Individual responses to novelty are associated with differences in behavioral and neurochemical profiles

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Dedicated to the memory of C. Spyraki.

Abstract

Experimental animals can be differentiated on the basis of their horizontal or vertical activity to high responders (HR) and low responders (LR) upon exposure to a novel environment. These individual differences have been associated with behavioral and neurobiological differences in a number of experimental procedures used for studying sensitivity to psychostimulants, anxiety, depression, and cognitive function. In the present study, we differentiated the rats to HR and LR based on their vertical activity upon exposure to a novel environment. Additionally, we ascertained whether HR and LR rats differ in a battery of tests such as passive avoidance (PA), object recognition (OR), and the water-maze (WM) that provide indices for cognitive function and the forced swim test (FST), an animal model of affective responsivity and antidepressant-like activity. Potential differences in neurochemical indices between the two phenotypes were also examined. HR rats displayed impaired non-spatial object recognition memory, but enhanced spatial performance, as compared to LR rats. FST induced “depressive-like” symptoms in both phenotypes that were differently manifested in HR versus LR rats. Neurochemical findings revealed distinct differences in serotonergic and dopaminergic activity in the striatum and the prefrontal cortex of HR as compared to LR rats. The above results show that HR and LR rats exhibit important differences in a battery of tests related to cognitive performance or affective responsivity, which may be associated with differences in certain neurobiological parameters.

Keywords: Open-field activity; High responders; Low responders; Rats

Experimental animals can be differentiated on the basis of their horizontal or vertical activity to high responders (HR) and low responders (LR) upon exposure to a novel environment [5,38,54,55]. These individual differences may be associated with behavioral or neurobiological differences in experimental procedures used for assessing sensitivity or vulnerability to psychostimulants or with respective differences in the animals’ reaction to the testing conditions [1,10,19,39,41,46].

Several studies have reported behavioral and/or neurobiological differences in both phenotypes when employed to experimental tasks related to stress, anxiety, emotional reactivity, depression and cognitive function [6,23,26,40,42,53,54]. Some of these studies have shown that HR rats are more anxious or hyper-responsive in stress-related tasks as compared to LR
This notion has been challenged given that HR most likely show an increased responsiveness to stress not coupled with a respective increase in their anxiety-like profile, as compared to LR rats [26,53]. Furthermore, Kabbaj et al. [25] have reported that basal differences in gene expression of key stress-related molecules such as hypothalamic mRNA CRH levels, and hippocampal expression of glucocorticoid (GR) but not mineralocorticoid receptors (MR) may play an important role in individual differences following exposure to stress and novelty. However, there have not been any substantial differences between HR and LR rats in relation to anxiety responses using various tasks such as the plus-maze and object-burying test [23]. Interestingly, White et al. [62] have reported some differences between HR and LR rats concerning their anxiety-like reactivity, but these differences were dependent on the specific animal anxiety model used. In particular, HR rats displayed less anxiety-like behavior, as deduced by the elevated plus maze and the defensive withdrawal test than LR, but the opposite effects were observed using the acoustic startle-induced vocalization test. In addition, HR did not exhibit any major differences as compared to LR rats in the depression-like reactivity as reflected by the forced swim test [23,62].

Although adult animals show phenotypic differences related to their reaction to novelty or to their specific responses recorded in various testing conditions, the potential association of these differences with tentative differences in attention, learning or memory procedures cannot be excluded. Interestingly, Cools et al. [9] have suggested that some differences between HR and LR, in the response to a simple four-arm radial maze, are not due simply to differences in motor activity, but to subtle yet important differences in the mode of learning. Moreover, our recent results [5] have shown that a phenotypic distinction between HR and LR rats probably reflects quantitative and qualitative differentiations that characterize their respective reaction to an experimental procedure and subsequently the specific type of a needed behavioral response in the respective task. On the other hand, minor phenotypic differences were yielded between HR and LR rats in some tasks related to learning and memory function [6,38].

