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Temozolomide treatment inhibits spontaneous motivation for exploring a complex object in mice: A potential role of adult hippocampal neurogenesis in "curiosity"

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Abstract

Intrinsic exploratory biases are an innate motivation for exploring certain types of stimuli or environments over others, and they may be associated with cognitive, emotional, and even personality-like traits. However, their neurobiological basis has been scarcely investigated. Considering the involvement of the hippocampus in novelty recognition and in spatial and pattern separation tasks, this work researched the role of adult hippocampal neurogenesis (AHN) in intrinsic exploratory bias for a perceptually complex object in mice. Spontaneous object preference tasks revealed that both male and female C57BL/6J mice showed a consistent unconditioned preference for exploring "complex"—irregular—objects over simpler ones. Furthermore, increasing objects' complexity resulted in an augmented time of object exploration. In a different experiment, male mice received either vehicle or the DNA alkylating agent temozolomide (TMZ) for 4 weeks, a pharmacological treatment that reduced AHN as evidenced by immunohistochemistry. After assessment in a behavioral test battery, the TMZ-treated mice did not show any alterations in general exploratory and anxiety-like responses. However, when tested in the spontaneous object preference task, the TMZ-treated

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mice did not display enhanced exploration of the complex object, as evidenced both by a reduced exploration time—specifically for the complex object—and a lack of preference for the complex object over the simple one. This study supports a novel role of AHN in intrinsic exploratory bias for perceptual complexity. Moreover, the spontaneous complex object preference task as a rodent model of "curiosity" is discussed.

KEYWORDS
anhedonia, chemotherapy, female mice, intrinsic exploratory bias, novelty

1 | INTRODUCTION

Intrinsic exploratory or attentional/perceptual bias refers to an innate (unconditioned) preference or aversion for exploring certain stimuli or attributes, which may be modulated by a variety of factors such as the context in which the stimulus is presented, previous experiences with the stimulus or the individual's personality or emotional state (reviewed in Biondi et al. (2015), Duque and Vázquez (2015)). Intrinsic exploratory bias often involves an ethological adaptive value and is relevant to guide behavior. In this way, it may influence decision-making, including the preference, approach, or aversion for one stimulus over others, and also the quantity of cognitive resources dedicated to the stimulus. For example, humans show an innate bias toward attending face-like and emotional stimuli over non-face-like or neutral ones (Dominguez-Borrás & Vuilleumier, 2013; Valenza et al., 1996). An intrinsic bias for visual complexity—which increases as a function of augmenting the number of stimuli, their heterogeneity, the asymmetry in their arrangement, and/or the irregularity of their physical shape—has also been described (Madan et al., 2017). As long as the level of complexity is not excessively high, humans rate visually complex pictures as more pleasant and physically arousing than simple ones (reviewed in Madan et al. (2017)). Likewise, animals such as birds may engage more time in exploring perceptually complex objects (Biondi et al., 2015).

In rodents, intrinsic exploratory bias has been extensively evidenced by preferences in spatial exploration. It is well known that rats and mice typically prefer staying in "safer" places such as those less illuminated and/or protected by walls—for example, the dark compartment of a light/dark box, the closed arm of an elevated plus maze or the periphery, and corners of an open-field or hole-board maze (Belzung & Griebel, 2001; Sampedro-Piquero et al., 2019). Conversely, they are also attracted by novelty that provides them with a motivation to explore unknown surroundings—for example, novel objects or novel environments—in order to obtain potential rewards or to avoid potential risks (Blaser & Heyser, 2015). Exploratory bias is on the basis of widely used behavioral paradigms that assess rodent's motor and exploratory activity, anxiety-like behavior, and memory. In this regard, the spontaneous object exploration paradigm is usually employed to assess memory for familiar objects over time, considering that preference for a novel object in comparison to a familiar one will only be displayed if animals remember the (familiar) object experienced before (Blaser & Heyser, 2015). However, object exploration tasks may also be used to evaluate which perceptual attributes of the objects are discriminated and/or intrinsically preferred (Angulo et al., 2017; Gámiz & Gallo, 2012). It has been described that rats spontaneously prefer exploring rounded objects over objects with cylindrical shapes (Winne et al., 2015), and objects that could be touched and climbed on are preferred over objects that could only be touched (Heyser & Chemero, 2012). Nevertheless, the existence of spontaneous preference for complex objects in rodents and its potential neurobiological basis have been scarcely investigated.

The hippocampus, mainly its anterior region, has been widely involved in the long-term consolidation and processing of declarative memory, which includes spatial and contextual information (Squire et al., 2015). In this regard, the hippocampus is a key region of the ventral visual–perirhinal–hippocampal steam, which acts as an interface between memory and visual perception involved in visual discrimination and object recognition (Bussey & Saksida, 2007). Midbrain dopaminergic signals converge on the hippocampus, allowing to detect novel—that is, not previously stored in memory—and motivationally significant stimuli while influencing long-term potentiation and learning (Kafkas & Montaldi, 2018). Conversely, the hippocampal-dependent processes of encoding and memorizing a stimulus guide its visual exploration ("viewing"). It has been proposed that both the hippocampal and exploratory demands increase as a function of a stimulus' complexity (Voss et al., 2017).

In rodents, the activity and function of the adult hippocampus are modulated by the generation and addition of new neurons (adult hippocampal neurogenesis, AHN). While the functional implications and even the existence of AHN in humans are still controversial (Leal-Galicia et al., 2021), the number of adult-born hippocampal neurons in rodents is generally directly related to their performance in hippocampal-dependent memory tasks, such as spatial navigation, contextual fear conditioning, discrimination of novel objects and places, and discrimination of highly similar visual stimuli (pattern separation) (Castilla-Ortega et al., 2011; McAvoy et al., 2015; Sekeres et al., 2021). However, in spite of the role of AHN in the processing of spatial and contextual information, its potential role in intrinsic exploratory bias—such as in the preference for complex stimuli—has not yet been investigated.

The present research includes four different experiments. The first set of experiments (1–3) was aimed to confirm a robust intrinsic preference for exploring complex objects in C57BL6/J mice of both sexes, as well as to identify some of the perceptual features of an object that may increase its "complexity." First, two potentially complex objects—that varied in their number of protrusions/intrusions, that is, irregularities—were tested against a simple—with plain surface—object in a spontaneous object exploration paradigm (Experiment 1). After demonstrating an intrinsic preference for the more irregular complex object, we aimed to assess whether increasing the number of copies of this object would influence the number of exploratory responses in the task (Experiment 2). The third experiment was aimed to confirm an innate preference for complexity—that is, object irregularity—in a new object pair (Experiment 3). In the final experiment, AHN was reduced by a pharmacological treatment (Castilla-Ortega, Blanco, et al., 2016; Sekeres et al., 2021) to test the role of the adult-born hippocampal neurons in mice's spontaneous preference for exploring a complex object (Experiment 4).

