

# Specific Effects of Characteristics of Enriched Environment on Innovative Problem Solving by Animals

Sha-Te Cheng<sup>1,2</sup>, Sha Liu<sup>1,2</sup>, Bo Ou-Yang<sup>1,2</sup>, Xin-Yu Dai<sup>1,2</sup>,  
and Liang Cheng<sup>1,2</sup> 

<sup>1</sup>School of Psychology, Central China Normal University, and <sup>2</sup>Key Laboratory of Adolescent Cyberpsychology and Behavior, Ministry of Education, Central China Normal University

## Abstract

Numerous studies have revealed that an enriched environment can enhance the survival-related behaviors and brain functions of animals. However, the effects and specific roles of the enrichment characteristics on animals' innovative capability, a cognitive ability crucial for survival in nature, are still not well known. In this study, we assigned mice to environment-manipulation groups ( $n = 15$  each) to investigate the specific effects of environmental novelty (novel vs. familiar) and environmental complexity (complex vs. normal) on innovative problem solving and its possible neural mechanisms. Results showed that mice in only the novel-environment group performed better at innovative-problem-solving tasks and showed greater numbers of novel explorations and dopaminergic projections from the ventral tegmental area to the nucleus accumbens in the brain. These findings indicate that an enriched environment has the potential to promote the innovative capability of mice by enhancing their novel exploratory motivation, which depends on the novelty of the environment but not its complexity.

## Keywords

enriched environment, novelty and complexity, innovative problem solving, novel-seeking motivation, dopamine

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*Environmental enrichment* traditionally involves customizing the environments of captive animals to enhance their physical and psychological well-being by providing stimuli that meet their species-specific needs (Baroncelli et al., 2010; Nithianantharajah & Hannan, 2006; Wolfer et al., 2004). In the laboratory setting, an enriched environment can be created by providing a cognitively and physically stimulating living space that enables spontaneous exploration (Kempermann, 2019; van Praag et al., 2000). Until now, numerous laboratory studies, in which the environment was created and controlled, have shown that animals maintained in an enriched environment show enhanced behavioral abilities. For example, they perform better in behavior tasks, engage in exploration more often, and demonstrate a higher level of activity (Gabriel et al., 2020; Pietropaolo et al., 2004; Schrijver et al., 2002; Sparling et al., 2020). The influence of an enriched environment on animals can also be seen in the context of brain-related functions,

in particular, cognition (Puurunen & Sivenius, 2002; Widman & Rosellini, 1990). For instance, Garthe et al. (2016) reported that mice raised in an enriched enclosure displayed better spatial cognition in the Morris water maze than mice raised in a barren environment. These findings revealed that environmental enrichment can enhance the behavior and related cognitive ability of animals, allowing animals to more rapidly collect and memorize environmental information and adapt to the environment more easily, ultimately improving their survival rate.

Innovation, an advanced form of cognition that refers to new or modified learned behavior and that introduces novel behavioral variants into a population's repertoire (Reader et al., 2016; Reader & Laland, 2003), is

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## Corresponding Author:

Liang Cheng, Central China Normal University, School of Psychology  
Email: chengl@ccnu.edu.cn

also of considerable significance to animals' survival in the wild. Animals with good innovation tend to collect plenty of information about the physical and social environment, create new behaviors (Sol et al., 2005), and adapt to and use new or unfamiliar resources efficiently (Hopper & Torrance, 2019; Lefebvre et al., 1997). However, the effects of an enriched environment on innovation capability have received little attention and are not yet elucidated. As described above, previous studies have demonstrated that animals living in enriched environments have higher levels of exploration activity (Genaro & Schmidek, 2000; Pietropaolo et al., 2004). Reportedly, there is a significant positive correlation between exploratory behaviors and innovative behaviors, which means that animals with more exploratory behaviors should have a higher success rate in problem solving (Keynan et al., 2015; Webster & Lefebvre, 2001). It may, therefore, be hypothesized that enriched environments improve animals' performance when solving innovative problems (innovation capability) by enhancing their exploratory behavior.

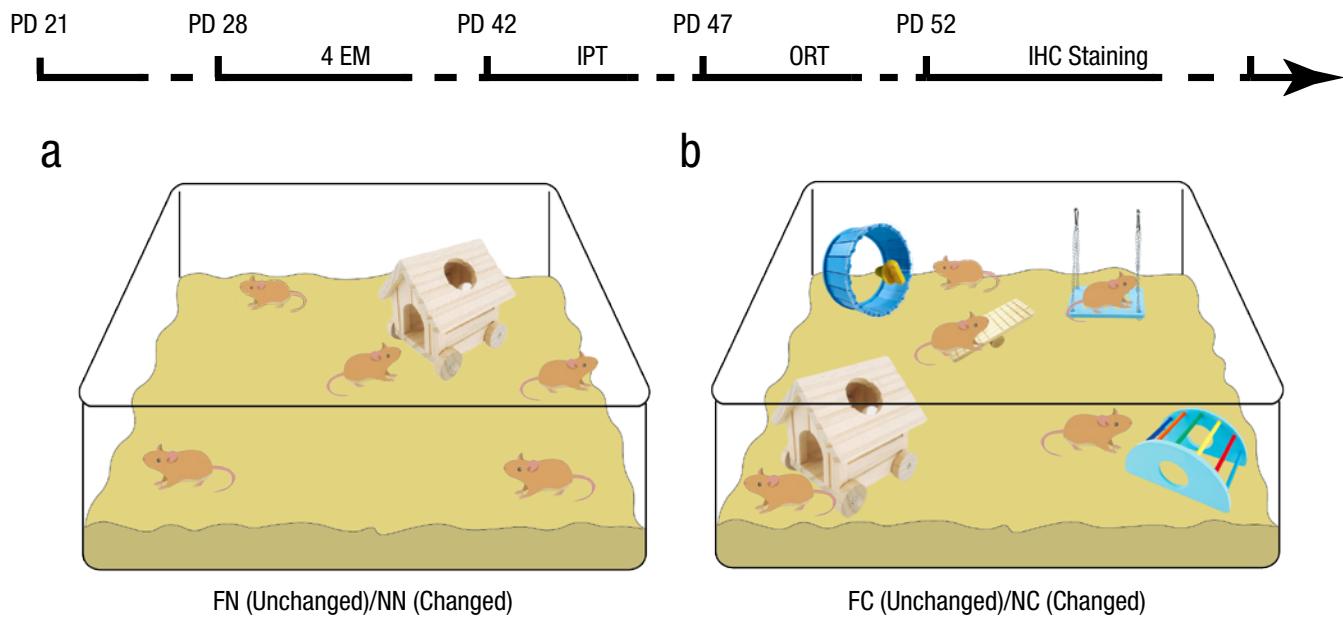
In most enrichment studies in rodents, the stimulus is provided in the form of a variety of objects or toys to stimulate the animals' attention and engagement in the environment (Cheng et al., 2014; Kempermann, 2019; Newberry, 1995). Examples include running wheels, puzzles, and accessories (e.g., mazes, toys, ropes, ladders, hanging objects, and platforms). Because animals provided with environmental enrichment are compared with controls in standard housing conditions, past studies have usually treated environmental enrichment as a whole, single condition and compared the various effects with those in the control group. However, environmental enrichment is a composite condition with various aspects, such as environmental complexity (multiple objects) and environmental novelty (objects in a new place or new objects; Garthe et al., 2016; Widman & Rosellini, 1990; Zeleznikow-Johnston et al., 2017). It is particularly essential for researchers to consider the experimental components of the environmental enrichment as well as the specific roles of the different characteristics. Thus, in the present study, enriched environments were arranged to facilitate the study of two separate characteristics, complexity and novelty, in order to explore the influence of environmental enrichment on murine innovative capability.

