has repeatedly been discussed before, that the outcome of nicotinic manipulations can be rather variable, and may depend on situational and subject-dependent factors (Picciotto, 1994). LRA and HRA rats were found to differ not only behaviorally, but also with respect to catecholaminergic and cholinergic activity in the brain (Cools and Gingras, 1998; Dellu et al., 1996; Thiel et al., 1998, 1999; Pawlak and Schwarting, 2002; Tuinstra et al., 2000; Feenstra et al., 1995). Thus, it can be assumed that the effects of post-trial metanicotine, which has pronounced effects on such transmitters (Summers et al., 1996), were due to neurochemical differences between HRA and LRA. Previously, we had found evidence for stronger psychomotor activation to cholinergic drugs like scopolamine and nicotine in HRA rats (Thiel et al., 1999; Pawlak and Schwarting, in press); we therefore expected them to respond more strongly than LRA rats to metanicotine. In contrast, we found that LRA, but not HRA rats showed an effect, indicating that previous psychomotor findings, which are usually attributed to dopaminergic function in the brain (e.g. Bardo et al., 1996), cannot simply be transferred to mnestic mechanisms and a drug which is selective for specific nicotinic receptors. In this context, it is important to note that a wealth of experimental evidence exists which shows that cholinergic agonist work best in tests of learning and memory when using animals with impaired or suboptimal cholinergic function (Levin and Simon, 1998). Our LRA rats may be similar to such rats, since their cholinergic activity in the hippocampus is lower than that of HRA rats (Thiel et al., 1998). Still, one might expect memory enhancement rather than impairment. This putative inconsistency might be explained by the fact, that only the highest dose of metanicotine was effective. Thus, lower doses of our nicotinic agonist may actually be ineffective in our paradigm, whereas increasing doses may act via a mechanism specific to LRA rats. It is known, that with increasing stimulation of nicotinic receptors, receptor desensitisation, and thus impaired cholinergic function becomes likely (Dani, 2001). LRA rats may be more vulnerable to such a dose-dependent effect, which led to impaired retention performance there. Again, this hypothesis is speculative and remains to be tested in future studies. Among others, one should test whether HRA and LRA rats differ physiologically with respect to specific nicotinic receptors, including density, affinity, or linkage to their neuronal targets in the brain. Furthermore, these rats should be tested in other tests of learning and memory, and in their responsiveness to post-trial administration of other cholinergic agonists (e.g. nicotine).

In sum, our findings add to previous evidence, which has shown that HRA and LRA rats, as defined in normal animals, which were not specifically bred for a certain

behavioral criterion, can differ substantially in behavior, physiology, and pharmacological reactivity. Previous work with HRA and LRA rats focused on drug effects on psychomotor activity, motivated behavior, and pre-trial treatments on learning, whereas the present work broadens the experimental evidence with respect to post-trial treatments and the phase of memory consolidation. Taking such inter-individual factors into account may help to explain the variability of drug effects, and might serve as one of several criteria, which could allow predicting the outcome of pharmacological treatments.

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