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DIFFERENTIAL NOVELTY DETECTION IN RATS SELECTIVELY BRED FOR NOVELTY-SEEKING BEHAVIOR

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Abstract

“Novelty-seeking” behavior describes the variability of rats’ locomotor response, namely High and Low Responders (HR and LR respectively), when exposed to a novel environment. Novelty-seeking in the rat is considered to model “sensation-seeking” in humans, a personality trait related to substance abuse. It is assumed that HR rats and LR rats differ in their emotional reactivity because of the disparate incentive value of contextual stimulus, thus differentially interacting with their environment. However, little is known about how HR and LR rats recognize novelty arising from the environment. The present study evaluates whether phenotype may affect spontaneous, non-spatial novelty discrimination. Selectively-bred HR and LR rats were submitted to the novel-object recognition test. The task involved a delay of three hours after a first encounter with an object (“old”), which had to be discriminated from a second object (“new”). Object discrimination was assessed minute-by-minute during a 3-min choice session. Amnesic effects of scopolamine (0.5 mg/Kg, intraperitoneal) were also analyzed. HR-bred rats showed sustained novel-object recognition throughout the 3-min choice session, whereas LR-bred rats began to discriminate between objects only in the last minute. Surprisingly, level of discrimination in scopolamine-treated HR-bred rats was significant during the first minute of the choice test and diminished thereafter, presumably because both objects became equally familiar as they were explored. Additionally, scopolamine induced changes in muscarine M₂ receptor gene expression in a phenotype-dependent manner. Because consistent object discrimination mainly arises during the first minute, these findings may reflect differential novelty detection in HR-bred respect to LR-bred rats.

Keywords

Muscarinic M₂ receptor; novelty-seeking behavior; scopolamine; novel-object recognition

INTRODUCTION

The term “novelty-seeking” portrays a behavior characterized by the vigor of the emotional response to environment-induced novelty. It is defined as the amount of activity displayed by an animal when exposed to a novel, inescapable environment [19]. Animals that exhibit a high level of exploration are termed high responders (HR), and those with little exploration are termed low responders (LR). These endophenotypes represent differences in

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vulnerability to substance abuse, namely that HR rats self-administer psychostimulants at higher rates than LR rats [16]. Interestingly, HR and LR rats exhibit important differences in tests related to cognitive performance that may well be linked with their affective responsivity [1,2,9]. Because brain cholinergic neurotransmission is involved in novelty arousal [6,20,21], this study evaluates the effects of the muscarinic antagonist scopolamine in selectively bred HR and LR rats [19] submitted to the novel Object Recognition (OR) test [13]. After behavioral testing, *in situ* hybridization experiments examined gene expression for the acetylcholine muscarinic M₂ receptor (M₂R), given that it facilitates cognitive processes and memory [18]. Unlike prior studies [1,2], this study is testing inbred lines of HR and LR rats in OR rather than rats categorized on the basis of their activity in a novel environment. It also differs from others [5] in the delay-dependent decline in memory retention and subsequent novel-object recognition [7]. It provides evidence for a role of the cholinergic system in the pattern of novelty arousal in the HR-LR paradigm.

METHOD

Subjects

The present study used selectively-bred Sprague-Dawley HR and LR male rats of the 15th generation from our breeding colony weighing 350–400g. A description of our breeding strategy and initial behavioral characterization has been published elsewhere [19]. Rats were housed in pairs on a 12 hr light/dark cycle (lights on at 7 am), with access to food and water *ad libitum*. Rats were never tested or trained before the OR task. Behavioral testing was conducted during the light cycle. The present studies followed the *Guidelines for the Care and Use of laboratory Animals* (National Institutes of Health, 1978). All efforts were made to minimize the number of animals used and their discomfort.

Object Recognition

The OR paradigm was originally described by Ennaceur et al. [13]. The apparatus was a traditional white open field (100×100×50 cm) divided into four zones by removable boards (base dimensions 0.25-m²; height 40-cm). Black lines drawn on the floor divided each zone into four virtual 25-cm² quadrants. Light intensity at floor level was 75 lux. The objects to be discriminated were made of a neutral (e.g., odorless) material and were different shapes. They were (sample-object) a ceramic cup (6-cm base × 8-cm high), and (novel-object) a plastic bottle (4.5-cm base × 12-cm high). In-house experiments demonstrated that rats showed no preference for a certain object.

One day before testing, all rats explored the open field apparatus for 10 min without any objects. On the test day, HR-bred and LR-bred rats were exposed to the Sample session followed by a delay period (3 h) and the Choice session. In the Sample session, rats were exposed to two identical copies of the same “sample-object” arranged diagonally in opposite corners. Three hours after completion of the Sample session, rats were returned to the open field (Choice session) where a copy of the “sample” objects remained and the other one replaced by a new (i.e., novel) object. The duration of every test session was 3 min [12]. Object positions were counterbalanced between rats to avoid location bias. The apparatus was thoroughly cleaned between trials with 70% alcohol.