2 | MATERIALS AND METHODS

2.1 | Animals

C5BL6/J mice were bred in-house (weaned at 21 days of age) and maintained in standard conditions (temperature: 22 ± 2°C; 12 h light/dark cycle; lights on at 8:00 a.m.) with nesting material and water and food provided ad libitum.

Experiments 1–3 used 96 adult mice of both sexes (Experiment 1: 24 males and 22 females; Experiment 2: 21 males and 21 females; Experiment 3: 4 males and 4 females). Experiment 4 used 20 male C5BL6/J mice. Mice were group-housed for Experiments 1–3 and single-housed for Experiment 4. In all cases, littermates were equally distributed among the different experimental conditions.

Procedures were performed according to the European and Spanish regulations for animal research (Directive 2010/63/UE, Real Decreto 53/2013, and Ley 32/2007) and were approved by the research ethics committees of the University of Málaga (code: CEUMA 104-2021-A) and Junta de Andalucía (code: 3/11/2021/170).

2.2 | Behavioral analysis

Mice were carried to a noise-isolated room (illuminated 120 lux) at 9:00 a.m., and they were habituated for at least 20 min before starting the behavioral assessment. A solution of 30% ethanol was used to clean the maze arena and eliminate odor cues.

Sessions were recorded, and the video tracking software EthoVision XT (Noldus, Wageningen, the Netherlands) was used both for the automatized analysis of spatiotemporal parameters—that is, locomotion and time in zone—and for the observational scoring of object exploration with EthoVision's Manual Score module. According to previous studies (reviewed in Mañas-Padilla et al. (2022)), object exploration was scored either when the mouse sniffed the object (i.e., by touching the object with its nose or by pointing its nose toward the object less than 1–2 cm away) or when the mouse actively touched the object with its forepaws. Behaviors, such as touching the object with other body parts, climbing, or chewing the object, were not considered exploration, in agreement with previous literature (Mañas-Padilla et al., 2022). Observational registries were performed by highly experienced observers who were blind to the mice's sex or experimental condition and had no previous assumptions about the outcome of the study.

2.3 | Specific methods for Experiment 1

This experiment was aimed to evaluate mice's intrinsic preference for exploring perceptually complex objects over simple ones. First, we selected a "simple" object and two potentially "complex" objects for comparison. The simple object consisted of one squared Rubik's cube (unbranded) made of black plastic (6×6 cm^2) made-up smaller cubes (2×2 cm^2) disposed in 3 rows × 3 columns (Figure 1a). "Complexity" was obtained by increasing the number of physical irregularities (i.e., edges and concavities) in the object (Biondi et al., 2015; Madan et al., 2017). Therefore, the two potentially complex objects were made from similar Rubik's cube—thus maintaining most of its perceptual properties (i.e., height, material, color, and texture)—but with a more "irregular" shape. The outer small cubes from the upper row were removed (complex object "A"; Figure 1a) or the middle row of Rubik's cube was rotated in 90° (complex object "B"; Figure 1a).

Mice were first placed in the center of a white squared open field (40×40 cm^2) with no objects for assessment and allowed to freely explore for 6 min for habituation. The spontaneous object preference session to evaluate bias for complexity took place 60 min later. Mice were placed back in the apparatus that now contained two objects located in its central zone (separated from each other ~12 cm and separated ~8 cm from the walls), and they were allowed to explore for 10 min. A copy of the "simple" object was compared either with a copy of the complex object "A" ($n = 22$ mice; 11 males and 11 females) or with a copy of the complex object "B" ($n = 24$ mice; 13 males and 11 females). The position of the complex object within the maze—left or right—was counterbalanced across mice.

2.4 | Specific methods for Experiment 2

This experiment assessed whether exploratory variables differed as a function of objects' complexity. Methods were performed as in Experiment 1, but three different conditions were compared in the spontaneous object preference session: "low" complexity (two copies of the simple object; $n = 5$ males and 5 females), "medium" complexity (a copy of the complex object "B" and a copy of the simple object; $n = 11$ males and 11 females), and "high" complexity (two copies of the complex object "B"; $n = 5$ males and 5 females). Each condition used naïve mice that did not participate in the previous experiment.