We were additionally interested in the neural mechanisms underlying the potential effects of environmental enrichment on the innovative capability of animals. Dopamine is an abundant neurotransmitter in the brain and is probably a component of innovative problem solving in animals. For example, studies in mice have demonstrated that blocking the dopamine receptor correspondingly decreased exploration of novel stimuli

### Statement of Relevance

Environmental stimulation has been considered an important factor affecting the brain development of human and animals. Investigating how the environment may impact brain functions, especially cognition, is beneficial for understanding characteristics of brain development and also opens the possibility of testing putative interventions for some problems in brain development. Here, we focused on mice's innovative capability, a cognitive ability crucial for survival in nature, and explored its alteration by environmental enrichment and the possible mechanisms underlying this capability. The results indicated that an enriched environment has the potential to promote the innovative capability of mice by enhancing their novel exploratory motivation, which depends on the novelty of the environment but not its complexity. These findings have substantial translational value because promoting the brain's cognitive ability through manipulated environmental conditions will aid in the advancement of treatment for cognitive disorders in clinical environments.

(Dulawa et al., 1999); similarly, dopamine injections increased novelty-seeking motivations (Flaherty, 2005). In agreement with this, the findings of Cui and colleagues have shown that dopamine and dopamine receptors in mice were associated with flexibility in strategy transformation, which is closely related to innovation (Cui et al., 2018; Gilhooly et al., 2007). Given these findings, a plausible assumption is that alterations in brain dopamine levels may account for the influence of enriched environmental stimuli on innovative problem solving in animals. To test this, we used immunohistochemical staining to identify alterations in response to environmental enrichment in the dopaminergic neurons or projecting nerve fibers of the ventral tegmental area (VTA), nucleus accumbens (NA), and prefrontal cortex (PFC), which regulate motivation and cognitive flexibility in the mouse brain. We also investigated the relationships between the changes and the innovative-problem-solving ability of mice (Chau et al., 2018; Floresco, 2013; Lammel et al., 2014). In this study, weanling mice were used as subjects because the developing brain, especially during the critical period, is more susceptible and shows greater plasticity to environmental influences in areas such as neurogenesis, cell survival, synapse maturation, compared with a more mature adult brain (Kempermann, 2019; Nithianantharajah & Hannan, 2006).



**Fig. 1.** Experimental arrangement and manipulation in the present study. The timeline shows the treatment schedule of the mice. After 1 week of adaptation to the housing, from Postnatal Day (PD) 21 to PD 28, four groups started the environmental manipulation (EM), which lasted for 14 days (from PD 29 to PD 42). Then, from PD 43 to PD 52, mice completed two behavioral tests: the innovative-problem-solving test (IPT) and object-recognition test (ORT). On PD 53, mouse brains were dissected and immunohistochemical (IHC) staining was used to determine the effects of environmental enrichment. Illustrations of the living conditions in different experimental groups are shown in (a) and (b). In the novel-normal (NN) group, one object was placed in the cage and changed every day; in the novel-complex (NC) group, five objects were provided and changed every day; in the familiar-normal (FN) group, one object was provided and not changed; and in the familiar-complex (FC) group, five objects were provided and not changed.

## Method

### Animals and grouping

As experimental subjects, we used 60 male Kunming mice (*Mus musculus*; 21 days old; 10–12 g in body weight), which were provided by the Hubei Experimental Animal Research Center. An a priori power analysis (G\*Power Version 3.1.9; Faul et al., 2007) suggested that a sample size of at least 48 total subjects would be necessary to detect an effect size ( $\delta$ ) of 0.4 with 80% power and an  $\alpha$  of .05 (O'Connor et al., 2014). Only male mice were chosen for experiments to avoid potential variability due to sex differences or behavioral changes in a mixed breeding condition, as well as to maintain a high level of exploration activities and risk-taking behaviors in the subjects (Palanza, 2001; Pisula & Siegel, 2005).

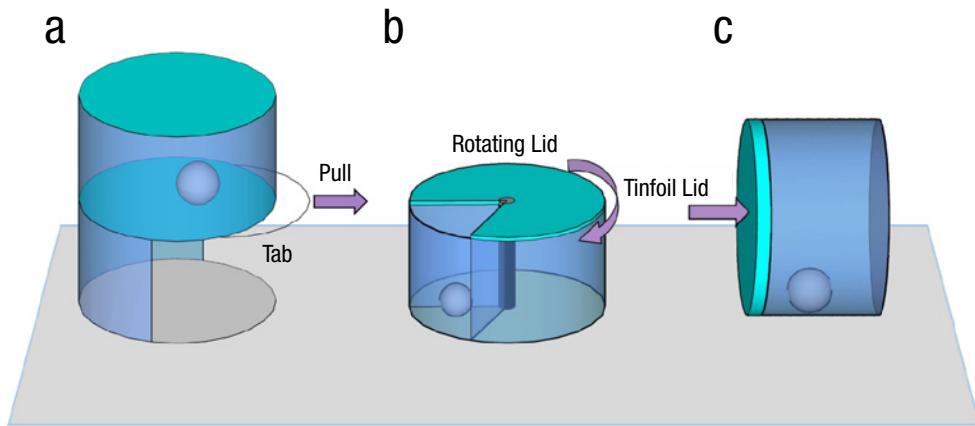
Groups of five mice were housed in each plastic cage, and sawdust covered the bottom of the cages. The mice were assigned randomly to four groups ( $n = 15$  for each group) according to the experiment's 2 (environmental novelty: novel vs. familiar)  $\times$  2 (environmental complexity: complex vs. normal) between-subjects design. In the novel-normal (NN) group, one object was placed in the cage and changed every day; in the novel-complex (NC) group, five objects were

provided and changed every day; in the familiar-normal (FN) group, one object was provided and not changed; and in the familiar-complex (FC) group, five objects were provided and not changed (Fig. 1).

The objects placed in the cage were mainly small-animal toys, such as a plastic track, running wheel, swing, or colorful ladder, which have been used for environmental enrichment in previous studies (Garthe et al., 2016; Widman & Rosellini, 1990; Zeleznikow-Johnston et al., 2017). All four groups were kept in the same room with a natural light cycle and allowed free access to food and water. The ambient temperature was maintained at 20 to 25 °C. The mice were allowed to reach a body weight of at least 25 g before food restriction was imposed at 2.5 weeks after weaning to maintain them at 85% of their free-feed body weight. After 1 week of adaptation to the housing, environmental treatment started at 2 weeks after weaning and lasted for 14 days, after which the mice were taken to the laboratory to become familiarized with the experimental environment before undergoing behavioral tests on the 15th day.

### Innovative-problem-solving tests

Three innovative-problem boxes, adapted from the work of Thornton and Samson (2012), were used in the present study to test the innovative-problem-solving



**Fig. 2.** Illustration showing the three boxes used to detect innovative problem solving (food was a reward in the box). In Task 1 (a), mice needed to pull out the tab to cause the food to drop. In Task 2 (b), mice needed to rotate the lid until the gap in the lid appeared over the food. In Task 3 (c), mice needed to poke through the tinfoil to get the food.

ability of mice by measuring the rate and time taken to successfully solve the problems (Fig. 2). In the tests, mice had to work out different ways to obtain food from the boxes. Box 1 (Task 1, push-pull task) was a transparent plastic cylindrical box with an opening at the bottom that allowed the mice access. Food was placed on a movable panel in the middle of the box, and to receive the food, the mice had to push or pull this panel, which resulted in the food dropping to the bottom. Box 2 (Task 2, rotating task) was a transparent plastic cylindrical box with a transparent plastic rotatable lid. The mice could get the food in the partitioned area of the box by rotating the lid to create an opening. Box 3 (Task 3, jar-breaking task) was a transparent plastic box covered with a tinfoil lid that the mice could poke through to get access to the food. Before the tests, the mice were taken to the laboratory and familiarized with the environment for 1 hr. Then each mouse was given 60 min (20 min for each box) to finish the three tasks. The mouse was immediately moved to the next task after finishing the previous one. If a mouse successfully solved the problem, the timer was stopped and the time recorded; if the mouse failed to solve the problem within 20 min, the time was recorded as 20 min.