It seems that despite the substantial work on the study of individual differences, inconsistent findings provide limited clarification on the respective behavioral variations. Notably, distinct differences probably predict respective phenotypes in subsequent behavioral paradigms related to drug abuse, anxiety, depression, or cognition, but these differentiations are dependent on both the screening procedure and the specific model used. There are few studies that have focused on differences in various behavioral and neurobiological responses using a set of experimental tasks, following a distinct classification of the rats under study. Spontaneous open filed activity includes a variety of responses that could be interpreted as indices of exploration, arousal, locomotion, anxiety and emotionality. In particular, increased locomotion (horizontal, forward or ambulatory activity) and vertical activity (rearing) mainly characterize the rat’s behavioral response to novelty. Rearing behavior reflects most of the aforementioned aspects of spontaneous open field activity and has been used apart from locomotion as a valuable variable for the rats’ phenotypic classification [5,23,38,55]. Thus, in the present study, the rats were differentiated into HR and LR, based on the counts of vertical activity during their exposure to a novel environment. Moreover, we ascertained whether HR and LR rats differ in a battery of tests such as passive avoidance (PA), object recognition (OR), and the water-maze (WM) that provide indices for different aspects of learning and memory function. In addition, a depression-like reactivity using the behavioral responses in the forced swim test (FST) was examined in both groups of rats. Moreover, possible basal neurochemical differences between the two phenotypes regarding dopaminergic and serotonergic brain function were examined by measuring tissue levels of the monoamines and their metabolites in distinct regions of the brain.

1. Materials and methods

Male Wistar rats (inbred in the Pasteur Institute of Athens), aged 70–90 days and weighing 250–300 g, at the beginning of the experiments were used. The animals were housed in groups of six or seven in plastic cages (57 cm × 35 cm × 20 cm) with food and water available ad libitum, under controlled laboratory conditions, i.e. 12-h light/dark with lights on at 06:00 h and a constant temperature of 21 ± 1 °C. All rats were accustomed to our laboratory conditions for a 2-week period. They were gently handled twice per week and their weight was routinely checked once per week.

All animal experiments were reviewed and approved by the local committee and all studies have been carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23; revised 1996). Efforts were made in order to use a minimal number of rats necessary for experiments and reduce their suffering.

1.1. Open field behavior—response to novelty

The behavioral testing was performed between 08:00 and 16:00 h. The rats were initially accustomed to the experimental room, for 1 h prior to the experiment. The rats were then introduced into the testing cage, a transparent plastic open field cage (40 cm × 40 cm × 40 cm) and behavior was recorded for a 15-min observation period. In brief, an observer recorded the behavioral responses, using a registration program based on Spruijt and Gispen [52]. The frequency and duration of each behavioral response were recorded at the end of each 15-min observation period [2–5]. The duration of this session was chosen because a short exposure to a novel environment captures clearly the reactivity of the rats to novelty [5,55,62]. Pilot studies in our laboratory have shown that the first 15-min period of novelty is the most important period for screening the rat’s reactivity. After the 15-min period the rats become habituated, they are not moving or sniffing around while their rearing behavior is declined. Thus, the following behavioral responses were recorded: standing (std) on all four feet, essentially motionless and not sniffing; moving (mov), walking on all four feet; sniffing (snf), not moving but sniffing parts of the walls or floor of the apparatus; rearing (rr), body inclined vertically with hindpaws on the floor of the activity cage and forepaws on the wall of the cage; grooming (grm), washing the face or any other body part with the forepaws; scratching (scr), raising of hindpaw to touch any body part; sniffing air (sna), rearing (rr) in the open field area of the activity cage. Then, all rats (n = 130) were ranked using the frequency of rr and sna and the upper half (score above the median) of the animals was designated as HR (n = 65), while the lower half of animals (score below the median) was indicated as LR rats (n = 65). The summation of rr and sna responses reflect vertical activity, which has been used apart from locomotion as a reliable criterion for assignment of rats into groups during their exposure to novelty [5,55]. After designation to HR/LR groups according to the aforementioned criterion, the rats were left undisturbed (except for some gentle handling) for a 10-day period. Subsets of these rats were then employed for testing at the PA, the OR, the WM or the FST. The coders were blind to rat classification.
Another subset of rats was sacrificed and dopaminergic and serotonergic activity was assessed in various brain regions. HR and LR rats were matched and divided in each subset of rats based on the median of vertical activity as previously described. Thus, each experiment contained similar distribution of HR and LR rats.

1.2. Passive avoidance test

A subset (n = 24, HR = 12, LR = 12) of these rats was used for PA test experiments. PA was conducted as described earlier by Misane and Ögren [32,33]. A standard shuttle box (Ugo Basile, Comerio-Varese, Italy), with two communicating (7 cm × 7 cm sliding door built in the separating wall) compartments of equal size and a stainless-steel bar floor was used. The right-hand compartment (shock compartment) was painted black to obtain a dark chamber. The left-hand compartment was illuminated by a bulb (24 V; 5 W) installed on the top Plexiglas cover.