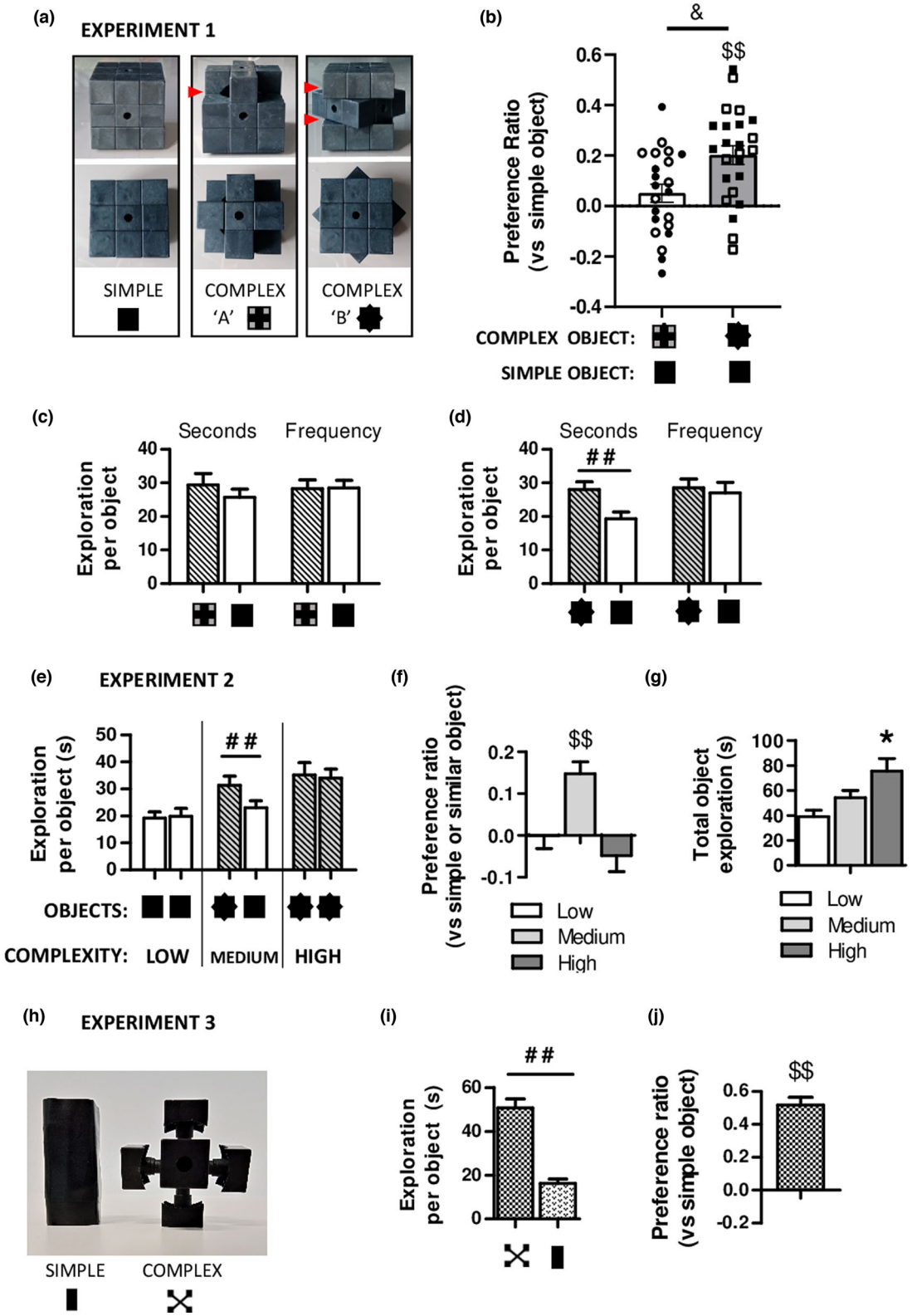

FIGURE 1 Intrinsic preference for exploring a complex object in both male and female mice: (a) lateral view (upper row) and overhead view (lower row) of the first set of objects tested. Rubik's cubes originally had stickers with different colors, which were carefully removed before the experiments started. Red arrows point irregularities (i.e., intrusions/protrusions) in complex objects "A" and "B," being the object "B" presumably the most complex. (b-d) In Experiment 1, mice preferred exploring the complex object "B" over the simple object. However, the complex object "A" was not successfully discriminated from the simple one). Male (black symbols) and female (empty symbols) mice are represented individually in (b). (e–g) In Experiment 2, separate groups of mice were exposed to different maze conditions that increased the degree of complexity as a function of increasing the number of copies of the complex object ("B"). Increasing the number of complex objects in the maze augmented total time of object exploration. The preference for the complex object as shown in (f) was maintained on a second session (24 h intertrial interval) for a group of mice

(Continues)

FIGURE 1 (Continued)
from the "medium" complexity condition—24 h data are not displayed in graphs; details in the main test. (h–j) Preference for complexity was demonstrated for a different pair of objects, where the "complex" object displayed a higher number of irregularities that the "simple" one. Student's *t*-test: difference versus zero: $^{\$\$}p < .001$ or versus the complex object: $^{\#\#}p < .001$. Analyses of variance (ANOVA) effect for complex object-type—reported in the main text: $^{\&}p < .05$. Post hoc least significant difference (LSD): difference of the high complexity group versus the other groups: $^{*}p < .05$. Data are expressed as mean ± *SEM*

The following day (i.e. 24 h interval), six male and six female mice from the "medium" complexity group were tested for the second session in identical experimental conditions, to study preference for the complex object in a familiar environment.

2.5 | Specific methods for Experiment 3

This experiment was aimed to demonstrate innate preference for complexity with a different object pair. For this, we used pieces from Rubik's cube to build two new objects of a comparable height and a similar surface to be explored. However, in one of the objects (complex), the elements were disposed to maximize the number of irregularities and concavities; however, the simple object was built in the shape of a column (Figure 1h). Eight naïve mice (4 males and 4 females) were tested with this pair of objects as reported for Experiments 1 and 2.

2.6 | Specific methods for Experiment 4

2.6.1 | TMZ and BrdU administration

To study the role of the adult-born hippocampal neurons on the intrinsic preference for a perceptually complex object, AHN was inhibited by temozolomide (TMZ). TMZ is a chemotherapy drug with high blood–brain barrier permeability that reduces cell proliferation in a quantity-dependent way (Sekeres et al., 2021). As a DNA-alkylating agent, TMZ induces cell cycle arrest at G2/M phase and eventually leads to cell death (Alonso et al., 2007). From the seminal study by Kempermann's laboratory (Garthe et al., 2009), a systemic administration of TMZ has been consistently used as a pharmacological approach to deplete AHN in mice, considering its minimal side effects for health. TMZ is commonly administered during 3–4 weeks in a multi-cyclic treatment paradigm that includes several days of resting between cycles. This seems sufficient for rodents to recover from the physiological disruptions induced by TMZ, which frequently include inflammatory responses or reduced body weight (Castilla-Ortega, Blanco, et al., 2016; Nokia et al., 2012; Sekeres et al., 2021).

For this study, a commonly used TMZ administration protocol that inhibits AHN without impairing general health in mice has been chosen (Castilla-Ortega, Blanco, et al., 2016; Garthe et al., 2009; Sekeres et al., 2021). During the 4 weeks before the behavioral assessment, naïve male mice (~12 weeks of age) received a weekly cycle of TMZ ($n = 10$) or similar administrations of vehicle (VEH, $n = 10$). Each treatment cycle consisted of a daily intraperitoneal administration of TMZ (25 mg/kg) for 3 consecutive days, followed by 4 days of recovery (Figure 2a). For its administration, TMZ (T2577, Sigma-Aldrich, Madrid, Spain) was first dissolved in dimethylsulfoxide (DMSO, D5879, Sigma-Aldrich) and then diluted in saline (0.9% NaCl) to a concentration of 10% DMSO.

Newly generated cells were labeled by the thymidine analog bromodeoxyuridine (BrdU). For hours after its administration, this compound can be incorporated in DNA of dividing cells during DNA synthesis (S-phase) and remains in their nuclei, thus allowing them to date their birth and monitor their fate (Taupin, 2007). Bromodeoxyuridine (Sigma-Aldrich) was dissolved in saline and administered weekly on the day after each TMZ administration cycle—days 4, 11, 18, and 25 (Figure 2a). All mice received two daily 75-mg/kg intraperitoneal BrdU administrations, separated by 4 h (Mañas-Padilla, Gil-Rodríguez, et al., 2021).

Animals were weighed, and their external appearance (e.g., the condition of the fur, whiskers and eyes, and the absence of injuries or swelling) was checked daily during intraperitoneal administrations. Neurological tests for sensory reflexes, limb reflexes, and limb coordination were performed on day 25 (Figure 2a) as previously described (Castilla-Ortega, Blanco, et al., 2016).