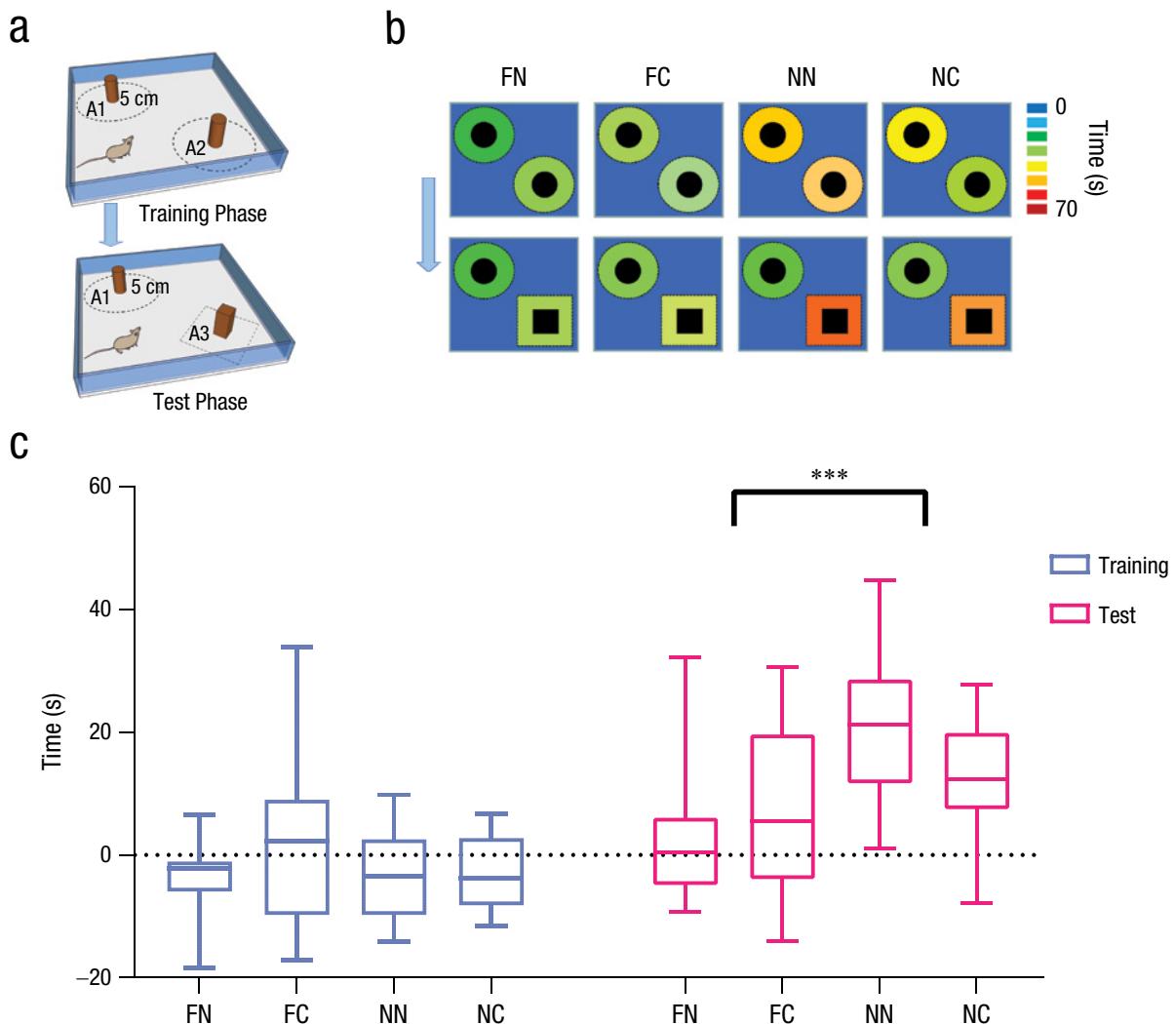
### Object-recognition test

To interpret the effect of novel environmental stimuli on innovative problem solving, we examined the novelty-seeking behaviors of mice using the object-recognition test, in which the mice were allowed to explore the objects freely. The test procedure in this experiment was divided into the training phase and the testing phase. In the training phase, two identical cylindrical objects (A1 and A2) were placed in symmetrical corners

of a square box (25 cm length × 25 cm width × 15 cm height; Fig. 3a). At the beginning of the test, each mouse was placed in a third corner of the box facing the wall so its nasal tip was roughly equidistant from the two objects. The mice were allowed to explore the two objects for 5 min, and the exploratory-behavior time was recorded. Exploratory behaviors were defined as sniffing, licking, or touching objects or staring at objects 5 cm or less away; lying motionless on the identified object was not considered exploratory behavior. All mice that completed this stage were moved to the next stage (test phase) after at least a 1-hr rest. In the test phase, one of the cylindrical objects was replaced with a completely new object, a cuboid (A3). Then the procedure described for the training phase was repeated, and the amount of time the mouse explored the two objects within 5 min was recorded. Finally, the difference in the exploratory-behavior times between the two objects ( $A_2 - A_1$  and  $A_3 - A_1$ ) was used as an indicator of novelty-seeking behavior in response to a new environmental stimulus.

### Immunohistochemical staining

Immunohistochemical staining of tyrosine hydroxylase, a key rate-limiting enzyme in dopamine synthesis, was used to examine alterations in the dopamine neurons and fibers of the VTA, NA, and PFC in response to the different external environments. Five mice chosen randomly from each group were used for the staining experiment. The staining procedures were basically the same as those in previous studies (Greenwell et al., 1991; Hecke, 2002; Jackson & Blythe, 2008). Briefly, after being deeply anesthetized with Nembutal, the mouse was perfused and washed with normal saline



**Fig. 3.** Design of and results from the object-recognition test. The experimental arrangement for the test is shown in (a). In the training phase, two identical cylindrical objects (A1 and A2) were placed in symmetrical corners of a square box. In the test phase, one of the cylindrical objects was replaced with a completely new object, a cuboid (A3). Average exploration time (within 5 cm around the objects) is shown separately (b) for the familiar-normal (FN), familiar-complex (FC), novel-normal (NN), and novel-complex (NC) groups. The top and bottom rows show exploration time of mice in the training and test phases, respectively. The graph (c) shows exploration time in the training phase (A2 – A1) and test phase (A3–A1), separately for each group of mice ( $n = 14$ ). In each box-and-whisker plot, the center line represents the median, the box indicates the first and third quartile (25th and 75th percentile) of the data, and the whiskers mark the upper and lower extremes of the data. The horizontal dashed line indicates zero difference between the two objects (A2 vs. A1, A3 vs. A1). Asterisks indicate a significant difference in performance between the novel and familiar groups (\*\*\*( $p < .001$ )).

through the left ventricle-aorta cannula and fixed with 4% paraformaldehyde for 1 hr. The brain was dissected and postfixed in 4% paraformaldehyde solution for 10 hr before dehydration with a graded series of ethyl alcohol at 50%, 70%, 85%, 95%, and 100% (2 hr). After treatment for transparency with a mixed solution of alcohol and xylene (2 hr), the brain tissue was embedded with paraffin wax and then cut into continuous slices (4–7  $\mu$ m) on a microtome (Finesse 325, Thermo Shandon, UK). The slices were dewaxed and rehydrated with alcohol and pure water and washed three times (5 min each time) with phosphate-buffered saline. The

endogenous peroxidases in the slices were blocked with 3% hydrogen peroxide at room temperature for 20 min. The brain slices were washed again with phosphate-buffered saline and sealed with 10% goat serum; they were then incubated with the primary antibody to tyrosine hydroxylase (PB0470, Boster, China) at 37°C for 12 hr. Immunoenzymatic reactions were performed with biotinylated (horseradish peroxidase-labeled) secondary antibody (SA1022, Boster, Wuhan, China) revealed with 3,3'-diaminobenzidine (DAB, a chromophoric reagent). After staining, the slices were observed under an upright microscope (CX31; Olympus,

Tokyo, Japan), and images were acquired with a digital camera (D5100; Nikon, Tokyo, Japan). The VTA, NA, and PFC were localized in the slices according to the *Mouse Brain Atlas* (Paxinos & Franklin, 2001). From this data, the amount of stained area was quantified pixel by pixel using *ImageJ* software (Version 1.8.0; Rasband, 1997). For the above analysis, five images per sample and five samples per group were included.

