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# Exploration of a novel object in late adolescence predicts novelty-seeking behavior in adulthood: Associations among behavioral responses in four novelty-seeking tests

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ABSTRACT

The sensation/novelty seeking behavioral trait refers to the exploration/preference for a novel environment. Novelty seeking increases during late adolescence and it has been associated with several neurobehavioral disorders. In this experiment, we asked whether inbred Roman high- and low-avoidance (RHA-I, RLA-I) rats (1) differ in novelty seeking in late adolescence and (2) whether late adolescent novelty seeking predicts this trait in adulthood. Thirty six male RHA-I and 36 RLA-I rats were exposed to a novel object exploration (NOE) test during late adolescence (pnd: 52–59; Dependent variables: contact latency, contact time, contact frequency). Head-dipping (hole-board, HB), time and visits to a novel-arm (Y-maze), and latency-in and emergence latency (emergence test) were registered in adulthood (pnd: 83–105). The results showed strain differences in all these tests (RHA-I > RLA-I). Factor analysis (RHA-I + RLA-I) revealed two clusters. The first one grouped HB and emergence test measures. The second one grouped NOE and Y-maze variables. Time exploring a novel object (NOE) was a significant predictor of novel arm time (RHA-I + RLA, RHA-I); contact latency was a significant predictor of novel arm frequency (RLA-I). Present results show consistent behavioral associations across four novelty-seeking tests and suggest that late adolescent novelty seeking predicts this genetically-influenced temperamental trait in adult Roman rats.



## 1. Introduction

In animal behavioral research, trait is defined as a pattern of specific behavior which differs among individuals, but which is relatively constant within subjects across time and situations (Gosling, 2001). Behavioral traits resemble types, temperaments or personalities in humans, and have been proposed as critical for individual adaptive capacity, as well as for vulnerability or resistance to various pathologies (Steimer and Driscoll, 2005).

Several methodological approaches have been developed to analyze inter-individual differences in behavior in non-human animals (see Pawlak et al., 2008, for review). One of the most commonly used involves selectively breeding animals on the basis of divergent behavioral dispositions such as addiction proneness, exploration, fearfulness or novelty seeking, among others. The results of this psychogenetic selection is the creation of strains of animals showing consistent and stable patterns of behaviors over generations, providing a useful tool to study the biological basis of personality (Driscoll et al., 2009; Steimer and Driscoll, 2005). One of the behavioral traits most extensively studied by using this approach is sensation seeking. In humans, it is described as the need for varied, novel, complex and intense sensations and experiences, and willingness to take physical and social risks for the sake of such experiences (Zuckerman, 1994). The parallel concept in

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research with nonhuman animals, novelty or sensation seeking, has been used to describe high levels of exploratory activity in response to novel environments and unknown objects or stimuli (Bardo et al., 1996). A distinction has been recently proposed between "sensation seeking" tests (based on exposure to inescapable novel environments) and "novelty seeking" tests (involving preference for novelty in free-choice tasks; see Flagel et al., 2014). The locomotor activity exhibited in a novel environment from which there is no escape (e.g., horizontal activation in a circular corridor or vertical activation—rearing-in an open field) is frequently measured in sensation seeking tests (Piazza et al., 1989; Pawlak and Schwarting, 2002). By contrast, novelty seeking is commonly defined as a preference for a novel context (or object) compared with a familiar context (Pelloux et al., 2004, 2006, 2015; Pisula, 2003; Dellu et al., 1996), thus usually involving two-trial testing procedures (and, thus, learning/habituation processes) and giving animals a choice to either approach or avoid novelty (Bardo et al., 2013; Meyer et al., 2010). Finally, the hole-board (HB) test consists in an open field apparatus with equidistant holes in the floor in which head-dipping behavior is used as an index of novelty reactivity (File and Wardill, 1975). This test has been conceptualized both as an inescapable and a free-choice-based novelty test (Bardo et al., 2013).

Mixing findings have been found in the literature with respect to the relationship between sensation and novelty seeking, suggesting that these tests may be measuring different aspects of novelty seeking that are mediated by partially different neurobiological processes (Beckman et al., 2011). In this regard (a) elevated reactivity to inescapable novelty (in terms of locomotor activity) is frequently unrelated to preference for novel environments (Cain et al., 2004; Beckman et al., 2011; Flagel et al., 2014; Meyer et al., 2010); (b) whereas exploratory behavior in forced novelty tests seems to be a good predictor of the initial proneness to take drugs (Belin et al., 2008; Flagel et al., 2014), preference for novelty in free-choice tasks correlates with compulsive drug taking and severity of addictive behavior (Belin et al., 2011; Belin and Deroche-Gamonet, 2012; Flagel et al., 2010); and (c) although both sensation and novelty seeking are related to the mesolimbic dopaminergic system (Bardo et al., 2013), only responses to inescapable novelty seem to elevate corticosterone levels and are associated with activation of the stress axis (Bardo et al., 1996; Kabbaj, 2006), suggesting that inescapable novelty may represent a stressful rather than positive incentive value experience in rodents (Norbury and Husain, 2015). Overall, this evidence suggests that novelty/sensation seeking is not a unitary neurobehavioral trait, but one that includes some behaviors differentially associated to a variety of neurobehavioral disorders (Duclot et al., 2011; Flagel et al., 2014; Norbury and Husain, 2015). Nevertheless, this conclusion deserves further investigation considering that discordant results with this sensation/novelty seeking distinction have also been reported (e.g., Dellu et al., 1996; Kabbaj, 2006; Kabbaj et al., 2000).