Rat behavior was videotaped using a videocamera mounted immediately above the open field, and monitored by hand by a trained observer masked to the experimental conditions. The time spent exploring each of the objects was used as the basic measure. “Exploration of an object” was defined as directing the nose to the object at a distance of less than 2 cm and/or touching it with the nose. Qualitative assessment of novel-object discrimination was determined by directly comparing the time spent investigating each object [2]. As suggested

by others [12], a discrimination ratio (d_r) was reckoned as the difference in time spent exploring both objects divided by the total object-exploration during each minute of the 3-min Choice session. Rearing and line crossings frequency was also monitored.

Drug administration

The non-selective muscarinic antagonist scopolamine (scopolamine hydrobromide, Tocris, Ellisville, MO) was prepared fresh (0.5 mg/ml) in physiological saline. Injections of either saline or of drug were administered (1ml/Kg) via intraperitoneal injection 30 min before the Sample session. Eight HR and eight LR animals received saline while eight HR and eight LR animals received scopolamine.

In situ hybridization histochemistry

Behaviorally tested rats were killed 48 h after the Choice session. Brains were quickly frozen and stored at -80°C . Coronal sections (10 μm) were thaw-mounted on poly-L-Lysine coated slides in 100 μm intervals. A detailed description of the in situ hybridization technique is published elsewhere [3]. The acetylcholine muscarine M_2 receptor (Chrm2 GenBank accession # MN031016) probe was a 835 bp sequence in the coding region (nucleotides 475–1310) cloned in the lab. Slides were exposed to Kodak XAR film (Eastman Kodak, Rochester, NY) eight days. The specificity of the hybridization signal was confirmed with sense probe controls for M_2R .

Image analysis of autoradiograms

Analysis of digital images relied on optical densitometry in the linear range of the gray levels using NIH Scion ImageTM (Scion Corporation, Frederick, MA). Digital images were captured from the X-ray Films using a CCD camera (TM-745; Pulnix, Sunnyvale, CA). Optical density measurements were multiplied by outlined area yielding integrated optical density as the final unit of radioactive hybridized mRNA signal. Samples collected from each region of interest for each rat were used to obtain optical density measures. Even though a total of eight rats were used per group, only brain samples located at the same distance from Bregma (a minimum of five) were used to estimate group averages.

Statistical analysis

Direct object exploration was analyzed using repeated measures ANOVA, with the interaction of Treatment (Scopolamine vs. Saline) and Phenotype (HR vs. LR) as between-subjects factors, and Object (Familiar vs. Novel) as within-subjects factor. Post hoc tests with Bonferroni correction were used to compare time spent interacting with the familiar vs. novel-object within each level of treatment and phenotype. Discrimination ratio (d_r) was analysed using a two-way factorial (Treatment $\times$ Phenotype) repeated measures ANOVA to see if discrimination varied within the 3 min of the Choice session. A series of one-sample t -tests determined whether d_r differed from zero value (no discrimination). Two-way factorial (Phenotype $\times$ Treatment) ANOVA followed by Student-Newman-Keuls test was applied to the rest of analyses. Alpha value was set at 0.05. Analyses were carried out using SAS-STAT[®].

RESULTS

OR test

Figure 1 shows the extent to which HR-bred and LR-bred rats explored copies of the sample-object 30 min after the injection of either saline or scopolamine. Neither main effects nor within-subject contrasts were significant (data not shown). Contaminant effects of experimental bias or artifacts were, then, ruled out.

Figure 2 represents object-directed exploration during the Choice session. Inter-subjects effects were not significant (data not shown). There was a significant Object (recognition) effect at one minute ($F(1,28) = 14.74, p < 0.01$), and in the 3-min interval ($F(1,28) = 6.00, p < 0.05$). An Object $\times$ Treatment interaction was significant for the first minute ($F(1,28) = 6.41, p < 0.05$) and also in the 3-min interval ($F(1,28) = 9.90, p < 0.01$). In the absence of drug, HR-bred rats, but not LR-bred rats, showed novel-object discrimination in the first minute (p -values: 0.000 and 0.202 respectively). When considered the entire Choice session, saline-treated HR-bred rats discriminated between objects ($p < 0.001$), whereas saline-treated LR-bred rats showed a non-significant trend (p -value: 0.093). Novel-object recognition in Scopolamine-treated HR-bred rats was observed in the first minute ($p < 0.05$), but was non-existent after 3-min session (p -value: 0.935).