PA training was conducted in a single session (day 1) during the light phase of a 12-h day/night cycle (09:00–16:00). Each rat was placed in the light compartment with no access to the dark compartment and allowed to explore for 2 min. When 2 min expired, the sliding door was automatically opened by pressing a pedal and the rat was allowed to cross over into the dark compartment. Once the rat had entered the dark compartment, the sliding door was automatically closed and an inescapable, constant current, scrambled shock (5 s, 0.6 mA) was delivered through the grid floor. Latency to cross into the dark compartment (training latency) was recorded. If a rat failed to move into the dark compartment within 300 s (cut-off latency), the door was reopened and the rat was gently moved into the dark compartment by the experimenter, where it received footshock. Following training, the rat was immediately removed from the PA apparatus. Retention performance was examined 24 h after training (day 2). The animal was placed in the light (safe) compartment, with access to the dark compartment (within 15 s) for a period of 300 s. The latency to enter the dark compartment with all four feet (retention latency) was automatically measured. If the rat failed to enter the dark compartment within 300 s, it was removed and assigned a maximum test latency score of 300 s.

1.3. Object recognition test

A subset (n = 30, HR = 15, LR = 15) of rats was used for OR test experiments. Rats were subjected to the object recognition task, according to the protocol described by Ennaceur and Delacour [18] with minor modifications. This paradigm has been widely used as a non-spatial paradigm that reflects working memory, based on spontaneous exploratory activity [37]. The task used consists of a 2-day habituation period. The rat was placed in the arena without any objects for 2 × 3 min trials on the first and second day, with a 60 min interval between trials. On the testing day (third day), each rat was subjected to 2 × 3 min trials in the presence of two objects. During Trial 1 (T1), two identical objects were placed in the arena and the time spent by the animal for objects exploration (rat nose at a distance <2 cm from the object) was recorded. During Trial 2 (T2), following a 60 min interval, one of the objects was replaced by a new, different one. The time spent exploring the familiar (F) and the novel (N) object was recorded and a discrimination index (D) was calculated as (N − F)/(N + F). This ratio represents the difference in exploration time between the two objects during the Trial 2 (T2), expressed as a proportion of the total time spent exploring these two objects. In addition, an index of habituation (H) to the familiar object, H = T1/T2 − F was also calculated. This index measures the difference between an average time spent in exploring one of the identical objects during Trial 1 (T1) and the time spent exploring the familiar object (F) during the Trial 2. The discrimination ratio and the index of habituation are related variables and widely used for the estimation of the rats’ performance in the OR paradigm [18,43]. A dark glass bottle and a plastic shaker filled with water were the different objects used in duplicates.

1.4. Water-maze test

A subset (n = 20, HR = 10, LR = 10) of the rats was used for the Morris WM paradigm with hidden platform, which is a valid test for investigating spatial learning and memory procedures. It is worthy to note that the rat performance during the memory procedure of this paradigm is dependent on procedural as well as working memory function [59]. The water-maze was a circular galvanized pool 140 cm in diameter and 50 cm deep, filled with opaque water (24 °C) to a depth of 30 cm. The pool was divided in four equal quadrants. A platform (13 cm × 13 cm) was placed in the center of one of the quadrants (target quadrant), 2 cm beneath the water surface. The present procedure consisted of a 2-day period, the learning and the memory test, respectively, as described by de Quervain et al. [15] with some minor modifications. During the first day of the WM paradigm (learning test), the animals were trained to find the position of the fixed, hidden platform and escape onto the platform, in a total number of eight trials. The rats were placed into the water, facing the wall of the tank, sequentially from four different entry points, equally set around the pool. All rats were subjected to the four entry points, following the same order while each entry was repeated twice. Each trial lasted 90 s and when a rat escaped onto the platform, it was left there for 20 s, to allow orientation to visual cues in the room, and then placed for 30 s in a dry holding cage, ready to proceed to the next trial after that. If a rat failed to locate the platform within the 90 s trial, it was gently guided to it by the experimenter. By the end of the training session all animals returned to their home cages. The following day, the rats were given a retention trial in the absence of the platform (memory test). A single 60 s trial was applied in which the rat was put into the water in a position equally distanced from the imaginary target and opposite quadrants [28]. The parameters recorded were escape latency, total distance swim and mean velocity during training test, as well as time spent and mean velocity in each quadrant, during the memory test (EthoVision V1.90, Noldus, Wageningen, The Netherlands).