2.6.2 | Behavioral protocol

Four days after the last intraperitoneal administration, mice were submitted to a behavioral test battery following previous methods published by our research group to evaluate exploration, anxiety-like responses, and intrinsic exploratory bias for objects and places (Mañas-Padilla, Ávila-Gámiz, et al., 2021; Mañas-Padilla, Gil-Rodríguez, et al., 2021; Mañas-Padilla et al., 2022; Sampedro-Piquero et al., 2019). The behavioral schedule is described below (Figure 2a):

- Elevated plus maze (day 30): The plus-shaped (+) apparatus consisted of two unprotected open and two enclosed arms (30×5 cm^2 each) connected by a central platform (5×5 cm^2). It was raised 47 cm above the floor. The mouse was released in the central platform, and it was allowed to explore freely for 6 min.
- Y maze (day 31): Mice were placed on one of the three enclosed arms (30×6 cm^2 each) of a Y-shaped apparatus and allowed to explore freely for 6 min. Spontaneous alternation behavior (SAB) was calculated as follows: [(number of spontaneous alternations)/(total the number of arm entries−2)]; where one spontaneous alternation was defined as three successive entries in different arms.
- Hole-board test (day 32): An automatic hole board (CIBERTEC, Madrid, Spain) was made up of a square white arena (45×45 cm^2) was surrounded with clear Plexiglass walls (25 cm high) and contained 16 equidistant holes (5.5 cm apart, 2.5 cm diameter, 3 cm depth). Mice were placed in the center of the apparatus and allowed

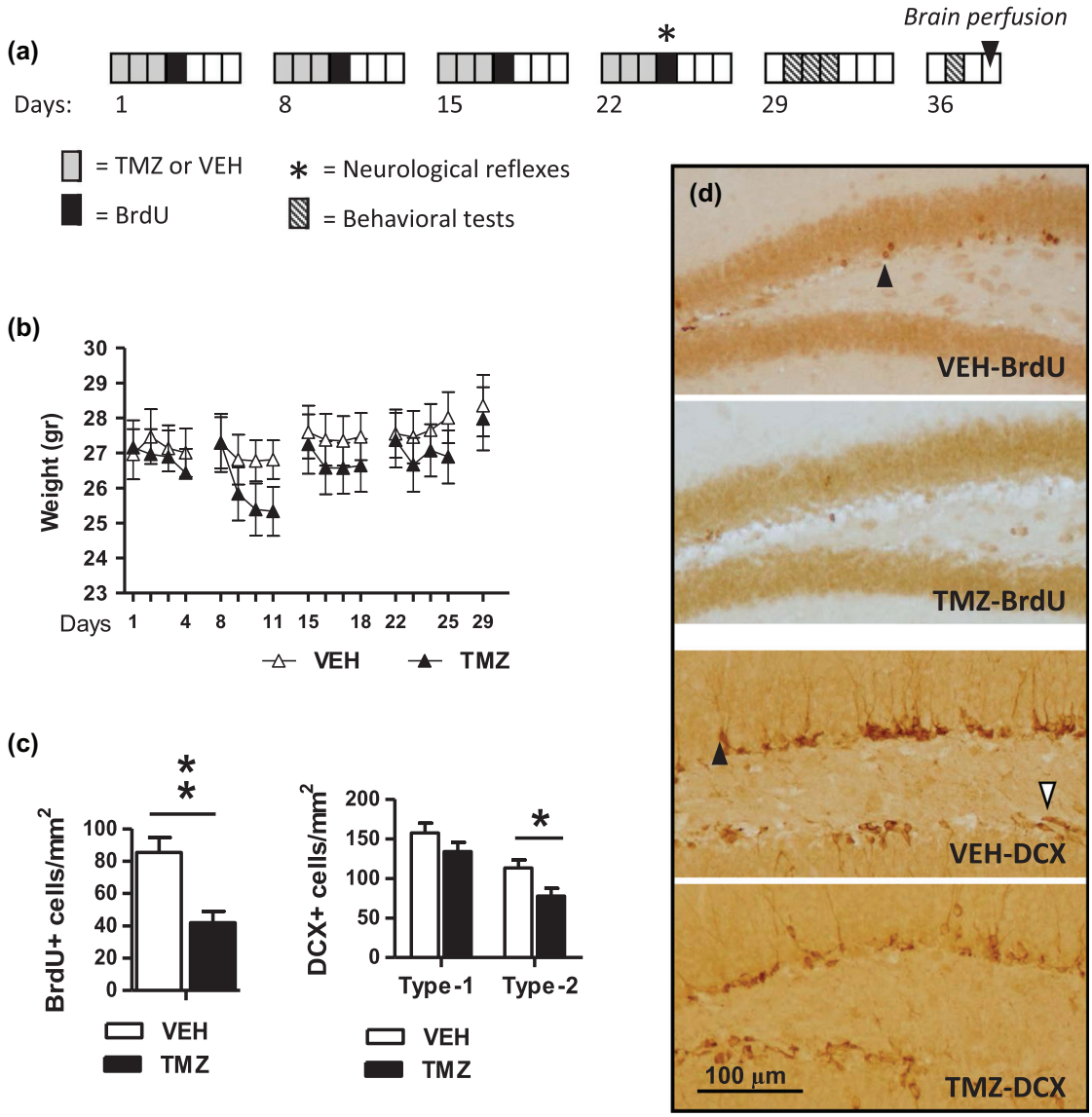

FIGURE 2 Temozolomide (TMZ) administration reduced adult hippocampal neurogenesis without impairing mice's general health: (a) experimental protocol; (b) transient weight loss induced by TMZ was resolved before starting the behavioral experiments; (c and d) TMZ administration reduced adult hippocampal neurogenesis (AHN)-related markers. Black arrow in (d) points a BrdU+ cell and a DCX+ cell with mature-like morphology (Type-2). White arrow in (d) points a DCX+ cell with immature-like morphology (Type-1). Between-groups differences by Student's *t*-test: $^{*}p < .05$; $^{**}p < .001$. Data are expressed as mean ± *SEM*

to explore for 6 min. Holes were classified as located in the "center," "corner," or "wall" as depicted in Figure 3c. Hole explorations were scored each time the mouse's nose crossed the infrared beans located inside the holes at a depth of 1 cm.

- Exploratory bias for a complex object (day 37): This task was performed in an open-field maze as described in Experiment 1. The object preference session included one copy of the simple object and one copy of the complex object "B" (Figure 3d).