Although substantial behavioral consistency across life cycle has been observed (Ray and Hansen, 2005), responses involving novelty seeking seem to reach a peak in adolescence (especially in the late period), and then decline (Laviola et al., 1999; Spear, 2002). Interestingly, adolescence constitutes a life cycle period in which different patterns of normal but maladaptive outcomes are common, and mental illnesses often manifest (Compas et al., 1995; Sturman and Moghaddam, 2011); for example, increased negative affect, higher sensitivity to stressful events, increased impulsivity, and a greater propensity to take risks, including the use of psychoactive agents (Milivojevik and Covault, 2013; Steinberg, 2004). Therefore, identifying individual differences in sensation seeking during adolescence could be used as a risk marker for vulnerability to psychopathology with basic and clinical implications.

In this experiment, we asked whether inbred Roman high- and low-avoidance (RHA-I, RLA-I) rats (1) differ in novelty seeking in late adolescence and (2) whether late adolescent novelty seeking predicts this trait in adulthood. Although initially selected and bred on the basis of their good (RHA-I) vs. poor (RLA-I) acquisition of the two-way active—shuttle box- avoidance response, the Roman rat lines/strains also show divergent profiles in a host of correlated behavioral traits, including anxiety/fearfulness (Driscoll and Bättig, 1982; Driscoll et al., 1998, 2009; Escorihuela et al., 1999; Fernández-Teruel et al., 1997; López-Aumatell et al., 2009), reactivity to frustration (Gómez et al., 2009; Rosas et al., 2007), impulsivity (Klein et al., 2014; Moreno et al., 2010), coping styles in novel/stressful environments (Díaz-Morán et al., 2012, 2013; Escorihuela et al., 1999; Estanislau et al., 2013; Fernández-Teruel et al., 1992a, 2002a; Giorgi et al., 2003; Piras et al., 2010, 2014; Pisula, 2003; Steimer and Driscollk 2003), consumption of palatable tastes (Fernández-Teruel et al., 2002a; Razafimanalina et al., 1996), vulnerability to addiction (Giorgi et al., 2007), sexual behavior (Sanna et al., 2015) and novelty seeking (Driscoll et al., 2009; Escorihuela et al., 1999; Fernández-Teruel et al., 1992a, 2002a; Giorgi et al., 2007; Guitart-Masip et al., 2006a). Within this respect, both outbred and inbred adult RHA rats exhibit more novelty seeking responses than their RLA counterparts in a variety of behavioral tests based on both inescapable and free-choice situations, including head-dipping in the HB, preference for a novel arm in the Y-maze, and preference for novelty introduced in a familiarized environment, among others (Escorihuela et al., 1999; Fernández-Teruel et al., 1992a, 2002a; Guitart-Masip et al., 2006a; Manzo et al., 2014; Pisula, 2003; Steimer et al., 1998). Strain differences have also been observed in young animals (pnd: 30–40) exposed to novelty-based situations, including the dark-light hexagonal tunnel maze and the timidity test (Escorihuela et al., 1999; Fernández-Teruel et al., 1991, 1992b, 2002b). Many of these strain differences have been shown to be enduringly abolished by neonatal handling (e.g., Fernández-Teruel et al., 1991, 1992a,b, 2002b; Río-Alamos et al., 2015), suggesting that genetically-influenced behavioral traits can be modulated by environmental events (Fernández-Teruel et al., 1997, 2002b).

Based on the evidence reviewed above, indicating behavioral consistency across age and sensation/novelty seeking tests in Roman rats, we predicted that (1) RHA-I rats would exhibit more novelty seeking responses than RLA-I when tested in late adolescence; and (2) novelty seeking in late adolescence would predict novelty seeking behavior in adulthood. Present study will also allow us to identify associations in behavioral responses across four novelty-seeking tests.