1.5. Forced swim test

Rats (n = 15, HR = 7, LR = 8) were individually placed in a cylindrical tank measuring 60 cm height × 38 cm width. The tank was filled with water (24 ± 1 °C) at a height of 40 cm and water was changed after each session. The animals were forced to swim for a 15-min period (pretest) and 24 h later were subjected to a 5-min swimming session (test) [44]. The total duration of floating (immobility), swimming, head swinging and climbing periods was measured for the first 5 min of each session (pretest and test). Rats were considered to show immobility (floating) when they floated without struggling and making only those movements necessary to keep their heads above water. Swimming was recorded when they actively swim around in circles. Head swinging was recorded when the rats exhibited headshake responses indicative of searching for an escape. Climbing was considered when the rats were climbing at the walls of the cylinder. It has been previously shown that the aforementioned behavioral parameters such as floating, swimming and climbing are sensitive to antidepressants [12,16]. Following swimming sessions, the rats were removed from the tank, carefully dried in heated cages and then returned to their home cages.

1.6. Neurochemical analyses

After assignment to LR/HR groups, the rats (n = 25, HR = 12, LR = 13) were left undisturbed for 4–5 days. They were then sacrificed, their brain was rapidly removed and discrete brain regions were dissected (hippocampus, hypothalamus, nucleus accumbens, dorsal striatum, prefrontal cortex and amygdala). After weighing, the dissected tissue was homogenized and deproteinized in 500 μl of 0.2N perchloric acid solution (Merck KgaA, Darmstadt, Germany) containing 7.9 mM Na2S2O5 and 1.3 mM Na2EDTA that were both purchased from Riedel-de Haen AG (Seelze, Germany). The homogenate was centrifuged at 14,000 rpm for 30 min and the supernatant was stored at −80 °C. The analytical measurements were performed by high-performance liquid chromatography (HPLC) with an electrochemical detector, as described by Sharp et al. [51] and Papadopoulou-Daifotis et al. [36] with some minor modifications [13,17]. Reversed phase ion pair chromatography was used in all analyses of dopamine (DA), 3,4-dihydroxyphenylacetate (DOPAC), homovanillic acid (HVA), serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA). The mobile phase consisted of an acetonitrile (Merck) and 50 mM phosphate buffer (10.59:1.5) pH 3.0, containing (300 mg/l) 5-oxylsulfate sodium salt (Merck) as the ion-pair reagent and (20 mg/l) Na2EDTA (Riedel-de Haen AG). Reference standards
were prepared in 0.2N perchloric acid solution containing 7.9 mM Na₂S₂O₃ and 1.3 mM Na₂EDTA. The sensitivity of the assays was always tested using external standards. The HPLC system consisted of a BAS-LC4B with an amperometric detector. The working electrode was glassy carbon; the columns were Hypersil, Elite C18 150 mm × 2.1 mm, 5 µm (Thermo Electron, Cheshire, UK) and the HPLC system was connected to a computer. Samples were quantified by comparison of the area under peaks with those of reference standards using an HPLC software (Chromatography Station for Windows). Additionally, the turnover ratios of DA (DOPAC/DA, HVA/DA) and 5-HT (5-HIAA/5-HT) were also calculated, in order to provide a better evaluation of dopaminergic and serotonergic functions [8,11].

1.7. Statistical analyses

One-way analysis of variance (ANOVA) was used for the comparison between HR and LR rats concerning all the behavioral responses registered during their reaction to the open field.

Specifically, one-way repeated ANOVA with novelty as “between-subjects factor” (HR versus LR rats) and session as “within-subjects factor” (training latency versus retention latency) was used in the PA test. Separate analyses were conducted when appropriate.

One-way ANOVA was used for comparison between HR and LR rats concerning discrimination index (D) and habituation index (H) in the OR task.

For the analysis of the WM training data, a repeated one-way ANOVA was used. Novelty was considered as “between-subjects factor” and trial as “within-subjects factor” for all available variables: escape latency scores, mean velocities and total distances swum registered during the 8 repeated trials. In addition, one-way ANOVA with novelty as factor (HR versus LR) was conducted to estimate possible differences for all available variables (time spent in target or opposite quadrant, mean velocity, total distance swum) registered during the memory retrieval test.

A repeated ANOVA was used for FST data, with between-subjects factor novelty (HR versus LR) and within subjects factor, the session (pretest versus test). Separate analyses were performed when appropriate.

For neurochemical measurements, a one-way ANOVA was used for comparison between HR and LR rats.

For post hoc comparisons, on factorial and repeated measures ANOVAs, Bonferroni’s tests were used for adjustment and control of the type I error rate. Significance was accepted when p < 0.05.