## Statistical analysis

All statistical analysis was calculated using SPSS (Version 22.0). Descriptive characteristics of group variables were expressed as means and standard deviations. The significance of variables between groups was assessed using  $\chi^2$  test, logistic regression (results are reported as odds ratios [*ORs*]), multivariable linear regression (results are reported as  $\beta$ s), and analysis of variance (ANOVA), and a Student-Newman-Keuls post hoc test was used when appropriate. The confidence interval (CI) was set to 95%, and the margin of error accepted was adjusted to 5%. Only  $p$  values less than .05 were considered significant.

## Results

### Innovative-problem-solving test

A three-problem-boxes paradigm was used to examine changes in the innovative-problem-solving performance of mice following different environmental treatments ( $n = 15$  for each group). The results showed that mice in the novel-normal and novel-complex groups performed significantly better in Task 1 than the familiar-normal and familiar-complex groups, as they displayed a higher success rate,  $\chi^2(1, N = 60) = 5.46, p = .02$  (Fig. 4a), and required less time to solve the problem ( $\beta = -0.59, p = .001, 95\% \text{ CI} = [-1.83, -0.52]$ , adjusted  $R^2 = .20$  (Fig. 4b). However, we found no significant difference between the complex and normal groups—success rate:  $\chi^2(1, N = 60) = 0.22, p > .05$ ; problem-solving time:  $\beta = -0.21, p > .05, 95\% \text{ CI} = [-1.07, 0.24]$ , adjusted  $R^2 = .20$ —both with novel and familiar environments—success rate:  $\chi^2(1, N = 30) = 3.33, p > .05, \chi^2(1, N = 30) = 2.14, p > .05$ , respectively; problem-solving time:  $\beta = 0.22, p > .05, 95\% \text{ CI} = [-0.43, 1.42]$ , adjusted  $R^2 = .20$ .

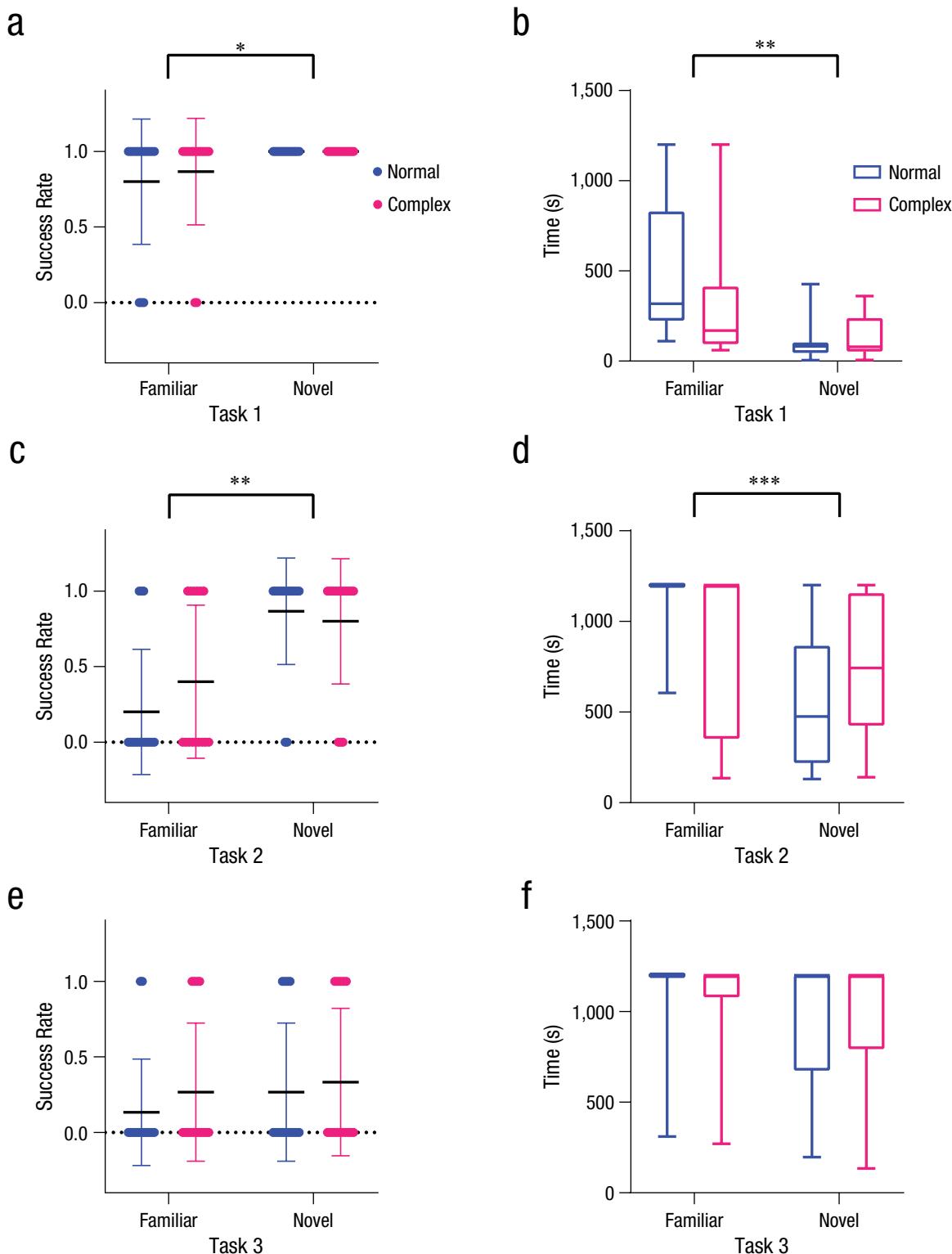
Similar to mice in Task 1, mice housed in the novel conditions also had a higher success rate in Task 2 (odds ratio [*OR*] = 26.0,  $p = .001, 95\% \text{ CI} = [-3.69, -183.42]$ , Nagelkerke  $R^2 = .38$ ; Fig. 4c) and a shorter problem-solving time ( $\beta = -0.70, p < .001, 95\% \text{ CI} = [-2.02, -0.75]$ , adjusted  $R^2 = .25$ ; Fig. 4d). There was no effect on performance of either environmental complexity (success rate: *OR* = 2.67,  $p > .05, 95\% \text{ CI} = [0.52, 13.66]$ ,

Nagelkerke  $R^2 = .38$ ; problem-solving time:  $\beta = -0.26, p > .05, 95\% \text{ CI} = [-1.16, 0.12]$ , adjusted  $R^2 = .25$ ) or its interaction with novelty (success rate: *OR* = 0.23,  $p > .05, 95\% \text{ CI} = [0.02, 2.95]$ , Nagelkerke  $R^2 = .38$ ; problem-solving time:  $\beta = 0.37, p > .05, 95\% \text{ CI} = [-0.06, 1.74]$ , adjusted  $R^2 = .25$ ). However, in Task 3, the breaking task, there was no significant difference among all four groups in either performance (novelty:  $\beta = -0.14, p > .05, 95\% \text{ CI} = [-1.01, 0.47]$ ; complexity:  $\beta = -0.02, p > .05, 95\% \text{ CI} = [-0.79, 0.70]$ ; interaction:  $\beta < 0.01, p > .05, 95\% \text{ CI} = [-1.05, 1.05]$ , adjusted  $R^2 = -.03$ ; Fig. 4f) or success rate (novelty: *OR* = 2.36,  $p > .05, 95\% \text{ CI} = [0.36, 15.46]$ ; complexity: *OR* = 2.36,  $p > .05, 95\% \text{ CI} = [0.36, 15.46]$ ; interaction: *OR* = 0.58,  $p > .05, 95\% \text{ CI} = [0.05, 6.72]$ , Nagelkerke  $R^2 = .04$ ; Fig. 4e).