## 2. Method

### 2.1. Subjects

The subjects were 72 inbred male rats (36 RHA-I, 36 RLA-I) obtained from the colony established at the Autonomous University of Barcelona, Spain. Animals were housed in pairs with free access to food and water throughout the experiment, in a room kept at 22–23 °C, and subjected to a 12:12 h light cycle (lights on at 08:00 h). Animals were tested between 9:30–13:00 a.m. The experiment was conducted following the European Union directive guidelines for the use of animals in research (2010/63/EU) and the Spanish Law (RD 53/2013).

### 2.2. Apparatus

In order to assess novelty seeking behavior during late adolescence (pnd: 52–59), a novel object exploration (NOE) test was conducted (see also Río-Alamos et al., 2015). This involved the assessment of the exploratory responses of Roman rats when a

novel object is introduced in their home cages. The test started by removing the food from the home cage. Five minutes later, the novel object (green-colored pen) was perpendicularly introduced in their home cages through the grid cover. Individual cages were pulled from the rack and placed on a counter for video recording in the same animal room.

The apparatus for the three novelty tests conducted when rats became adults (pnd: 83–105) were placed in a sound-attenuated room under dim illumination. Numerous visual cues were placed on the walls of the testing room and were kept constant across tests. As described elsewhere (Manzo et al., 2014), the HB apparatus was a square white 66 × 66 × 47 cm wooden box divided into 16 equal squares, containing four holes (diameter: 3.7 cm) in the floor. Partially hidden objects (small metallic toys) were located below the holes; this procedure has been reported to specifically induce novelty-seeking, rather than exploratory behavior or locomotor activity (Escorihuela et al., 1999).

The Y-maze apparatus was similar to the one previously described by Dellu et al. (1992). The Y-maze was made of acrylic; arms were 50 × 32 × 16 cm ($L \times H \times W$). The floor was black and the walls were transparent. The floor of the maze was covered with odor-saturated sawdust.

The apparatus used for the emergence test (adapted from Dellu et al., 1992) consisted in a box with two equal compartments measuring 27 × 28 × 25 cm ($L \times H \times W$). A door (9 × 9 cm) enabled the rats to pass from one compartment to the other. One of the compartments was completely enclosed by black opaque plastic sides with a lid of the same material, while the other was made of white plastic and had no lid. The white compartment was illuminated by a 60 W lamp placed 70 cm above it.

### 2.3. Procedure

The four sensation/novelty-seeking tests were conducted in the early part of the light cycle, between 09:30–13.00 h, to reduce the possible influence of diurnal variation in activity. The order of the three tests conducted in adulthood was counterbalanced across rats. There were 7 days between successive tests. Dependent variables for each test were video recorded and then processed with JWatcher (http://www.jwatcher.ucla.edu) by two observers. Frequency variables were measured on a ratio scale with an absolute zero and unbounded upper limit. Time variables were measured in seconds with the software JWatcher.

The NOE test was conducted in pairs of rats located in their home cage during one minute. The dependent variables were contact latency (time spent until the first exploration of the novel object), contact frequency (number of times the animal contacted the object with the snout) and contact time (total time spent exploring the pen).

In the HB test, animals were placed in the corner located closest to the door of the experimental room and were given 6 min to explore the board. Animals were returned to their home-cages at the end of the test, while the apparatus was cleaned with a 70% alcohol solution. A single test was administered. The dependent variables were the number of crossings (moving all 4 paws over a line dividing adjacent squares), head-dipping time and frequency (introducing the nose into a hole to the level of the eyes), and grooming time (face washing, licking, or scratching any part of the body).

The Y-maze test consisted in two trials separated by a 2-min interval. In the first trial, one arm of the Y-maze (counterbalanced across rats) was closed. Rats were placed in an arm, their head pointing away from the center of the maze, and they were allowed to visit the two arms for 5 min. During the second trial, animals had free access to the three arms, and were allowed to explore the maze again for 5 min. Then they were returned to their home-cages, while the apparatus was cleaned with a 70% alcohol solution. The dependent variables were novel arm entries and time (entering an arm with its four paws).

In the emergence test, rats were placed in the illuminated compartment facing the wall opposite to the door. Two dependent variables were registered in this test: (a) latency-in (the time it took the animal to come into the dark compartment from the illuminated compartment with its four paws); and (b) the emergence latency (the time it took the animal from the moment it placed all four paws into the dark compartment to the moment in which all four paws were back into the illuminated compartment). If a rat did not emerge from the dark compartment within 10 min after entering it, the trial was stopped and an emergence latency of 600 s was assigned to that animal.

### 2.4. Statistical analyses

The dependent variables registered in the NOE test conducted during the late adolescence were subjected to Student's *t*-tests for independent samples. The dependent variables registered in the three novelty tests conducted in adulthood were separately subjected to a multivariate analysis of variance with two factors, test Order (first, second or third), and Strain (RLA-I vs. RHA). For all statistical analyses, alpha was set at .05. Given that no interaction between Order and Strain was obtained for any dependent variable, statistical data are reported only for significant results involving Strain main effects.