2.6.3 | Analysis of AHN-related markers

Histological and immunohistochemical procedures were carried out to confirm a reduction of the newly born hippocampal cells by TMZ. For that purpose, the methodology described in our previous publications was employed (Castilla-Ortega, Blanco, et al., 2016; Ladrón de Guevara-Miranda et al., 2017; Ladrón de Guevara-Miranda et al., 2019; Mañas-Padilla, Gil-Rodríguez, et al., 2021). On day 39, mice were deeply anesthetized with pentobarbital (200 mg/kg) and sacrificed by intracardiac perfusion with 0.1 M phosphate-buffered saline pH 7.4 (PBS) and 4% paraformaldehyde. After postfixation for 48 h in paraformaldehyde, the left-brain hemisphere was arbitrarily used and cut into 40-μm coronal vibratome sections distributed into six equivalent tissue series. Free-floating sections received endogenous peroxidase blocking solution (80% PBS, 10% methanol, and 10% hydrogen peroxide) in the dark for 20 min. Bromodeoxyuridine immunohistochemistry was preceded by a denaturation of the DNA in 2N HCl for 20 min at 37°C and subsequent neutralization in 0.1 M boric

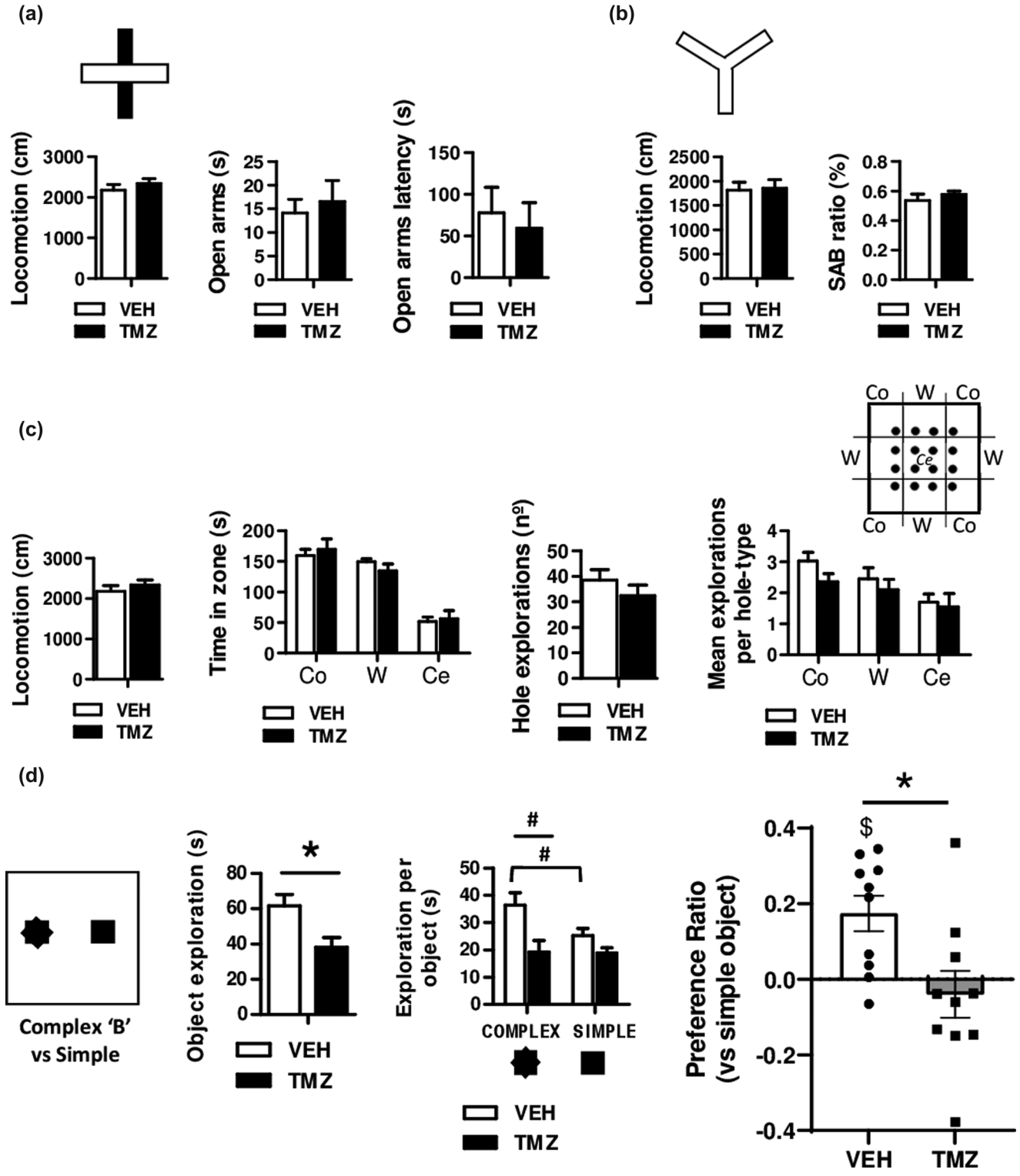

FIGURE 3 Temozolomide (TMZ) administration reduced intrinsic preference for complexity and exploration elicited by a complex object. (a–c) Mice with reduced adult hippocampal neurogenesis (AHN) by TMZ did not show any alterations in parameters of general exploratory activity and anxiety-like behavior in the elevated plus maze (a), the Y-maze test for spontaneous alternation (b), or the hole-board test (c). (d) In the spontaneous object preference task, the TMZ-treated mice showed a reduced exploration specifically of the complex object, which was not preferred over the simple object as found in the vehicle (VEH)-treated mice. The spontaneous preference ratio for the complex object is represented individually for each mouse (black symbols). Between-groups differences by Student's *t*-test: $^{*}p < .05$; difference by least significant difference (LSD) post hoc analysis: $^{\#}p < .05$; Student's *t*-test versus zero: $^{\$}p < .05$. Data are expressed as mean ± *SEM*

acid (pH 8.5) for 10 min, prior to incubation in the primary antibody. The primary antibodies used were rat Anti-BrdU (ab6326, Abcam, Cambridge, UK; diluted 1:500)—to detect 35–14 days old cells generated during the TMZ/VEH treatment that survived until the end of the experiment—and rabbit anti-doublecortin (DCX; ab18723, Abcam, diluted 1:400)—an endogenous marker expressed in immature neurons up to 3–4 weeks of age (Brown et al., 2003). The following day, sections were incubated with biotin-conjugated secondary antibodies (Dako, Glostrup, Denmark; diluted 1:500) for 90 min and with peroxidase-conjugated extravidin (Sigma-Aldrich, 1:1000 in PBS) in the dark for 60 min. The staining solution contained 0.1 ml of diaminobenzidine (DAB) previously diluted at 5% in distilled water, 10 μl hydrogen peroxidase, and 10 ml PBS. Every step was followed by PBS rinses.

Cell quantification was carried out in the dentate gyrus in one of every six dorsal hippocampal sections (bregma−1.06–3.08 mm). Photographs were captured with an Olympus BX51 microscope equipped with an Olympus DP70 digital camera (Olympus, Glostrup, Denmark) and quantified with the software ImageJ (National Institutes of Health, Maryland, USA). For the quantification of the DCX+ neurons, two categories on the basis of their morphological features were distinguished: neurons with immature-like morphology that may still undergo proliferation (Type-1: with absent or short dendritic processes), and presumably older postmitotic neurons displaying a mature-like morphology (Type-2: with at least one prominent apical dendrite penetrating the granule cell layer) (Castilla-Ortega, Blanco, et al., 2016; Mañas-Padilla, Gil-Rodríguez, et al., 2021; Plümpe et al., 2006).