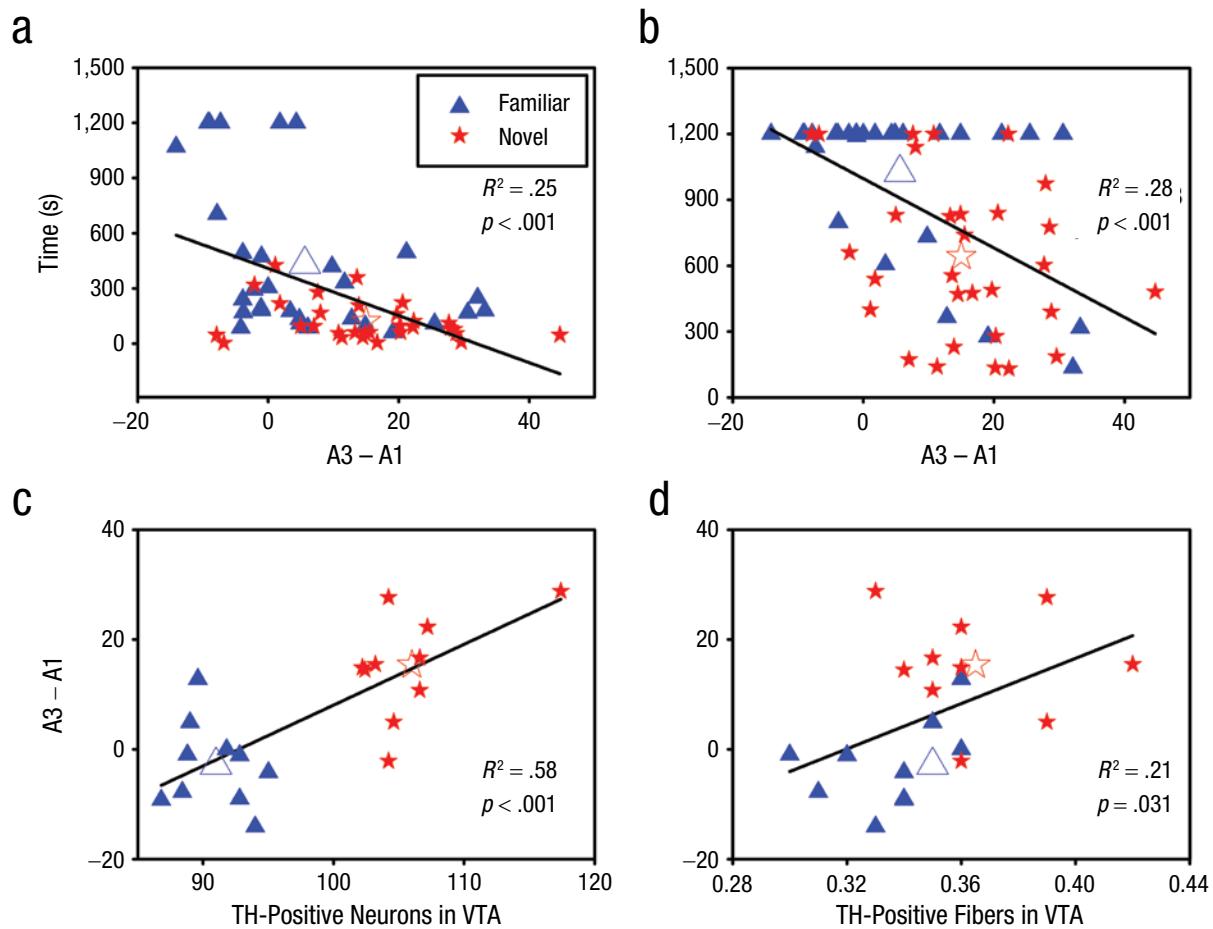
All these results suggest that the novelty, not the complexity, of the enriched environment promoted the innovative-problem-solving ability of mice. For analyses, two sets of linear regression models—the logistic regression and multivariable linear regression—were conducted. The dependent variables for the models were success rate and time spent solving the problems, and the independent variables were environmental novelty, environmental complexity, and their interactions. No other variable was controlled as a covariate. Note that for success-rate analysis in Task 1, a  $\chi^2$  test was used instead of logistic regression (performed in Task 2 and Task 3), because the data distribution of this task was not suitable for the logistic regression model.

### Object-recognition test

To further explore the underlying reasons for the effect of a novel environment on innovation problem-solving ability, we tested the novel exploratory behavior of mice in an object-recognition test (Fig. 3a). Note that the data for only 56 ( $n = 14$ ) of the 60 mice were included in the analysis because four mice (one in each group) showed discomfort or died before or during the test (Postnatal Days 47–52). The results showed that, in the training phase, the exploration behaviors of mice in all groups were quite similar for the two objects (close to 0), but in the test phase, mice spent more time exploring the new object A3 (A2 – A1 vs. A3 – A1),  $F(1, 52) = 57.11, p < .001, \eta^2 = .52$  (Figs. 3b and 3c). Further statistical analysis showed that, in the test phase, the novel group showed higher exploration levels with the new object than the familiar group,  $F(1, 52) = 6.55, p = .013, \eta^2 = .11$ , but no significant difference was found between the complex and normal groups,  $F(1, 52) = 0.05, p > .05, \eta^2 < .01$ , either in novel or familiar conditions—no interaction,  $F(1, 52) = 2.60, p > .05, \eta^2 = .05$ . The results indicated that the novelty, not the complexity, of the enriched environment enhanced the novel exploration



**Fig. 4.** Effects of environmental novelty (novel vs. familiar) and environmental complexity (complex vs. normal) on performance of mice in the innovative-problem-solving tasks. The graphs on the left show success rate as a function of environmental novelty and environmental complexity in (a) Task 1, (c) Task 2, and (e) Task 3. Horizontal black bars and colored error bars show means and standard deviations, respectively. Horizontal dashed lines indicate a 0% success rate. The colored ovals show the data distributions for each group, separately for mice who successfully completed the task (ovals at 1.0) and those who did not (ovals at 0.0). The box-and-whisker plots on the right show task-completion time as a function of environmental novelty and environmental complexity in (b) Task 1, (d) Task 2, and (f) Task 3. In each box-and-whisker plot, the center line represents the median, the box indicates the first and third quartile (25th and 75th percentile) of the data, and the whiskers mark the upper and lower extremes of the data. Asterisks indicate significant differences between the familiar and novel conditions (\* $p < .05$ , \*\* $p < .01$ , \*\*\* $p < .001$ ).



**Fig. 5.** Correlation among innovative-problem-solving ability, novel exploration levels, and midbrain dopaminergic projections for mice in familiar and novel environments. The top row shows correlations for the difference between exploratory-behavior time for the square (A1) and circular (A3) objects in the test phase of the object-recognition test (*x*-axes) and time to complete innovative problem solving (*y*-axes), separately for mice in the familiar and novel groups of (a) Task 1 and (b) Task 2. The bottom row shows correlations between midbrain dopaminergic projections (*x*-axes) and the difference between exploratory-behavior time for the square (A1) and circular (A3) objects in the test phase of the object-recognition test (*y*-axes), separately for mice in the familiar and novel groups. Dopaminergic projections are shown separately for (c) tyrosine hydroxylase (TH)-positive neurons in the ventral tegmental area and (d) TH-positive fibers in the nucleus accumbens. Solid lines show best-fitting regressions.