To test associations among behavioral measures across behavioral tests and to identify underlying novelty-seeking dimensions, Pearson's correlation coefficients were calculated and factor analyses were performed (direct oblimin rotation). These analyses incorporated all the novelty-seeking-related variables, as well as number of crossings (HB) as an index of locomotor activity (Escorihuela et al., 1999), and grooming time (HB) and "latency-in" as indexes of anxiety-related responses (Estanislau et al., 2013; Steimer and Driscoll, 2003). Three out of the eleven dependent variables (i.e., Contact latency –NOE-, Grooming time –HB- and "Latency-in") did not fit with normal distribution (according to Kolmogorov–Smirnov tests of normality applied to data from the whole rat sample, $n = 72$), and so they were square root transformed to achieve normality. As all the analyses including these three transformed variables produced exactly the same results as using their raw scores, we present here analyses with these raw measures. Finally, multiple regression analyses (forward stepwise method) were conducted in order to complete the study of relationships among variables from different tests.

## 3. Results

### 3.1. Strain comparisons

#### 3.1.1. NOE test

A Strain analysis including contact latency, contact time and contact frequency showed a Strain effect for contact frequency, $t(1, 70) = 2.21$, $p < 0.030$, with RHA-I animals showing higher contact frequency scores than RLA-I animals (Table 1).

#### 3.1.2. HB test

A Strain analysis including the dependent variables registered in the HB test revealed Strain significant differences in grooming time, $F(1,70) = 23.111$, $p < 0.0001$ (RLA-I > RHA-I), head-dipping frequency, $F(1,70) = 15.005$, $p < 0.0001$ (RHA-I > RLA-I), and number of crossings, $F(1, 70) = 33.247$, $p < 0.0001$ (RHA-I > RLA-I) (Table 1).

**Table 1**
Strain differences in novelty seeking behaviors in late adolescence (a) and adulthood (b–d).

| Dependent variables and tests | RHA-I | RLA-I |
|---|---|---|
| (a) Novel object exploration test | | |
| Latency to first contact (s) | 8.79 ± 1.58 | 10.27 ± 1.37 |
| Contact frequency | 5.28 ± .36 | 4.14 ± .37* |
| Time exploring object (s) | 13.29 ± 1.52 | 12.61 ± 1.36 |
| (b) Hole-Board | | |
| Head-dipping frequency | 10.39 ± 0.74 | 6.83 ± .55*** |
| Time spent head-dipping (s) | 20.43 ± 1.50 | 18.86 ± 1.88 |
| Crossings | 106.39 ± 4.42 | 73.86 ± 3.51*** |
| Grooming time (s) | 3.10 ± .64 | 10.91 ± 1.49*** |
| (c) Y-maze | | |
| Novel arm time (s) | 79.21 ± 6.55 | 61.04 ± 4.36* |
| Novel arm frequency | 5.17 ± .29 | 4.19 ± .26* |
| (d) Emergence test | | |
| Emergence latency | 6.42 ± .47 | 14.78 ± 2.09*** |
| Latency-in | 7.67 ± .39 | 6.20 ± .26** |

Means and SEM are shown. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ (Student's t-tests).

#### 3.1.3. Y-maze test

A Strain analysis including the dependent variables registered in the novel arm of the Y-maze showed Strain significant effects for novel arm time, $F(1, 70) = 5.333$, $p < 0.024$ (RHA-I > RLA-I), and novel arm frequency, $F(1, 70) = 6.381$, $p < 0.014$ (RHA-I > RLA-I) (Table 1).

#### 3.1.4. Emergence test

A Strain analysis including the dependent variables registered in the emergence test revealed statistically significant differences in latency-in, $F(1,68) = 10.826$, $p < 0.002$, with the RHA-I strain showing higher latency-in scores than the RLA-I strain; and in emergence latency, $F(1, 68) = 15.214$, $p < 0.0001$, with the RHA-I strain showing lower emergence latency scores than the RLA-I strain (Table 1).

### 3.2. Correlations, factorial, and regression analyses

Pearson's correlation coefficients among variables are shown in Table 2 for all animals pooled. There were significant correlations among variables within the same test, ranging from $r = -0.48$ to $r = 0.41$ in the NOE test, from $r = -0.30$ to $r = 0.75$ in the HB test, and $r = 0.49$ in the Y-maze test (Table 2). Interestingly, correlations among measures from different tests were also found, some of the most relevant for novelty seeking being: $r = 0.29$ ($p < 0.014$) between contact time (NOE) and novel arm time (Y-maze), and $r = -0.25$ ($p < 0.039$) between contact frequency (NOE) and emergence latency. No significant correlations were observed among novelty-seeking measures from the HB test (i.e., head-dipping time and head-dipping frequency) and NOE, Y-maze or emergence test. However, grooming time was negatively correlated with contact frequency (NOE; $r = -0.30$, $p < 0.012$) and positively correlated with emergence latency ($r = 0.29$, $p < 0.013$), whereas number of crossings (HB) positively correlated with latency-in (Table 2).