Figure 3 displays a detailed minute-by-minute analysis of the d_r ratio. Repeated measures ANOVA showed a significant Phenotype $\times$ Bin interaction ($F(2,27) = 3.55, p < 0.05$). In Saline-treated LR-bred rats began to recognize the novel-object just in the last minute ($t(7) = 3.80; p$ -value: 0.007) whereas Scopolamine-treated HR rats discrimination changed from significant (Bin 1: $t(7) = 2.82; p$ -value: 0.026) to non-existent (Bin 3: $t(7) = 0.15; p$ -value: 0.88) over the Choice phase. Conversely, significant novel-object discrimination remained invariably across the Choice phase in Saline-treated HR rats (Bin 1: $t(7) = 8.16, p$ -value: 0.000; Bin 2: $t(7) = 5.71, p$ -value: 0.001; Bin 3: $t(7) = 5.54, p$ -value: 0.001).

The table illustrates general exploration. Rearings in HR-bred rats were higher than in LR-bred rats in the Sample and Choice phases ($F(1,28) = 14.42, p < 0.001$ and $F(1,28) = 26.46, p < 0.001$ respectively). A Phenotype effect was significant for line crossings (HR-bred rats higher than LR-bred rats) in the Sample phase ($F(1,28) = 18.17, p < 0.001$), whereas Phenotype and Treatment effects were significant for the Choice phase ($F(1,28) = 32.36, p < 0.001$ and $F(1,28) = 8.42, p < 0.01$ respectively). Scopolamine-treated HR-bred rats crossed more lines than Scopolamine-treated LR-bred ($p < 0.01$) and Saline-treated HR-bred rats ($p < 0.05$).

Treatment effects on M₂R gene expression

A Treatment effect was significant for the M₂R mRNA signal in the medial septum-diagonal band complex ($F(1,12) = 10.64, p < 0.01$; Fig. 4a). Also, a Treatment effect and Treatment $\times$ Phenotype interaction were significant in the medial orbitofrontal cortex ($F(1,20) = 8.19, p < 0.01$ and $F(1,20) = 4.34, p < 0.05$ respectively; Fig. 4b). Expression of M₂R in the medial septum-diagonal band was higher in Saline-treated HR-bred rats when compared to Saline-treated LR-bred rats ($p < 0.05$). In Scopolamine-treated LR rats, M₂R gene expression was significantly elevated relative to Saline-treated LR-bred rats ($p < 0.01$), thus removing the difference with Scopolamine-treated HR-bred rats. As to the medial orbitofrontal cortex, M₂R mRNA in Scopolamine-treated HR-bred rats was higher than Saline-treated HR-bred rats ($p < 0.01$). Significant main effects were not found in the agranular insular cortex (Fig. 4c).

DISCUSSION

The major finding of this research is that the behavioral and molecular changes induced by scopolamine may reflect differential novelty recognition in HR-bred respect to LR-bred rats due to regulation of acetylcholine.

The present study used two selectively-bred strains of Sprague-Dawley rats showing remarkably differences in novelty-seeking behavior [19] to examine the effects of scopolamine on novelty discrimination. Using the OR test, a task which is thought to involve spontaneous recognition memory, it was observed that performance was dependent

on the HR-LR phenotype. In the Choice session, saline-treated HR-bred rats consistently recognized the novel object as opposed to saline-treated LR-bred rats which did not. To this end, it is important to note that this study is not comparable to others claiming a lack of correlation between “preference” for novel objects and novelty-seeking behavior [5], since these authors conducted their experiments with no time delay. HR-bred and LR-bred rats’ OR performance accords well with the evidence that learning abilities may reflect individuals’ propensity for novelty seeking [14]. It is, however, in contradiction of previous findings demonstrating that outbred HR rats, but not outbred LR rats, show impaired novel-object recognition memory [1,2]. This suggests that, although there may exist a cognitive dimension underlying novelty-seeking behavior [1,9,11,14], this relationship is genotypically complex and confounded with other factors, notably general activity and stress.

The use of rats exhibiting extreme HR and LR phenotypes following genetic enrichment [19] may explain the above discrepancy. Considering that general activity was not equal between phenotypes, the OR differences were not the result of better spontaneous memory, but could be due to more exhaustive investigation of the exploration box. This could give HR-bred rats an advantage over LR-bred rats in encoding contextual information [9], thereby facilitating memory acquisition. Although the OR paradigm relies on memory processing for objects, novelty detection is also implicit in this ‘recognition memory’ [7]. The discrimination ratio in saline-treated HR-bred rats was very high ($d_r = 0.6$) compared to what is reported for the first minute ($d_r = 0.2$) in the rat [12]. A dose of 0.5 mg/Kg of scopolamine is enough to impair memory for previously encountered objects [6,22]. However, it did not fully removed novel-object recognition in HR-bred rats. Instead, exploratory preferences in Scopolamine-treated HR-bred rats varied over the 3-min retention test, whereas differential familiarity with objects waned as expected [12]. While it could be argued that scopolamine effects were the consequence of some level of hyperactivity because of the relatively high dose used in this study [17], the fact that rearings were not affected by scopolamine does not support this view. In addition, 0.5 mg/Kg-1 of scopolamine was not sufficient to alter vision in rats [10]. Dosage of scopolamine was based on one known to be effective in inhibiting attention [4] and learning [20]. In this regard, scopolamine and phenotype effects on OR performance may denote a disparity in novelty detection and memory resources from which LR-bred and HR-bred rats benefited.