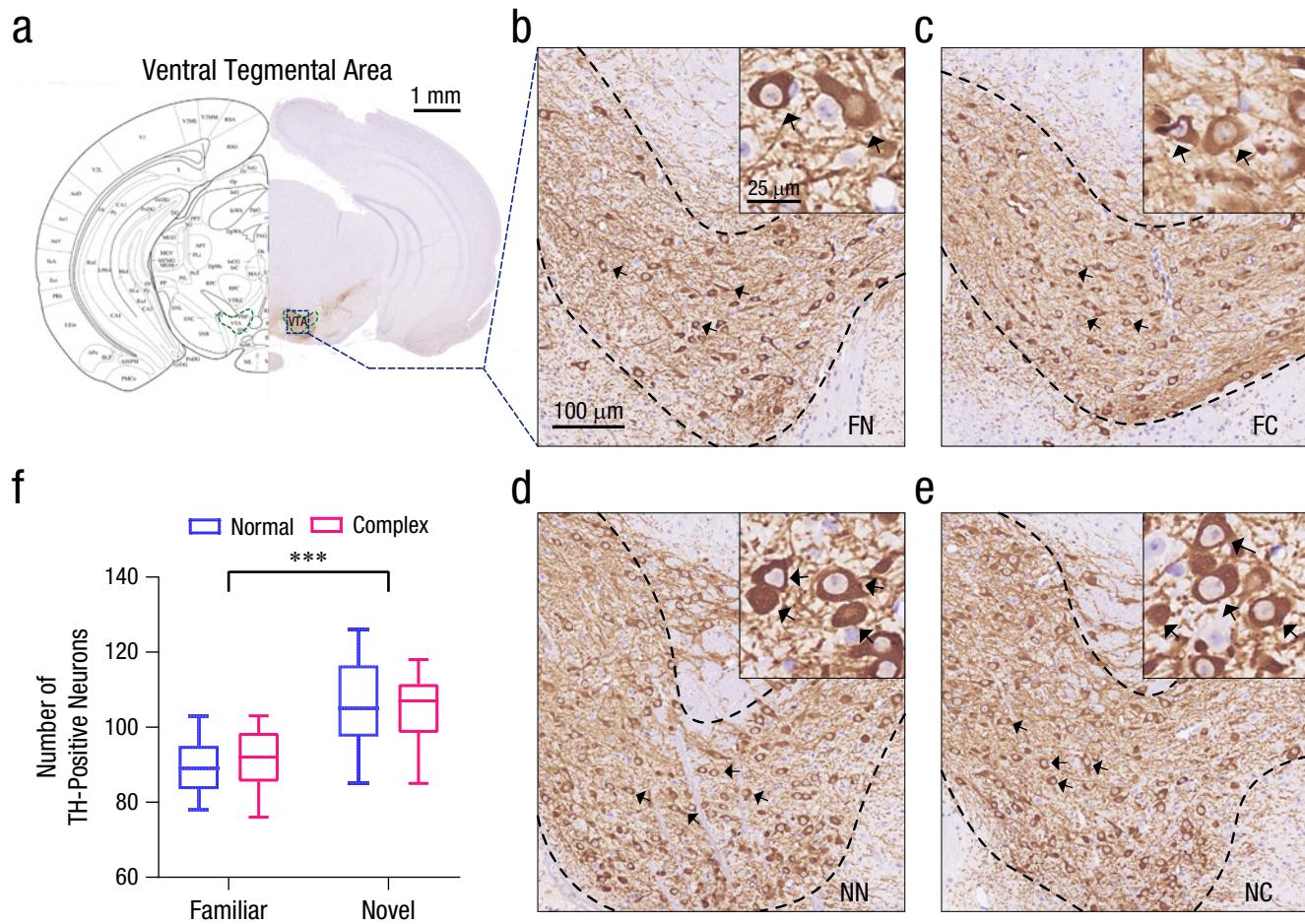
and seeking behavior of mice. This finding is consistent with the results of the innovative-problem-solving task. Regression analyses further demonstrated that, as novel exploration and seeking levels increased, the mice's innovative-problem-solving ability gradually increased ( $R^2 = .25$ ,  $p < .001$  for Task 1;  $R^2 = .28$ ,  $p < .001$  for Task 2; Figs. 5a and 5b). All our findings suggest that novel environmental stimuli promoted the innovative ability of mice by inducing their novel exploration and seeking behavior.

### Immunohistochemical staining

To provide more in-depth evidence that the enhancement of novel exploration and seeking motivation by environmental novelty mediated the innovative ability

of mice, we examined the dopamine neurons or fibers of the VTA, NA, and PFC in the brain, which are closely related to motivation (for the analysis, five images per sample and five samples per group were included). Dopamine neurons in the VTA were stained and counted (Fig. 6a), and we found that the number of dopamine neurons differed among the four groups (Figs. 6b–6e). The VTA of mice in the novel groups had much more positive dopamine neurons compared with that of the familiar groups,  $F(1, 96) = 75.50$ ,  $p < .001$ ,  $\eta^2 = .44$  (Fig. 6f). However, there was no significant difference between the complex and normal groups,  $F(1, 96) < 0.01$ ,  $p > .05$ ,  $\eta^2 < .01$ ; there was no interaction effect as well,  $F(1, 96) = 1.75$ ,  $p > .05$ ,  $\eta^2 = .02$  (Fig. 6f).

The dopamine fibers in the NA showed similar results to dopamine neurons in the VTA (Fig. 7a). A statistically



**Fig. 6.** Tyrosine hydroxylase (TH)-positive neurons in the ventral tegmental area (VTA) of mice in the four groups. Green dashed lines show the location of the VTA in a coronal slice of the mouse brain (a) at ~3.2 mm posterior to the bregma. The black bar shows the scale of the image. The enlargements show representations of TH-positive neurons in the VTA of mice in the (b) familiar-normal (FN), (c) familiar-complex (FC), (d) novel-normal (NN), and (e) novel-complex (NC) groups. Black arrows indicate the TH-positive neurons, and the black bars show the scale of the images. The graph (f) shows the number of TH-positive neurons in the VTA of mice in each of the four groups. In each box-and-whisker plot, the center line represents the median, the box indicates the first and third quartile (25th and 75th percentile) of the data, and the whiskers mark the upper and lower extremes of the data. Asterisks indicate a significant difference between the novel and familiar groups (\*\* $p < .001$ ).

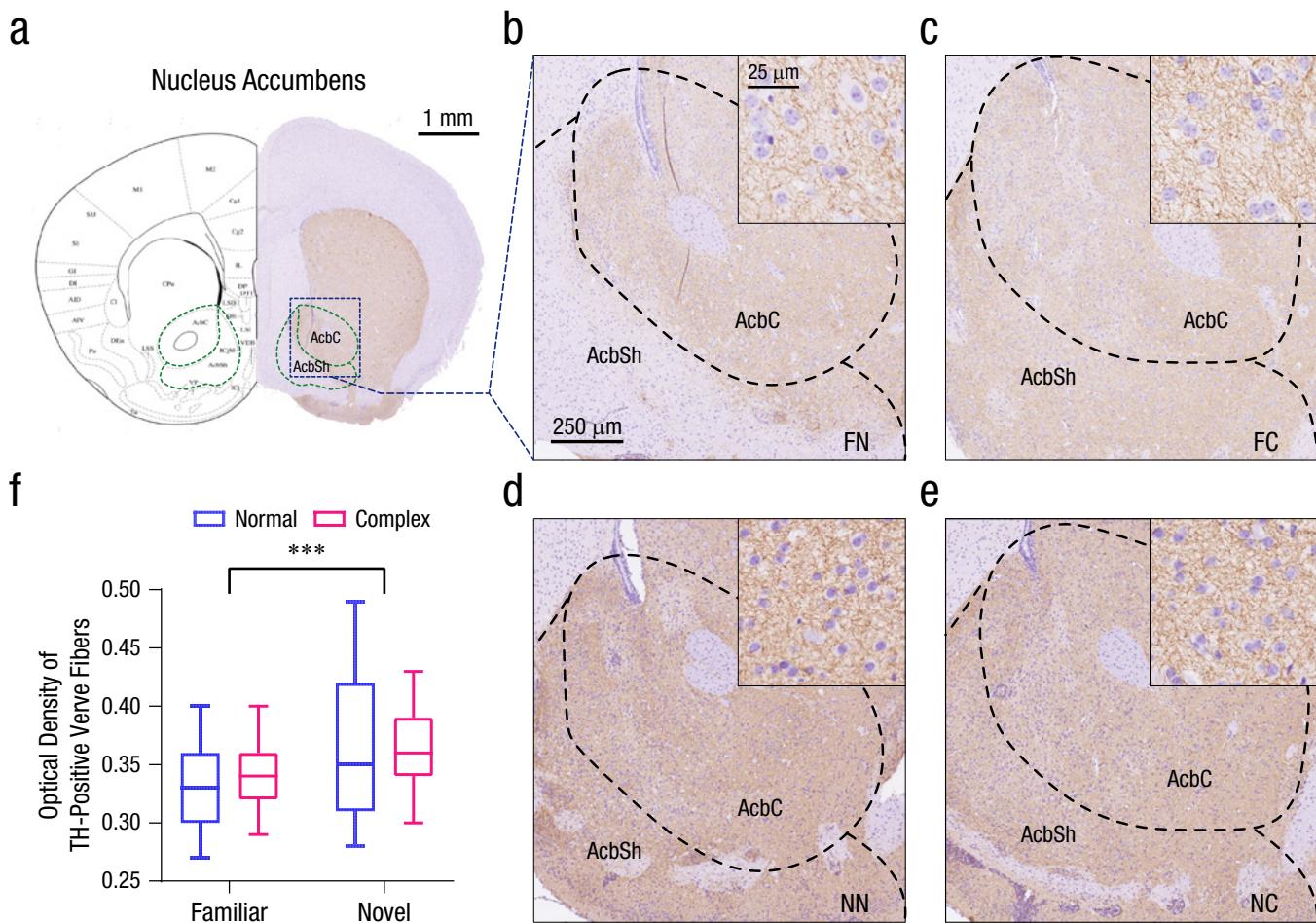
significant difference was found only between the novel and familiar groups—novelty effect:  $F(1, 96) = 14.10$ ,  $p < .001$ ,  $\eta^2 = .13$ ; complexity effect:  $F(1, 96) = 0.08$ ,  $p > .05$ ,  $\eta^2 < .01$ ; interaction effect:  $F(1, 96) = 0.64$ ,  $p > .05$ ,  $\eta^2 < .01$  (Fig. 7f), although the optical density of positive dopamine fibers in the NA differed among the groups (Figs. 7b–7e).