To further analyze the associations among variables and to identify underlying novelty-seeking dimensions, we performed factor analyses (direct oblimin rotation) for all rats pooled. These analyses incorporated all the novelty-seeking-related variables as well as number of crossings (HB) as an index of locomotor activity (influenced by novelty, i.e., by anxiety; see Escorihuela et al., 1999), grooming time (HB) as an index of anxiety (see Estanislau et al., 2013) and latency-in as another possible index of anxiety (influenced by activity; Steimer and Driscoll, 2003). The first analysis produced a 4-factor solution explaining 65.8% variance (Table 3), in which the four dimensions were essentially test-related factors: the first factor appears to be an anxiety/horizontal exploration (mainly grouping grooming time -HB-, number of crossings and latency-in), the second factor reflects NOE test measures, the third is a head-dipping (HB) factor, and the fourth is a Y-maze factor. Thus, we next tried to get a factor solution that would better reflect the underlying dimensions of the different tests/measures by applying the Catell's scree test (see Aguilar et al., 2002; López-Aumatell et al., 2008), which allows us to force the solution to two factors, as there is a relatively large decrease of eigenvalues between the first and second factors, while between the second factor and the third the fall of eigenvalues is very similar to the decrease observed between the third and fourth factors.

The 2-factor solution (direct oblimin; Table 3) revealed two essentially independent factors (as the correlation between them was very low), with the first grouping HB measures (note that the more the head-dipping time and frequency, the less grooming time). Interestingly, emergence latency loaded with the opposite sign to head-dipping time and frequency (i.e., the more head-dipping time and frequency −0.58 and 0.77-, the shorter emergence latency −0.52-). As number of crossings and latency-in also loaded positively (0.67 and 0.53) in this first factor, it would seem to be a mixed novelty-seeking/activity/anxiety dimension. The second factor appeared to be a rather pure novelty-seeking factor, not influenced by activity or anxiety, as it presented high loadings of the three NOE test measures and Y-maze variables.

Two-factor analysis (direct oblimin, Table 3) for RHA-I rats revealed a first factor grouping NOE test measures and novel arm time, while number of crossings (HB) loaded negatively (i.e., higher activity was not associated with higher exploration of the novel arm -Y-maze- nor with the novel object exploration in the NOE test). The second (independent) factor grouped only head-dipping variables from the HB test. In the case of RLA-I rats, the 2-factor analysis reflected a first factor dominated by head-dipping measures (time −0.82, frequency −0.84) but, importantly, there were also important loadings of other novelty-seeking responses: novel arm time (0.59), novel arm frequency (0.47) and contact time (0.43). The second factor represented also novelty-seeking combined with anxiety, as suggested by loadings of 0.68 and −0.86 of contact frequency and contact latency (respectively), 0.45 of novel arm frequency and −0.43 of grooming time (HB). Of note, number of crossings (HB) did not appear to be associated to any of these factors (Table 3).

Finally, multiple regression analyses (forward stepwise method; Table 4), aimed at detecting associations among variables from different tests, essentially confirmed some aspects (or underlying dimensions/associations) suggested by these factor analyses. Contact time (NOE) was a significant predictor of novel arm time (Y-maze) both in the whole rat sample ($n = 72$) and in RHA-I rats ($n = 36$), while contact latency (NOE) was a significant predictor of novel arm frequency (Y-maze) in RLA-I rats ($n = 36$).

## 4. Discussion

This experiment was designed to test the association among behavioral responses from four novelty-seeking tests in the RHA-I/RLA-I rat strains and, importantly, to test the predictive value of a particular novelty seeking test (i.e., the NOE test) administered in late adolescence on novelty seeking behavior measured in adulthood. Three predictions were made: First, RHA-I rats would exhibit increased preference for novel objects or environments in the four tests. Second, preference for novelty in the NOE test administered in late adolescence would be predictive of some novelty seeking responses during adulthood. Third, there would be associations (according to factor and regression analyses) among novelty seeking measures from the different tests. As far as we know, it is the first time that a battery of four novelty seeking tests is administered to rats of the Roman strains, and it is also equally novel the