2. Results

2.1. Open field behavior

As expected, there were statistically significant differences between HR and LR rats concerning the frequency and duration of rearing [F(1,125) = 99.45;45.8, p < 0.001] and sniffing-air [F(1,125) = 66.5;68.8, p < 0.001] behavior. Furthermore, HR rats exhibited increased frequency and duration of moving [F(1,125) = 39.3;32.1, p < 0.001] and sniffing [F(1,125) = 49.5;13.9, p < 0.001], but decreased duration of standing [F(1,125) = 64.8, p < 0.001], as compared to LR rats (Fig. 1).

2.2. Passive avoidance test

There was not any statistically significant difference in PA performance between HR and LR (Fig. 2). One-way ANOVA with repeated measures did not reveal any difference concerning training or retention latency scores between HR and LR rats, although there was a tendency for lower training and higher retention latency scores in HR, as compared to LR rats (Fig. 2). On the other hand, this analysis revealed a session effect [F(1,22) = 40.8, p < 0.001] and separate repeated analyses showed an increase in retention latency scores for HR and LR rats [F(1,123) = 21.64;19.5, p < 0.001, respectively] (Fig. 2).

2.3. Object recognition task

HR rats displayed a lower discrimination index (D), but a higher habituation index (H) as compared to LR rats (Fig. 3). One-way analysis revealed a decreased ability to discriminate (index D) between novel and known objects [F(1,28) = 5.7, p < 0.02] for HR as compared to LR rats (Fig. 3). In addition, the index H, which reflects habituation to the object, was higher in HR as compared to LR rats [F(1,25) = 4.7, p < 0.05] (Fig. 3). Moreover, time spent exploring the familiar and novel object (T2) was not different between both groups of rats, while time spent by the animal for exploration of identical objects (T1) was increased in HR in comparison with LR rats [F(1,29) = 6.2, p < 0.01] (Fig. 3).

2.4. Water-maze test

HR displayed enhanced spatial memory in the WM task as compared to LR rats (Fig. 4). In the training (learning) session of the water-maze, one-way repeated ANOVA revealed only a trial effect [F(7,112) = 9.7, p < 0.001] for escape latency. In particular, escape latency was decreased over time in both HR and LR rats. Subsequent analysis showed that HR displayed a shorter escape latency score as compared to LR rats in trial 2 (p < 0.05) (Fig. 5A). This difference was also observed in Trial 3 but it did not reach any statistical significance (Fig. 5A). The same analysis revealed that total distance swum was decreased over trials in both HR and LR rats [F(7,112) = 4.87, p < 0.001]. Similar to escape latency, HR rats have shown decreased total distance swam as compared to LR rats in trial 2 (p < 0.05) and trial 3, but it did not reach significance (Fig. 5B). Mean velocity was changed over trials in both HR and LR rats [F(7,112) = 3.02, p < 0.01]. In particular, HR rats exhibited higher mean velocity than LR rats in trial 6, 7 and 8 but this increase was statistically significant only in trial 6 (p < 0.05) (Fig. 5C).

During the memory retrieval test, one-way ANOVA revealed that HR rats spent more time in the target quadrant as compared to LR rats ([F(1,17) = 13.05, p < 0.001] (Fig. 4A). Additionally, HR rats spent more time in the target quadrant as compared to the opposite quadrant [F(1,17) = 19.14, p < 0.001], an effect that was not observed in LR rats (Fig. 4A). Total distance swam in target as well as in opposite quadrant was not different between HR and LR rats (data not shown). HR rats displayed higher mean velocity in the opposite quadrant as compared to LR rats [F(1,17) = 5.1, p < 0.05] (Fig. 4B). Moreover, mean velocity in the opposite quadrant was higher than that observed in the target quadrant only in HR rats [F(1,17) = 6.29, p < 0.05] (Fig. 4B).

2.5. Forced swim test

HR and LR rats showed a “depressive-like” behavior in the FST paradigm which was differently profiled between each phenotype (Fig. 6). Repeated measures ANOVA revealed a session effect [F(1,13) = 17.9, p < 0.001], on floating duration.
In particular, separated repeated ANOVA analyses revealed an increase in floating behavior in the test session as compared with the pretest session, in both HR and LR rats $[F(1,7) = 10.2; 8.1, p < 0.01; p < 0.05$, respectively]. Repeated measures ANOVA revealed a session effect $[F(1,13) = 6.1, p < 0.05]$, on swimming duration. Separate repeated ANOVA analyses revealed a decrease in swimming duration $[F(1,7) = 7.1, p < 0.03]$ only for HR rats (Fig. 6). Repeated measures ANOVA revealed a session effect $[F(1,13) = 5.9, p < 0.05]$, on head swinging duration. Separate repeated ANOVA analyses revealed a decrease in head swinging duration $[F(1,8) = 11.3, p < 0.01]$ only for LR rats (Fig. 6).