Dopamine fibers in the PFC were also examined (Figs. 8a–8e), but no significant differences were observed among the four groups—novelty effect:  $F(1, 96) = 0.14$ ,  $p > .05$ ,  $\eta^2 < .01$ ; complexity effect:  $F(1, 96) = 0.06$ ,  $p > .05$ ,  $\eta^2 < .01$ ; interaction effect:  $F(1, 96) = 0.38$ ,  $p > .05$ ,  $\eta^2 < .01$ ; Fig. 8f). Regression analyses further demonstrated that the increase in dopamine neurons or fibers in VTA and NA had a positive relationship with novel exploration behaviors ( $R^2 = .58$ ,  $p < .001$ ;  $R^2 = .21$ ,  $p = .031$ ; Figs. 5c, 5d). The results indicated that

environmental novelty enhanced the novel seeking motivation of mice, but complexity had a null effect. These immunohistochemical findings perfectly coincide with the results of the object-recognition test and further confirm that the promotion of innovative ability by a novel, not a complex, environment probably occurred because only environmental novelty affected the novel exploration and seeking motivation of mice.

## Discussion

The aim of the research was to provide evidence for specific effects of environmental characteristics on the innovative capability of mice. As shown by the results, the novel groups, but not the complex groups, demonstrated higher success rates and took less time to complete Tasks 1 and 2 compared with the respective controls,

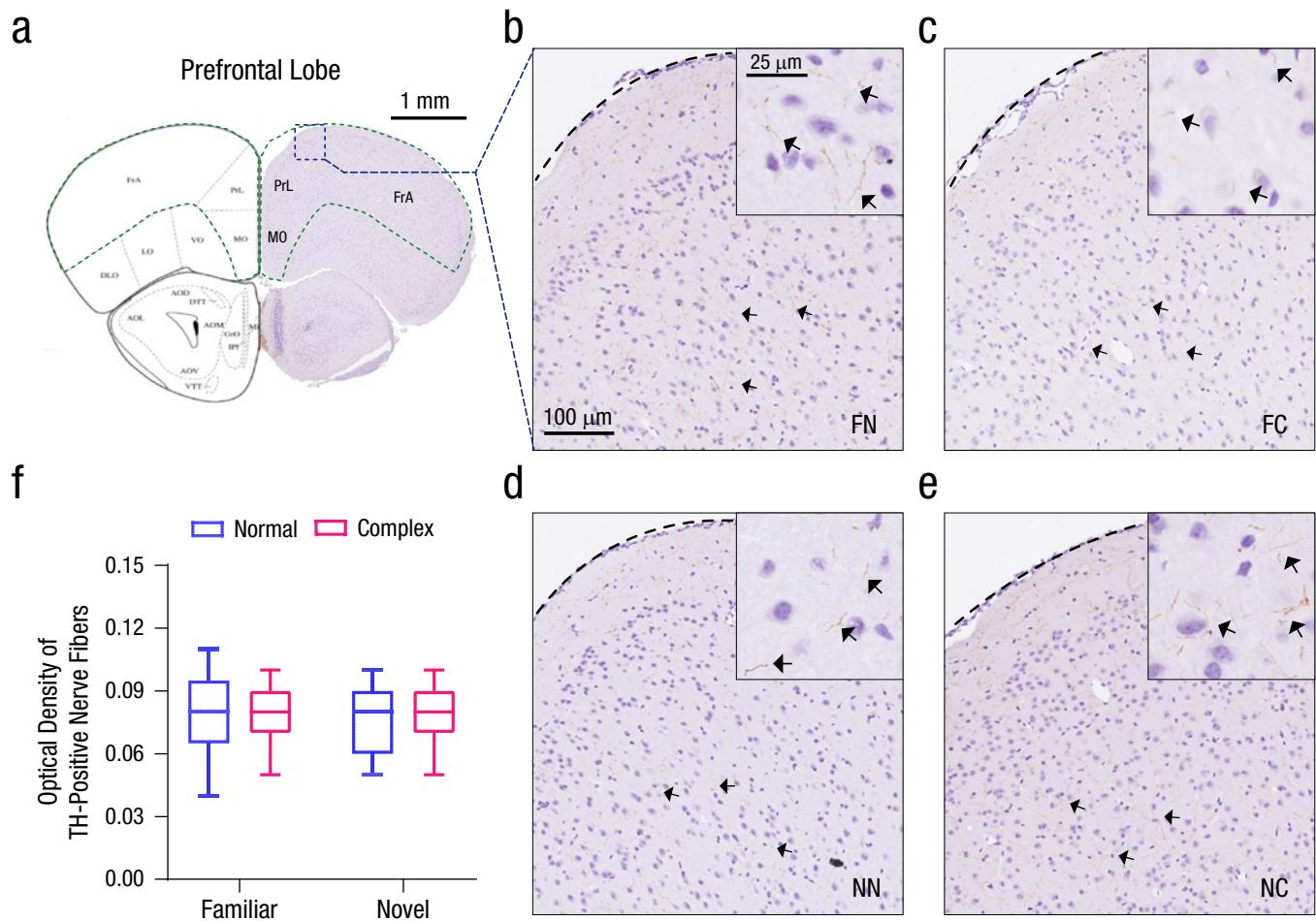


**Fig. 7.** The optical density of tyrosine hydroxylase (TH)-positive nerve fibers in the nucleus accumbens (NA) of mice in the four groups. Green dashed lines show the location of the NA in a coronal slice of the mouse brain (a) at ~1.4 mm anterior to the bregma. The black bar shows the scale of the image. The enlargements show representations of TH-positive nerve fibers in the NA of mice in the (b) familiar-normal (FN), (c) familiar-complex (FC), (d) novel-normal (NN), and (e) novel-complex (NC) groups. The black bars show the scale of the images. The graph (f) shows the number of TH-positive neurons in the NA of mice in each of the four groups. In each box-and-whisker plot, the center line represents the median, the box indicates the first and third quartile (25th and 75th percentile) of the data, and the whiskers mark the upper and lower extremes of the data. Asterisks indicate a significant difference between the novel and familiar groups (\*\*p < .001). AcbSh = nucleus accumbens shell; AcbC = nucleus accumbens core.

which suggests that environmental novelty, but not complexity, had the potential to significantly promote the innovative-problem-solving capability of mice. However, the ability to complete Task 3 did not vary among the mice; the absence of differences among groups was probably due to the level of difficulty (Kurz et al., 2017; Liebherr et al., 2018). Task 3 seemed to be too difficult for the mice (success rates in each group were low) for any significant influence of the environmental conditions to be apparent. In future studies, therefore, the task should be subdivided into tasks of varying difficulty to further explore the specific effects of the environment on the innovative capabilities of animals.