**Table 2**
Pearson correlation coefficients among the main behavioral variables ($n = 72$).

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Contact latency (1) | – | | | | | | | | | | |
| Contact frequency (2) | −.48*** | – | | | | | | | | | |
| Time exploring object (3) | −.12 | .42*** | – | | | | | | | | |
| Head dipping frequency (4) | −.04 | .14 | −.03 | – | | | | | | | |
| Time spent head-dipping (5) | −.01 | .05 | .01 | .75*** | – | | | | | | |
| Grooming time (6) | .21 | −.30* | −.13 | −.20 | −.05 | – | | | | | |
| Crossings (7) | .04 | .05 | −.02 | .31** | .07 | −.43*** | – | | | | |
| Novel arm time (8) | -.01 | .08 | .29* | .01 | −.17 | .01 | .13 | – | | | |
| Novel arm frequency (9) | −.15 | .09 | .09 | .16 | .02 | −.07 | .07 | .49*** | – | | |
| Latency-in (10) | −.08 | .06 | −.20 | .11 | −.04 | −.16 | .50*** | −.06 | .14 | – | |
| Emergence latency (11) | .17 | −.25* | −.01 | −.18 | −.12 | .30* | −.19 | −.10 | −.10 | −.21 | – |

*p < 0.05, **p < 0.01, ***p < 0.001.

approach of measuring novelty seeking in late adolescence in order to assess whether it is predictive of behavior later in adulthood.

Accordingly, relative to RLA-I rats, RHA-I rats made more exploratory contacts (i.e., contact frequency) with the novel object in the NOE test, showed more head-dipping behavior (as well as more crossings—activity- and less self-grooming-anxiety-) in the HB test, explored more the novel arm of the Y-maze test, and showed shorter emergence latency and longer "latency-in" in the emergence test. These findings are in agreement with results from previous works, in which some of these tests have been separately used with the Roman rat strains/lines (e.g., Fernández-Teruel et al., 1992a, 1997, 2002a,b; Escorihuela et al., 1999; Manzo et al., 2014; Steimer et al., 1998).

To the best of our knowledge the NOE test was first used by Fernández-Teruel et al. (2002b) as a "group test" for "timidity" and using much younger rats, but this is the first time that the present version of the NOE test (administered to rats caged in pairs but taking individual measurements) has been studied in association with three relatively well-validated novelty seeking tests. The most obvious difference between the NOE test and all the other novelty-seeking tests is that animals are tested in their home cage (thus minimizing contextual novelty) in the former, whereas all other tests are carried out in environments (cages, boxes, apparatus) different from their home cage and in rooms different from the animal room. Thus, at variance with these other tests, it is just the new object placed in the home cage what introduces the main novelty to the situation/context in the NOE test. Thus, it seems reasonable to propose that the NOE test has more pure novelty-seeking components (i.e., is less confounded or influenced by other factors −like emotionality, anxiety, activity—that are triggered by contextual novelty) than the other tests.

Some of the present results provide support to that contention: (1) the final two-factor solution for the whole rat sample (RHA-I and RLA-I rats pooled) showed a pure novelty-seeking (second) factor, in which NOE measures were associated with Y-maze variables, but not with activity (number of crossings −HB-) nor anxiety (grooming time −HB- and latency-in) (Table 3B); (2) the two-factor solution for RHA-I rats showed no association (in the first factor) among NOE measures and grooming time −HB-, and an inverse association of NOE variables and novel arm time (Y-maze) with number of crossings (HB), thus indicating that activity in the HB was inversely (rather than positively) associated with novelty-seeking behavior in the NOE and Y-maze tests (Table 3B); (3) for RLA-I rats, the two-factor solution revealed no association among NOE or Y-maze measures with activity (number of crossings −HB-) or anxiety (grooming time −HB-) in the first factor, with only a weak association being observed among grooming time (HB; −0.43) and the other two NOE variables (contact frequency and contact latency), novel arm frequency (Y-maze) and both emergence test parameters (i.e., emergence latency and latency-in) in the second factor (Table 3C); (4) regression analyses revealed that NOE test scores significantly predicted novelty seeking in the Y-maze test, but there were no significant relationships among activity (number of crossings −HB-) or anxiety (grooming time −HB-) and any of the novelty-seeking measures from the other tests according to regression models.

The present study also revealed that different novelty-seeking tests may be measuring different aspects of that behavioral trait. In this context, it seems remarkable that the HB test, which has accumulated evidence supporting its validity as a novelty-seeking test (e.g., Escorihuela et al., 1999; Fernández-Teruel et al., 1992a, 2002a; File and Wardill, 1975; Guitart-Masip et al., 2006a; Manzo et al., 2014; Steimer et al., 1998; Tournier et al., 2013), is associated with emergence test measures only when considering the whole rat sample (RHA + RLA) and one statistical analysis (factorial), but shows no association with NOE or Y-maze behaviors in any instance, as shown by separate factor analyses for each rat strain or by regression models. Conversely, the most consistent relationships observed in the present study concerned NOE and Y-maze behaviors, as both separately for each strain or in the whole rat sample NOE test measures were associated with Y-maze scores, as indicated by both factorial and regression analyses. This result is novel and may be important, as it suggests that a very simple novelty seeking test (NOE) administered at late adolescence in rats may be predictive of some novelty seeking behaviors/traits in adulthood.