Similar analyses did not reveal any statistically significant effects on climbing behavior. Interestingly, separate one-way ANOVA with novelty as between factor for pretest and test sessions, revealed decreased head swinging and increased swimming behavior in HR as compared to LR rats during the pretest session of FST $[F(1,14) = 5.9; 6.1, p < 0.05]$ (Fig. 6).
Fig. 2. Passive avoidance test. Mean values ± S.E. of the training latency (T1) and retention latency (T2) scores in LR and HR rats. ***$p<0.001$ (retention latency score vs. training latency score in LR and HR rats, respectively).

Fig. 3. Object recognition task. Total exploration time of trial 1 (T1) and trial 2 (T2) displayed by LR and HR rats (A). Discrimination index (D) performance of LR and HR rats in the trial 2 of the testing day (B). Habituation index (H) performance of LR and HR rats (B). Data are expressed as mean values (±S.E.) *$p<0.05$, **$p<0.01$ (HR vs. LR rats).

Fig. 4. Water-maze test. Mean values ± S.E. of time spent in quadrants (A) and mean velocity in quadrants (B) of LR and HR rats during the memory test of water-maze paradigm. *$p<0.05$; ***$p<0.001$ (HR vs. LR rats). *$p<0.05$; +++$p<0.001$ (target vs. opposite quadrant in HR rats).
2.6. Neurochemical measurements

HR rats displayed lower 5-HT turnover ratio in the prefrontal cortex, while they displayed higher tissue concentrations of DA, HVA, 5-HIAA and 5-HT turnover ratio in the striatum, as compared to LR rats (Table 1). Moreover, higher tissue concentrations of DOPAC in the hypothalamus were observed in HR in comparison with LR rats (Table 1). Specifically, in the prefrontal cortex, a one-way ANOVA revealed reduced 5-HT turnover ratio (5-HIAA/5-HT), which is an index of serotonergic activity, in the HR as compared with LR rats \( F_{(1,24)} = 4.5, p < 0.05 \) (Table 1). There were not any statistically significant differences in dopaminergic or serotonergic activity in the nucleus accumbens between HR and LR rats. A tendency for increased DA levels was only observed in HR compared with LR rats (Table 1). In the dorsal striatum, an increase in DA and HVA levels was observed \( F_{(1,22)} = 5.6;6.5, p < 0.05; p < 0.01 \) in HR as compared to LR rats (Table 1). In addition, 5-HIAA levels and the 5-HT ratio (5-HIAA/5-HT), were increased in the HR compared with LR rats \( F_{(1,21)} = 7.5;5.1, p < 0.01; p < 0.05 \) (Table 1). In the hypothalamus, an increase in DOPAC levels was observed \( F_{(1,23)} = 6.5, p < 0.05 \) for HR compared with LR rats (Table 1). Furthermore, a tendency \( p = 0.1 \) for increase of the DA ratio (DOPAC/DA) was observed in HR as compared to LR rats (Table 1). There were not any statistically significant neurochemical differences in the studied indices of the hippocampus and the amygdala in HR compared with LR rats (data not shown).

3. Discussion

The present study aimed to identify differences in the behavioral profiles, using a variety of tests, as well as in neurochemical indices of male rats exhibiting high and low rearing behavior upon exposure to a novel open field. HR displayed an increased locomotor/exploratory activity as compared to LR rats. Interestingly, HR rats seemed to display impaired object recognition memory, but enhanced spatial performance, as compared to LR rats. Affective responsivity, i.e., “depressive-like” behavior in the FST was differently manifested in HR compared with LR rats. Concerning the neurochemical evaluation, distinct differences in striatal and cortical serotonergic and dopaminergic activity were observed between the phenotypes.

3.1. Behavioural analysis

HR exhibited decreased standing but increased moving and sniffing behavior in the novel open field compared with LR rats. Apparently, HR also displayed an increase in rearing behavior as compared to LR rats. These findings are in agreement with our previous findings [5] and with those by Thiel et al. [55] who have also reported that rats with more rrs also display higher levels of locomotion. Based on the aforementioned studies and on our findings, it is suggested that rats with a high frequency of rearing behavior display an enhanced motor activity profile similar to the HR profile derived from differentiation of rats on the basis of locomotor response to novelty [10,39].