2.7 | Statistical analysis

A "preference ratio" was calculated to assess exploration bias for a complex object: [(time exploring the complex object–time exploring the simple object)/total time exploring both objects].

In all experiments, statistical analyses were performed either by Student's *t*-tests for independent or dependent samples (when appropriate) or by analyses of variance (ANOVA) followed by post hoc comparisons (Fisher's least significant difference; LSD) when required. One-sample Student's *t*-tests were used to compare the object preference ratios versus zero (which would indicate absent preference for any object-type). Significance was considered at $p \leq .05$. For transparency, both significant and nonsignificant statistical comparisons are reported. Data are expressed as means ± *SEM*.

3 | RESULTS

3.1 | Experiment 1

3.1.1 | Both male and female mice showed intrinsic exploratory bias for a complex object

In this experiment, two potentially complex objects ("A" and "B") were tested against a simple object. Mice did not significantly discriminate the complex object "A" from the simple object (one-sample Student's *t*-test vs. zero: $t(21) = 1.411$, $p = .173$), but they preferred exploring the complex object "B" over the simple one (one-sample Student's *t*-test vs. zero: $t(23) = 5.426$, $p < .001$; Figure 1b). A factorial ANOVA ("sex" × "complex object-type") showed that the preference ratio obtained for the complex object "B" was significantly higher than for the complex object "A" ("complex object type": $F(1, 42) = 7.930$, $p = .007$); however, no effects were found regarding "sex" ($F(1, 42) = 0.006$, $p = .939$) nor the "sex × complex object-type" interaction ($F(1, 42) = 0.700$, $p = .407$) (Figure 1b).

Accordingly, with the abovementioned observations, analyses of the total time exploring each object revealed that the complex object "A" was not significantly explored more time than the simple object (Student's *t*-test for dependent samples: $t(21) = 1.813$, $p = .084$; Figure 1c); however, the complex object "B" was clearly explored more time than the simple one ($t(23) = 4.938$, $p < .001$; Figure 1d). Nevertheless, there were no differences in the frequency of exploration of either complex object compared to the simple object ($t(21) = 0.042$, $p = .967$ for Figure 1c; $t(23) = 0.901$, $p = .377$ for Figure 1d). This shows that the preference for the complex object "B" could not be attributed to a higher exploration frequency but to a longer duration of the explorations.

3.2 | Experiment 2

3.2.1 | Higher number of complex objects augmented total object exploration time

Mice were tested in three separated conditions differing in the number of complex objects. The total object exploration time increased as objects' complexity increased, mainly in the condition with two complex objects (one-way ANOVA: $F(2, 39) = 4.784$, $p = .014$; post hoc LSD is shown in Figure 1g). Total locomotion was not significantly altered (one-way ANOVA: $F(2, 39) = 1.202$, $p = .311$; means ± *SEM*: "low" complexity: 2685.357 ± 271.909; "medium": 2671.024 ± 163.419; "high": 3161.224 ± 469.488 cm).

According to the previous experiment, mice facing the simple object versus the complex object "B" (i.e., "medium" complexity condition) spent more time exploring the latest (Student's *t*-test for dependent samples on object exploration times: $t(23) = 5.426$, $p < .001$, Figure 1e). The preference ratio in this group was significantly greater than zero by a one-sample Student's *t*-test: $t(21) = 5.230$, $p < .001$ (Figure 1f).

Twenty-four hours later, a sample of 12 mice of both sexes from the "medium" complexity condition was retested to study whether the preference for the complex object "B" was maintained in a familiar environment. The preference ratio for the complex object was similar in the first and second sessions for that group of mice (first session: 0.148 ± 0.042; second session: 0.213 ± 0.069; Student's *t*-test for dependent samples: $t(11) = 1.144$, $p < .277$), and it was significantly greater than zero (one-sample Student's *t*-test for the first session: $t(11) = 3.568$, $p = .004$; second session: $t(11) = 3.076$, $p = .011$).

No sex differences were found in preference ratios thorough the experiments.

3.3 | Experiment 3

3.3.1 | Preference for complexity was demonstrated with a different object pair

For this experiment, we tested mice with a new object pair that maximized the number of irregularities in the complex object compared to the simple one. Mice tested in this condition showed a strong intrinsic preference for exploring the complex object, as evidenced both by the total object exploration time (Student's *t*-test for dependent samples: $t(7) = -9.216$, $p < .001$, Figure 1i) and by the preference ratio (one-sample Student's *t*-test vs. zero: $t(7) = 10.960$, $p < .001$; Figure 1j).

3.4 | Experiment 4

3.4.1 | TMZ administration reduced adult hippocampal neurogenesis without impairing mice's general health

Administration of TMZ induced weight loss (repeated measures ANOVA on days 1–24: effect for "TMZ": $F(1, 18) = 0.432$, $p = .519$; "day": $F(15, 270) = 21.209$, $p = .000$; "TMZ × day": $F(15, 270) = 5.686$, $p < .001$; Figure 2b) but only transitorily, since weight was recovered after each resting period and before starting the behavioral assessment (Student's *t*-test for independent samples on day 28: $t(18) = 0.297$; $p = .770$; Figure 2b). Furthermore, the maximum weight loss registered in the TMZ-treated mice during the experiment was about 7.15% (−1.95 g on day 11 vs. day 8), which is far from a common endpoint for chemotherapy in mice set at >15% weight loss (Aston et al., 2017). TMZ-treated mice did not show any external signs of health impairment at any point in the experiment, and the neurological reflexes were preserved in all mice.

As expected, TMZ treatment notably reduced AHN-related markers, as shown by the numbers of both BrdU+ cells (Student's *t*-test: $t(18) = -3.710$; $p = .001$; Figure 2c) and Type-2 DCX+ neurons ($t(18) = -2.545$; $p = .020$; Figure 2c). In this study, the BrdU+ cells proliferated the day following each TMZ administration cycle and survived until the end of the experiment. The Type-2 DCX+ neurons—that showed a mature-like morphology—were also presumably generated during the previous weeks, while TMZ was being administered. Conversely, more immature Type-1 DCX+ neurons were not significantly affected ($t(18) = -1.378$; $p = .185$; Figure 2c). Importantly, these neurons were presumably younger and therefore generated closer to the end of the experiment, after TMZ administration had been discontinued.