Following the first experiment, we were interested in how environmental novelty promotes innovative problem solving in mice. In previous studies, the

innovative ability of animals was significantly and positively correlated with exploration behaviors—that is, animals who showed more exploration behaviors were more successful at innovative problem solving (Fei et al., 2019; Keynan et al., 2015; Morand-Ferron et al., 2011; Morand-Ferron & Quinn, 2011; Webster & Lefebvre, 2001). Therefore, we speculated that, in the present study, mice raised in the novel environment paid more attention to the novel objects and spent more time exploring, which improved their innovative-problem-solving performance (Ennaceur & Delacour, 1988; Modlinska et al., 2019). The results of the object-recognition test confirmed our conjecture and showed that the time spent exploring the novel objects was significantly longer in the novel groups than in the familiar group, which suggests that environmental novelty



**Fig. 8.** The optical density of tyrosine hydroxylase (TH)-positive nerve fibers in the prefrontal cortex (PFC) of mice in the four groups. Green dashed lines show the location of the PFC in a coronal slice of the mouse brain (a) at ~2.6 mm anterior to the bregma. The black bar shows the scale of the image. The enlargements show representations of TH-positive nerve fibers in the PFC of mice in the (b) familiar-normal (FN), (c) familiar-complex (FC), (d) novel-normal (NN), and (e) novel-complex (NC) groups. Black arrows indicate the TH-positive neurons, and the black bars show the scale of the images. The graph (f) shows the optical density of TH-positive nerve fibers in the prefrontal lobe cortex of mice in each of the four groups. In each box-and-whisker plot, the center line represents the median, the box indicates the first and third quartile (25th and 75th percentile) of the data, and the whiskers mark the upper and lower extremes of the data.

enhanced the exploration level of mice to further promote their innovation capacity (Ennaceur & Delacour, 1988; Ennaceur et al., 2005). The different motivations of mice following environmental treatment could probably account for the differences in the exploration levels between groups (Burnett et al., 2016; Düzel et al., 2010). For the novel group, the environment continually changed, leading to a stronger motivation to explore in order to adapt to the changes (Modlinska et al., 2019). By contrast, because of their long-term exposure to a constant environment, mice in the familiar group showed much lower exploratory motivation than those in the novel group (Düzel et al., 2010).

An examination of the neural mechanisms behind the effects of novelty on mice's innovate problem-solving ability provided further evidence for the involvement of exploratory motivation. The numbers of dopamine

neurons and VTA to NA dopaminergic projections (a neural functional circuit that regulates novelty seeking and motivation in the mouse brain; Flaherty, 2005; Griffin & Guez, 2014; Kaufman et al., 2011; Legault & Wise, 1999) increased in response to environmental novelty, but not environmental complexity, similar to previous studies in rats that demonstrated the increased release of dopamine in the NA in response to novel stimuli (Hooks & Kalivas, 1995; Legault & Wise, 2001). These results further indicated that only the novelty (relative to the complexity) of the enrichment environment had the potential to enhance novel exploratory motivation, which promoted the innovative capability of mice. Therefore, a possible pathway for the effect of environmental enrichment on the innovation performance of mice could be proposed: The novelty of the environment promotes the function of brain dopamine

systems, especially in the NA and VTA, which enhances the novelty-seeking motivation of mice, thus increasing exploration behaviors and, ultimately, improving their innovative-problem-solving performance (Flaherty, 2005; Griffin & Guez, 2014; Kaufman et al., 2011; Kelly et al., 1975; Legault & Wise, 2001).

Interestingly, the immunohistochemical results also showed no significant difference in dopaminergic neurons of the prefrontal region between the novel and familiar groups (Fig. 8), indicating that the novel environment was not likely to be consequential to changes in the dopaminergic projections from the VTA to the prefrontal lobe that influenced innovation performance. Although previous studies have found that prefrontal dopamine plays a vital role in innovative problem solving by promoting and maintaining persistence and flexibility when mammals are seeking new solutions (Boot et al., 2017; Floresco, 2013; Goldman-Rakic, 1992; Winter et al., 2009), prefrontal dopamine did not seem to be a major factor in the improvement of innovative performance of subjects reared in a novel environment. Additionally, the staining results also indicated no significant difference in the number of dopaminergic neurons in the VTA, NA, and PFC between the complex and normal groups, which suggests that the complexity of the environment did not promote brain dopamine function or the resulting innovative-problem-solving performance.

However, these results are inconsistent with those of some previous studies finding that animals in a complex environment performed better in corresponding tasks than those in a simple environment because of their accumulation of experience and knowledge and explorations of other animals or environmental objects (Ennaceur & Delacour, 1988; Modlinska et al., 2019; Zimmermann et al., 2001). One possible cause of the inconsistency is the different environmental manipulations included. Because techniques to study enrichment behaviors involve making the environment more complex than standard housing conditions, previous studies have used a single environmental condition involving a mixture of novelty and complexity (Garthe et al., 2016; Widman & Rosellini, 1990; Zeleznikow-Johnston et al., 2017). Actually, the novelty, rather than the complexity, in these manipulations of complex environments promoted the exploration and related behaviors of the animals. Specifically, if the degree of environmental complexity and enrichment is particularly high, the environment itself will be beyond the short-term cognitive capacity or exploration ability of the animals, who will be unable to become familiar with all environmental factors within a short period of time. This leads to the complexity and enrichment of the

environment appearing novel to the animals and thus promotes the motivation of exploration as well as performance in the corresponding tasks. Zimmermann et al. (2001) found a similar result in mice; they described how a group housed in a low-complexity environment showed fewer exploratory behaviors than a group in a much more complex environment.

Another possible cause of the inconsistency could be related to the different characteristics of the tasks. In the present study, the experimental paradigm of innovative-problem boxes was adopted to investigate the innovative-problem-solving ability of mice (Thornton & Samson, 2012). These tasks place more emphasis on the mice's cognitive capacity to explore and solve new problems, which is more dependent on the level of novelty seeking or exploration motivation (Brudzynski & Gibson, 1997; Keynan et al., 2015; Legault & Wise, 1999; Webster & Lefebvre, 2001). Thus, it is conceivable that the task performance of mice in our study was more affected by environmental-novelty factors (Hooks & Kalivas, 1995; Kaufman et al., 2011; Legault & Wise, 2001). In contrast, the tasks used in the previous studies mainly included the detection of cognitive functions other than innovation, such as experience, spatial learning, and memory, and environmental complexity has been shown to influence these cognitive functions (Ennaceur & Delacour, 1988; Garthe et al., 2016; Rodewald et al., 2011; Zeleznikow-Johnston et al., 2017). However, more studies in animals are needed to investigate behavioral changes caused by environmental complexity.

Although it was found, in the present study, that the promotion of dopamine-circuit functions in the VTA and NA probably mediated the environmental-enrichment (novelty) enhancement in exploratory behaviors and innovative problem solving of mice, other potential neural mechanisms thought to underlie the cognitive enhancement of environmental enrichment may also be involved and should be explored in future studies. These include (a) hippocampus functions and associative structures, (b) attention-related structures and functions, and (c) neurotransmitter or signaling systems responsible for the arousal response in animals confronted with a novel and complex environment (Kempermann, 2019; VanElzakker et al., 2008; van Praag et al., 2000). Moreover, detailed analysis of the contribution of each stimulus element, such as motor, sensory, and cognitive stimulation, to environmental-enrichment enhancement will also be needed in future work because of the potential specific effects of each element in driving environmental-enrichment enhancement (Garthe et al., 2016; Kempermann, 2019), even though these elements are not independent of each other and usually have to be considered together and in their interactions to

explain how environmental enrichment works (Fabel & Kempermann, 2008; Olson et al., 2006).

## Transparency

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*Author Contributions*

The first two authors contributed equally to this work. L. Cheng developed the study concept. L. Cheng, S.-T. Cheng, and S. Liu contributed to the study design. S. Liu performed the testing and data collection. S.-T. Cheng, S. Liu, B. Ou-Yang, and X.-Y. Dai analyzed and interpreted the data under the supervision of L. Cheng. S.-T. Cheng and L. Cheng drafted the manuscript and provided critical revisions. All authors approved the final manuscript for submission.

### Declaration of Conflicting Interests

The author(s) declared that there were no conflicts of interest with respect to the authorship or the publication of this article.

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### Open Practices

Data and materials for this study have not been made publicly available but can be requested via email from L. Cheng (chengl@ccnu.edu.cn). The design and analysis plans for the study were not preregistered.

## ORCID iD

Liang Cheng  <https://orcid.org/0000-0003-1536-0941>

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