HB behavior has also been shown to be dissociated from Y-maze novelty-seeking behavior in a recent study (Manzo et al., 2014). Moreover, factor analysis in that study, including ethanol preference scores, produced a two-factor solution, one grouping preference for low ethanol concentrations with HB head-dipping and grooming, and the other factor grouping preference for high ethanol concentrations with HB activity (number of crossings) and novel-arm time in the Y-maze test (Manzo et al., 2014). Globally, these results and the present study consistently suggest that different -and commonly used- novelty-seeking tests/measures in rats (1) may be defining several, sometimes even independent, subtraits or components of the novelty/sensation seeking trait, and that these components (2) may be in turn related (or associated) with different aspects of other novelty/incentive-seeking-related behaviors, such as alcohol (or drug) use or abuse (e.g., Manzo et al., 2014 and references therein).

The observed dissociation between head-dipping in the HB test and NOE/Y-maze measures is consistent with the contention that the latter two tests, both of which involve previous processes of habituation/learning, are measuring novelty seeking, whereas the HB test (which is inescapable and rats have not habituated to it, i.e., it involves a relatively higher degree of novelty) would be measuring other aspects of the sensation/novelty seeking trait (see Section 1, and references therein). Moreover, it is likely that

**Table 3**
Factor analysis with the main behavioural variables. (A) First, unforced factor analysis (oblimin direct) with the main behavioural variables (rats from both strains pooled; $n = 72$).[a] (B)Second factor analysis (oblimin direct), forcing a two-factor solution, with the main behavioural variables (rats from both strains pooled; $n = 72$).[b] (C) Factor analyses (oblimin direct), forcing two-fold solutions (see text), on behavioural measures from each rat strain.[c]

(A)

| | Factor | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| NOE test | | | | |
| Time exploring object | – | −.51 | – | .47 |
| Contact frequency | – | −.86 | – | – |
| Contact latency | – | .76 | – | – |
| Hole board test | | | | |
| Time spent head-dipping | – | – | .93 | – |
| Head dipping frequency | – | – | .93 | – |
| Crossings | .81 | – | – | – |
| Grooming time | −.64 | – | – | – |
| Y-maze | | | | |
| Novel arm time | – | – | – | .90 |
| Novel arm frequency | – | – | – | .78 |
| Emergence test | | | | |
| Latency-in | .77 | – | – | – |
| Emergence latency | −.45 | – | – | – |

(B)

| | Factor | |
|---|---|---|
| | 1 | 2 |
| NOE test | | |
| Time exploring object | – | −.60 |
| Contact frequency | – | −.69 |
| Contact latency | – | −.56 |
| Hole board test | | |
| Time spent head-dipping | .58 | – |
| Head dipping frequency | .77 | – |
| Crossings | .67 | – |
| Grooming time | −.55 | – |
| Y-maze | | |
| Novel arm time | – | −.62 |
| Novel arm frequency | – | −.51 |
| Emergence test | | |
| Latency-in | .53 | – |
| Emergence latency | −.47 | – |

(C)

| | RHA-I rats (n = 36) | | RLA-I rats (n = 36) | |
|---|---|---|---|---|
| | Factor | | Factor | |
| | 1 | 2 | 1 | 2 |
| NOE test | | | | |
| Time exploring object | .58 | – | .43 | – |
| Contact frequency | .64 | – | – | .68 |
| Contact latency | −.48 | – | – | −.86 |
| Hole board test | | | | |
| Time spent head-dipping | – | .96 | −.82 | – |
| Head dipping frequency | – | .94 | −.84 | – |
| Crossings | −.64 | – | – | – |
| Grooming time | – | – | – | −.43 |
| Y-maze | | | | |
| Novel arm time | .48 | – | .59 | – |
| Novel arm frequency | – | – | .47 | .45 |
| Emergence test | | | | |
| Latency-in | −.64 | – | – | .56 |
| Emergence latency | – | – | – | −.44 |
| Total explained variance= | 39.6% | | | 40% |
| Correlation between factors= | −.002 | | .028 | |

[a] Loadings > 0.40 are shown. Total explained variance = 65.8%.
[b] Loadings > 0.40 are shown. Correlation between factors = −0.116. Total explained variance = 39.3%.
[c] Loadings > 0.40 are shown.