The analysis of the M_2R gene expression was of interest because it has been recently shown to play a role in facilitating cognitive processes [18]. Phenotype-dependent differences in postsynaptic M_2R mRNA were not found in sub-regions of the prefrontal cortex, although scopolamine induced a significant enhancement of M_2R mRNA in the medial orbitofrontal cortex of HR-bred rats. The medial orbitofrontal cortex is involved in perseverative behaviors [8]. Improved cholinergic input into the medial orbitofrontal cortex could contribute to lowering novelty responsiveness in scopolamine-treated HR-bred rats. The pre-synaptic M_2R is expressed in cholinergic cells where it works as an autoreceptor [23]. In the medial septum-diagonal band complex, the major cholinergic input into the hippocampus [15], levels of presynaptic M_2R transcript in saline-treated HR rats were found to be significantly higher than that in saline-treated LR rats, whereas scopolamine curtailed this difference. These findings warrant further studies aimed at characterizing the differences in cholinergic function in HR and LR phenotypes, which could result in a better understanding of how the gathering of environmental information influences novelty-seeking behavior.

In summary, scopolamine and strain effects on OR denoted a distinctive pattern of novelty detection in HR-bred respect to LR-bred rats. Phenotypic specificity of the molecular changes induced by scopolamine may be indicative of differential cholinergic transmission in HR-bred and LR-bred rats.

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Figure 1. Effects of scopolamine (0.5 mg/Kg) on object exploration in HR-bred and LR-bred rats during the Sample session

The figure portrays exploration time (seconds) of both copies of the “sample-object” in the first minute and in the entire 3-min test session. Data are in mean $\pm$ S.E.M.; $N = 8$.

Figure 2. Effects of scopolamine (0.5 mg/Kg) on the recognition of the novel-object in HR-bred and LR-bred rats during the Choice session

The figure portrays exploration time (seconds) of both the “novel” (black) and the “sample-object” (white) in the first minute and in the entire 3-min test session. Data are in mean $\pm$ S.E.M.; * $p < 0.05$, *** $p < 0.001$, compared to the time investigating the sample-object in the same group; ++ $p < 0.01$ compared to the sample-object in Saline-treated HR-bred rats; $N = 8$.

Figure 3. Discrimination ratio (d_r) averages over the Choice session

The figure displays d_r values in 1-min bins. Data are in mean $\pm$ S.E.M.; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, mean is different from zero (i.e., novel-object discrimination); $N = 8$.

Figure 4. Effects of scopolamine (0.5 mg/Kg) on M₂R mRNA levels

Transcript levels are displayed as integrated optical density (IOD) units. Data are in mean $\pm$ S.E.M.; ** $p < 0.01$ relative to same-phenotype Saline-treated group; + $p < 0.05$ relative to Saline-treated HR group; $N = 5-8$. Abbreviations: medial septum-diagonal band complex (*mSDB*), medial orbitofrontal cortex (*MO*), and agranular insular cortex (*AI*).

Table

Effects of scopolamine (0.5 mg/Kg) on the general activity in HR-bred and LR-bred rats in the OR test.

	<i>Sample session</i>	<i>Choice session</i>
<i>Rearing frequency</i>		
Saline-HR	17.0 ± 1.2	14.1 ± 1.8
Scopolamine-HR	17.3 ± 2.9	16.5 ± 0.9
Saline-LR	10.6 ± 1.5	7.1 ± 1.6
Scopolamine-LR	7.8 ± 2.3	7.8 ± 1.6
<i>Line crossings</i>		
Saline-HR	25.6 ± 2.0	19.6 ± 2.8 ^{*++}
Scopolamine-HR	29.0 ± 3.6	29.8 ± 2.9 ^{**}
Saline-LR	17.1 ± 0.9	11.3 ± 1.0
Scopolamine-LR	17.8 ± 1.9	13.6 ± 1.2

Data are in mean ± S.E.M.;

^{*}
 $p < 0.05$,^{**}
 $p < 0.01$, compared to same-treatment LR;⁺⁺
 $p < 0.01$ compared to Scopolamine-treated HR, $N = 8$.