HR and LR rats displayed similar performance in the PA task, although a shorter training latency and longer retention
latency was observed in HR rats. The PA task is one of the most frequently used animal models for studying learning and memory processes based on aversive learning [35]. Similar to our findings, Borta and Schwarting [6] reported that HR and LR rats showed comparable step-in retention latencies, 24 h after the training procedure, using different shock intensities. Notably, these authors observed that HR displayed an impaired step-through PA retention, 72 h after the training procedure, especially when using the higher shock intensity. Moreover, Ho et al. [23] did not report any difference between HR and LR rats using the two-way avoidance paradigm. Based on our findings and on the aforementioned studies, it seems that these phenotypes display subtle differences in terms of reaction to an aversive stimulus or aversive learning procedures.

When the two groups of rats were subjected to the object recognition test, impairment in object recognition was observed in HR as compared to LR rats. In particular, HR exhibited a higher time for exploration of both identical objects (T1 session), along with a tendency for higher time for exploration of both novel and familiar objects (T2 session), a higher familiarization index (H), but a lower discrimination index (D). This behavioral performance could be related to the increased motor/exploratory activity, observed in this phenotype of rats. However, based on the higher H index displayed by the HR rats, which could be attributed to their higher exploration time of identical objects (T1), it is assumed that HR rats displayed an enhanced familiarization to the object as compared to LR rats due to their exaggerated first (basal) reaction to the object during the T1. On the other hand, Pawlak and Schwarting [38] reported that HR rats showed higher exploration of the new instead of the familiar object in the object recognition task. It is worth mentioning that these authors have not indicated the discrimination index (D) for HR and LR rats, while the intertrial interval was shorter as compared to that used in the present study. Notably, playground maze along with object recognition task have been used for assessment of novelty seeking and categorization of animals as either HR or LR rats [30,34]. Relevant studies have not shown any correlation between the horizontal activity in an inescapable novel environment and the scores in playground maze [29,30]. However, the object recognition task used in the present study has been considered as a non-spatial paradigm based on spontaneous exploratory activity that reflects working memory [37,43]. It is worthy to note that hyperactivity has been associated with deficits in short-term memory in spontaneously hypertensive rats [45,48]. Therefore, based on our findings and the aforementioned studies it could be argued that the higher locomotor response to novelty, might be contributing to an impaired non-spatial performance.

On the other hand, HR rats showed a better spatial related performance, as revealed in the WM paradigm. Thus, HR rats learn to navigate to a hidden platform faster than LR rats, as deduced by escape latency scores and total distance swum in the initial set of trials, during the learning phase of the WM procedure. Additionally, HR rats showed a clear preference for target quadrant along with a reduced velocity in this quadrant, compared with LR rats, during the learning test of WM paradigm. It should be pointed out that differences in the learning phase were not as robust as those observed in the memory phase, supporting the notion by Jakubowska-Dogru et al. [24] that memory acquisition and memory performance reflect independent processes. The WM with hidden platform has been used for elucidating hippocampal-dependent spatial learning and memory abilities. In accordance with our findings, HR displayed a better performance in the radial maze task, albeit of more working errors related to memory function as compared to LR rats [22]. Interestingly, these authors reported that this differential pattern between HR and LR rats is based on differences in motivational and cognitive status. Moreover, Cools et al. [9] have suggested that differences between HR and LR rats in response to a simple four-arm radial maze are not due simply to differences in motor activity, but to minor yet important differences in the mode of learning. However, Topic et al. [56] have reported a relationship between reactivity to novelty and thigmotaxis in the WM which may reflect behavioral traits and may bias spatial learning. This behavioral differentiation is probably characterized by cognitive and/or non-cognitive factors, as reported by several studies [22,56]. Based on these studies and on our findings it seems that differences in spatial memory and learning abilities between the HR and LR rats are probably related to their differentiated reaction to novelty and not simply attributed to their respective differences in motor activity.