3.4.2 | TMZ administration did not influence mice's general exploratory and anxiety-like behavior

No differences in locomotor activity ($t(18) = 0.865$; $p = .399$) nor in typical anxiety-related behaviors such as the time spent in the open arms ($t(18) = 0.463$; $p = .649$) and the latency to enter an open arm ($t(18) = -0.425$; $p = .676$) (Figure 3a) were found in the elevated plus maze. When exploring the Y-maze, both groups were similar in locomotor activity ($t(18) = 0.168$; $p = .869$) and in the frequency of SAB ($t(18) = 0.834$; $p = .415$) (Figure 3b). The TMZ treatment did not influence total locomotor activity ($t(18) = -0.850$; $p = .407$) or hole exploration ($t(18) = -1.048$; $p = .309$) in the hole-board maze. In addition, that treatment did not modulate the intrinsic spatial bias for exploring different maze zones (repeated measures ANOVA for time spent in different zones ("TMZ × zone-type"): "TMZ": $F(1, 18) = 0.671$, $p = .423$; "zone-type": $F(2, 36) = 10.822$, $p < .001$; "TMZ × zone-type": $F(2, 36) = 0.342$, $p = .712$) or for different hole locations (repeated measures ANOVA for exploration of different hole types ("TMZ × hole-type"): "TMZ": $F(1, 18) = 0.411$, $p = .530$; "hole-type": $F(2, 36) = 19.308$, $p < .001$; "TMZ × hole-type": $F(2, 36) = 0.071$, $p = .932$) (Figure 3c).

3.4.3 | TMZ inhibited increased exploration and intrinsic preference for a complex object

In the object preference session (complex object "B" vs. simple object), the VEH mice spend more time exploring objects than the TMZ mice (Student's *t*-test for independent samples: $t(18) = -2.729$; $p = .014$; Figure 3d). Specifically, the VEH mice showed more exploration of the complex object (repeated measures ANOVA [TMZ × object-type] compared to the TMZ-treated mice: "TMZ": $F(1, 18) = 7.450$, $p = .014$; "object-type": $F(1, 18) = 5.852$, $p = .026$; "TMZ × object-type": $F(1, 18) = 5.101$, $p = .037$; LSD post hoc analysis is shown in Figure 3d) and a significantly higher preference ratio for the complex object-type ($t(18) = -2.729$; $p = .014$; Figure 3d). Accordingly, the preference ratio for the complex object was significantly higher than zero in the VEH mice; however, the TMZ mice explored both object-types equally (one-sample Student's *t*-test: VEH: $t(9) = 3.702$; $p = .005$; TMZ: $t(9) = -0.644$; $p = .536$—non significant; Figure 3d).

Groups were no different in locomotion—cm—neither in the object preference session (VEH: 2862.756 ± 251.308; TMZ: 3395.259 ± 469.488; Student's *t*-test for independent samples: $t(18) = 1.000$; $p = .331$) nor during the previous habituation session (locomotion: VEH: 1822.780 ± 110.413; TMZ: 1755.213 ± 215.977; Student's *t*-test for independent samples: $t(18) = -0.279$; $p = .784$).

4 | DISCUSSION

A main result of the present work is that adult C57BL6/J mice of both sexes display a robust innate bias to explore a complex object when

tested in a spontaneous object exploration task. The term "complexity" was used here to refer to irregular objects shaped with edges and concavities (Biondi et al., 2015; Madan et al., 2017), which were compared to objects with a smooth surface. The first experiment tested two potentially complex objects. Only one of them (complex object "B") resulted preferred by mice over the supposedly simpler object. A likely explanation for this outcome is that the complex object "A" could still be perceived visually as cubic and regular-shaped despite its four concavities (Figure 1a). Conversely, the complex object "B" (star-shaped) had four protruding edges that could be more easily differentiated—either visually or through tact—and, thus, it could result in more appealing. Preference for this object was maintained in a second session, performed 24 h after. In a different experiment (Experiment 3), the complex object displayed highly evident protrusions compared to the simple one (Figure 1h), which resulted in a high intrinsic preference. Therefore, the salience of an object's irregularities seems a relevant feature to influence its amount of exploration. Interestingly, additional data (Experiment 2) revealed that increasing an object's complexity may not only favor an exploratory bias for such an object but may also stimulate mice's overall motivation to explore objects, increasing the total time they invest in object exploration—while not affecting locomotor activity. This behavior could have an ethological adaptive value considering that the details of complex objects seem more difficult to be discriminated (Gámiz & Gallo, 2012); so an increased time of object exploration may facilitate its cognitive processing (i.e., encoding and recognition).

Both the spontaneous preference and the increased exploratory drive could be attributed to the object's "complexity" since other key perceptual factors—such as height, color, brightness, or texture—and the opportunities to climb or rear on the objects—"affordance" (Heyser & Chemero, 2012)—were similar for all object-types. It should be highlighted that all objects were equally novel to the mice. This distinguishes the spontaneous object preference task from the widely used spontaneous "novel object recognition paradigm" (NOR) used to assess memory, in which a novel object should be preferred over a familiar one (Ennaceur, 2010). In this regard, in order to evaluate the effect of novelty unambiguously, the object types employed for NOR should have a comparable level of intrinsic appeal (Ennaceur, 2010; Heyser & Chemero, 2012). While some authors stress the importance of minimizing potential intrinsic bias in the objects used for NOR experiments (Ennaceur, 2010; Heyser & Chemero, 2012; Lueptow, 2017), few studies have focused on researching which specific features determine that an object may result more or less intrinsically motivating for rodents. In fact, while "complexity"—intended as the irregularity of the object or the number of elements in the object—is proposed as a key extrinsic dimension to influence exploration (Biondi et al., 2015; Hughes, 1997), this has been scarcely researched in mice and rats and should be taken into account when selecting objects for NOR.

Interestingly, we revealed that both increased exploration elicited by complexity, and the intrinsic preference for a complex object, were inhibited in mice treated with TMZ. Chemotherapeutic drugs such as TMZ are widely used agents to deplete AHN in preclinical rodent models due to their potent antiproliferative activity with minimal side effects for health. A preserved health condition is mandatory in order to attribute the lasting cognitive deficits in chemotherapy-withdrawn rodents specifically to impaired AHN (Castilla-Ortega, Blanco, et al., 2016; Egeland et al., 2017; Sekeres et al., 2021). Previous experiments showed that TMZ treatment hindered body weight gain (Castilla-Ortega, Blanco, et al., 2016; Nokia et al., 2012), though we reported weight loss, which is a common effect observed for chemotherapy drugs (Aston et al., 2017). Nevertheless, it should be noted that body weight loss was only moderate, and it was recovered after a few days of resting from TMZ administration. In agreement with previous observations (Castilla-Ortega, Blanco, et al., 2016; Egeland et al., 2017), the TMZ-treated mice did not display any neurological alterations, signs of discomfort nor general behavioral abnormalities such as in their anxiety-like responses nor in their motor exploratory activity and intrinsic spatial bias at the time of behavioral testing. This fact supports that diminished exploration and preference for the complex object induced by TMZ could hardly be attributed to residual health effects, increased anxiety—or neophobia—nor to a reduced interest in the surroundings. In fact, even the simple object-type was equally explored by VEH and TMZ mice, as their difference was specific to the complex object exploration. Overall, results may be explained by a reduced intrinsic motivation for complexity after AHN reduction.