**Table 4**
Multiple regression analyses (forward stepwise method) among behavioral variables.

| Dependent variable | Step | Predictor variable | *R* | Square *R* | *p* |
|---|---|---|---|---|---|
| RHA + RLA ($n = 72$) | | | | | |
| Novel arm time (Y-maze test) | 1 | Time exploring object (NOE test) | .324 | .105 | .006 |
| – | | | | | |
| RHA ($n = 36$) | | | | | |
| Novel arm time (Y-maze test) | 1 | Time exploring object (NOE test) | .373 | .139 | .025 |
| – | | | | | |
| RLA ($n = 36$) | | | | | |
| # Entries in novel arm (Y-maze test) | 1 | Contact latency (NOE test) | −.43 | .185 | .011 |

anxiety also plays a (confounding) role in both the HB and emergence tests, as actually suggests the association between self-grooming, head-dipping and emergence latency (see Table 3B).

As said earlier, the observed between-strain differences in novelty-seeking behavior are consistent with results from previous studies carried out either with the outbred Roman rat lines (e.g., Driscoll et al., 1998, 2009; Fernández-Teruel et al., 1992a, 2002a; Steimer et al., 1998; Tournier et al., 2013) or with the inbred Roman strains (e.g., Driscoll et al., 2009; Escorihuela et al., 1999; Estanislau et al., 2013; Fernández-Teruel et al., 2002b; Guitart-Masip et al., 2006a; Manzo et al., 2014; Río-Alamos et al., 2015). A large body of evidence suggests that the mesolimbic dopaminergic system plays a role in novelty/incentive-seeking behaviors, including addiction liability (Bardo et al., 1996; Hooks et al., 1994; Kabbaj et al., 2004; Tournier et al., 2013; Tverdic and Kocevski, 2008). Interestingly, the Roman rat strains/lines are known to differ in terms of this reward system (e.g., Driscoll et al., 2009; Giorgi et al., 2007; Guitart-Masip et al., 2006b; Tournier et al., 2013). For example, RHA rats show higher levels of dopamine turnover in the caudate nucleus and greater response to the nonselective dopamine agonist apomorphine (Driscoll et al., 1985, 1990; Giménez-Llort et al., 2005); a greater expression of dopamine receptors $D_1$ and $D_3$ in the nucleus accumbens (Giorgi et al., 1994; Guitart-Masip et al., 2006b, 2008a); and higher basal levels of dopamine, noradrenaline, and serotonin in the nucleus accumbens and striatum, some of which correlate with RHAs' impulsive behavior (Moreno et al., 2010). Some drugs of abuse (e.g., morphine, amphetamine, cocaine, ethanol), lead to greater activation of the mesotelencephalic dopaminergic pathway in RHA than in RLA rats (Corda et al., 2014; Giorgi et al., 2005, 2007; Lecca et al., 2004). The RHA strain also shows behavioral sensitization in response to repeated administration of amphetamine, cocaine, and morphine (Corda et al., 2005; Giorgi et al., 2007; Guitart-Masip et al., 2008b; Piras et al., 2003), a crucial phenomenon for identifying vulnerability to addiction in both animals and humans (Everitt and Wolf, 2002). Importantly, results from a recent study suggest a link between low $D_2$ auto-receptor availability, increased midbrain presynaptic DA tone and enhanced novelty seeking in RHA rats (Tournier et al., 2013, and references therein). Furthermore, outbred male RHA rats exhibit higher levels of sexual motivation and performance than their RLA counterparts (Sanna et al., 2014a). Such higher levels of sexual motivation and performance of RHA rats are associated with a more robust functional tone of the mesolimbic dopaminergic system, as indicated by studies with apomorphine and haloperidol (Sanna et al., 2014b) and by a larger dopamine output assessed by brain microdialysis in the nucleus accumbens of RHA vs RLA rats during both the appetitive and the consummatory phases of sexual activity (Sanna et al., 2015). These neurochemical and pharmacological differences seem to be related to the different behavioral and (in particular) novelty-seeking profiles in the Roman strains, which may be due, at least in part, to strain-dependent differences in the functional properties of their mesolimbic (and possibly hypothalamic) dopaminergic pathways (for reviews see Driscoll et al., 2009; Giorgi et al., 2007; Tournier et al., 2013).

## 5. Conclusions

Altogether, factorial and regression analyses lend support to the face and construct validity of the present NOE procedure as a novelty-seeking test with significant ability to predict other novelty-seeking trait scores. The possible advantages of this test are that it is of very easy administration, fast and inexpensive (as it involves only the introduction of a pencil in the home-cage). In addition, given the neurobiological connection between novelty seeking and proneness to drug taking (Bardo et al., 1996), identifying individual differences in novelty/sensation seeking during late adolescence that continue throughout adulthood will allow us to analyze its potential usefulness as a risk marker for vulnerability to drug use and abuse.

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