| Table 1 | Neurochemical evaluation of dopaminergic and serotonergic activity in the prefrontal cortex, nucleus accumbens, dorsal striatum and hypothalamus of high responders (HR) and low responders (LR) rats |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                  | **Prefrontal**  | **Nucleus accumbens** | **Dorsal striatum** | **Hypothalamus** |
|                  | LR              | HR              | LR              | HR              |
| DA               | **0.26 ± 0.04** | **0.14 ± 0.01** | **2.75 ± 0.60** | **3.98 ± 0.60** |
|                  | **2.93 ± 0.04** | **2.39 ± 0.06** |
| DOPAC            | **0.05 ± 0.001**| **0.04 ± 0.002**| **1.41 ± 0.33** | **1.91 ± 0.30** |
|                  | **1.74 ± 0.04** | **1.62 ± 0.44** |
| HVA              | **0.05 ± 0.001**| **0.04 ± 0.002**| **0.40 ± 0.07** | **0.44 ± 0.04** |
|                  | **0.70 ± 0.20** | **1.65 ± 0.20** |
| DOPAC/DA         | **0.21 ± 0.03** | **0.28 ± 0.03** | **0.53 ± 0.08** | **0.50 ± 0.04** |
|                  | **0.19 ± 0.05** | **0.23 ± 0.04** |
| HVA/DA           | **0.13 ± 0.02** | **0.19 ± 0.02** | **0.17 ± 0.03** | **0.13 ± 0.02** |
|                  | **0.04 ± 0.01** | **0.08 ± 0.02** |
| 5-HT             | **1.51 ± 0.22** | **1.60 ± 0.23** | **1.19 ± 0.19** | **1.21 ± 0.23** |
|                  | **0.40 ± 0.04** | **0.54 ± 0.08** |
| 5-HIAA           | **0.52 ± 0.04** | **0.52 ± 0.04** | **0.65 ± 0.08** | **0.63 ± 0.09** |
|                  | **0.36 ± 0.06** | **0.80 ± 0.15** |
| 5-HIAA/5-HT      | **0.52 ± 0.04** | **0.37 ± 0.05** | **0.58 ± 0.07** | **0.62 ± 0.11** |
|                  | **0.84 ± 0.16** | **1.43 ± 0.18** |

Levels of DA, its metabolites (DOPAC and HVA), 5-HT and its metabolite (5-HIAA) tissue concentration as well as the dopaminergic turnover ratios (DOPAC/DA and HVA/DA) and the serotonergic turnover ratio (5-HIAA/5-HT) are expressed as mean values (±S.E.). *p<0.05; **p<0.01 (HR vs. LR rats).
Both HR and LR rats exhibited increased floating behavior and decreased active behaviors during the test session of FST, properties that reflect a well-known “depressive-like” behavior. In accordance with Ho et al. [23] and White et al. [62] these rats do not exhibit any essential differentiation in relation to their “depression-like” status. Notably, HR rats exhibited less swimming behavior while LR rats displayed less head-swinging behavior during the test session of the FST paradigm. During the first exposure to the water (pretest), HR rats were swimming more than LR rats while LR rats were head-swinging more than HR rats. Therefore, HR and LR rats exhibited increased passive behavior and decreased active behavior in the FST paradigm, but active behavior was differently manifested in the two phenotypes in both the pretest and test FST session. HR and LR rats developed a different behavioral FST pattern characterized by a common behavioral state of “despair”, but distinct active behavioral reactions expressed either during the first or during the second exposure to the water.

Taken together, the results of previous studies [14,46,57] as well as our present findings suggest that the two behavioral phenotypes studied reveal differences in a variety of behavioral tasks due to differential responsiveness to the test conditions and the diversity in the repertoire of the responses studied in behavioral paradigms that assess novelty, habituation, reaction to an aversive stimulus or a stressful situation, and cognitive or affective reactivity.

3.2. Neurochemical analysis

Concerning the neurochemical findings, higher DA and HVA concentrations in the striatum were found in the HR compared to the LR rats. Although the exact impact of these differences on the overall state of dopaminergic neurotransmission in the forebrain is not clear, they are probably related to the behavioral differences seen between the two phenotypes. Similar to previous studies, dopaminergic activity in striatal tissue seems to be necessarily involved in the differentiation of animals exposed to a novel environment [41]. In particular, Piazza et al. [42] have reported a positive correlation between dopaminergic activity in the nucleus accumbens and motor activity in a novel environment, while this correlation was negative concerning the dopaminergic activity in the prefrontal cortex. Moreover, Sagi-gusa et al. [49] and Van der Elst et al. [58] have suggested an association between response to novelty and dopaminergic status in the nucleus accumbens. Similar to our findings, Thiel et al. [55] failed to show a clear increase in dopaminergic function in the ventral striatum (that includes the nucleus accumbens we sampled in our study) of HR rats, but they showed an increased DA function in the neostriatum of these rats. In accordance to our results, Shakil et al. [50] reported an increased DA ratio in the striatum of HR mice.

A decreased cortical serotonergic activity as well as an increased striatal serotonergic activity, as reflected by the 5-HIAA/5-HT turnover ratio, was observed in the HR as compared to LR rats. These results are slightly different concerning the cortical serotonergic activity as compared to the respective findings by Thiel et al. [55], who found reduced 5-HT tissue levels with-
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References