Regarding AHN data, the administration of TMZ halved the numbers of BrdU+ cells that proliferated during the antimitotic treatment—that is, labeled one day after each TMZ cycle. Though the phenotype of the BrdU+ cells was not directly examined here, it is well known that the vast majority (~70–90%) of the cells generated in the subgranular zone of the adult rodent hippocampus differentiate into neurons (Brown et al., 2003; Cameron et al., 1993). Importantly, the effect of TMZ on AHN is transitory since the hippocampus gradually recovers its proliferative activity once the antimitotic drug is withdrawn (Castilla-Ortega, Blanco, et al., 2016). This was evidenced in the present study by the DCX data. Doublecortin is an endogenous immature neuron marker expressed during a wide temporal window by the newly generated neurons: from 2 h after their generation to ~4 weeks of age (Brown et al., 2003). The number of DCX+ neurons with immature-like morphology—which are presumably younger and thus proliferated closer to the end of the experiment—was similar for both TMZ and VEH mice; but DCX+ neurons with a more mature-like morphology—which were more likely ~1–4 weeks of age at the time of brain perfusion—were reduced by TMZ (Brown et al., 2003; Plümpe et al., 2006). Therefore, the mature-like DCX population partially overlaps with the pool of BrdU+ cells, which were aged ~2–5 weeks old at the time of sacrifice and object preference testing. The population of newly born neurons affected by TMZ in this study is at an appropriate age to contribute to hippocampal function. While full maturation of the adult-generated neurons may not be reached until ~4–8 weeks of age, immature neurons may be functionally integrated in the dentate gyrus circuit from the second week. At such immature stage, they show increased excitability and enhanced plasticity in response to the environmental demands and may be functionally involved in hippocampal-dependent tasks (Castilla-Ortega et al., 2011; Nakashiba et al., 2012).

Considering the abovementioned, our results support a role of AHN within the hippocampal circuits responsible of intrinsic preference for visually complex stimuli. However, it is not possible to know whether the inhibition of AHN affected cognitive and/or motivational factors only with the present data. On the one hand, because AHN has been involved in contextual and spatial perception (Castilla-Ortega et al., 2011; Nakashiba et al., 2012), the inhibition of AHN may have influenced the discrimination of the physical attributes of the complex object. On another hand, a decreased interest for a naturally appealing complex stimulus also could be interpreted as an anhedonic-like response. The hippocampus receives innervations from the dopaminergic midbrain regions, and, conversely, it regulates dopamine liberation through different polysynaptic circuits (reviewed in Castilla-Ortega, Serrano et al. (2016)). These dopaminergic pathways are part of the brain network involved in selecting which stimuli are relevant (salient) and, thus, should receive additional exploration (Deyoung, 2013; Kafkas & Montaldi, 2018). For example, the hippocampus guides exploration toward novel stimuli (Kafkas & Montaldi, 2018) and to contextual stimuli previously associated with rewarding drugs (Castilla-Ortega, Serrano, et al., 2016). It is then possible for AHN to modulate exploration of both stimuli and contexts. Specifically, AHN has been linked to the exploratory responses in a novel environment (van Dijk et al., 2016). Furthermore, despite mice with reduced AHN show depression-like anhedonia toward naturally rewarding stimuli (Egeland et al., 2017), they are more prone to self-administer addictive drugs—such as cocaine (Deroche-Gamonet et al., 2019)—and persist more in exploring drug-associated contexts (Castilla-Ortega, Blanco, et al., 2016).

Interestingly, intrinsic reward for complexity is highly related to "curiosity." "Curiosity" may be understood both as a transitory state and as a personality trait that entails experiencing pleasure through the act of seeking and learning new information which is not overly simple—that is, it should entail at least an intermediate level of complexity—and/or it is highly anticipated (Gruber & Ranganath, 2019; Kidd & Hayden, 2015). "Curiosity" enhances both exploratory activity and hippocampal-dependent encoding and memory processes, likely by stimulating the dopaminergic and hippocampal pathways that could partially overlap with those activated by "novelty" (Gruber & Ranganath, 2019). Nevertheless, the neurobiological mechanisms of "curiosity" are still poorly understood, and there are currently no rodent models that distinguish specific "curiosity"-related responses from general exploratory activity (Gruber & Ranganath, 2019). Hence, considering that the TMZ-treated mice lacked a particularly enhanced motivation for complexity but showed normal exploratory responses to both environments and (simple) objects, we propose the "spontaneous preference for a complex object task" as a new model to study a specific type of "curiosity."

In conclusion, this work will allow to start a new line of research on the potential role of AHN on intrinsic exploration bias for visual complexity. Nevertheless, it is not exempt of limitations that are detailed as follows: (1) The potential adaptive implications of the intrinsic preference for complex objects—for example, to facilitate its subsequent long-term encoding or discrimination—and the consequences of inhibiting this behavior were not investigated. (2) In relation to this, it is not possible to conclude whether cognitive and/or motivational factors explained absent preference for complexity in conditions of reduced AHN. Future experiments aimed to confirm whether the visual discrimination of complex objects is preserved after AHN depletion may serve to disentangle cognitive from motivational variables. (3) It should also be noted that the methodological strategy employed here to reduce AHN is highly unspecific, because chemotherapy drugs induce a range of systemic and brain side effects—for example, neuroinflammation—and they target the division of glial cells. More sophisticated genetic or virus-based approaches may be employed to provide stronger conclusions (Leuner & Gould, 2010). (4) Finally, while we used both male and female mice for the complex object preference experiments, and both sexes showed a similar preference for complexity, the study of AHN reduction was performed in males only. Unfortunately, sex differences have not been traditionally researched in AHN studies, existing a strong bias to research in male rodents (Yagi & Galea, 2019). The available data support that the basal levels and maturational dynamics of the adult-born neurons, as well as its modulation by external factors such as stress or learning, may differ between sexes (Yagi & Galea, 2019). Therefore, sex differences on the functional implications of AHN should be thoroughly investigated in future studies.

AUTHOR CONTRIBUTIONS

Estela Castilla-Ortega conceived and designed the study. Sonia Melgar-Locatelli, Sara Gil-Rodríguez, Lucía Vicente, and María del Carmen Mañas-Padilla conducted the behavioral experiments. Patricia Rivera, Sonia Melgar-Locatelli, and María del Carmen Mañas-Padilla conducted the histological study. Sonia Melgar-Locatelli, María del Carmen Mañas-Padilla, and Estela Castilla-Ortega analyzed the data. Sonia Melgar-Locatelli, Celia Rodríguez-Pérez, Estela Castilla-Ortega, and María del Carmen Mañas-Padilla wrote the manuscript; all authors critically reviewed the manuscript; all authors approved the final version.

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CONFLICT OF INTEREST

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

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