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Development of an "Object Category Recognition" Task for Mice: Involvement of Muscarinic Acetylcholine Receptors

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Recent research suggests that rats are capable of object categorization-like processes. To study whether mice possess similar abilities, we developed a spontaneous one-trial object category recognition (OCR) task. Based on the spontaneous object recognition paradigm, mice discriminated between two otherwise equally novel objects, one from a novel category and one from a studied category. During the sample phase, mice were exposed to two different exemplars from the same category. After a retention delay, they explored a third (i.e., novel) object from that sampled category and an object from a novel category in a choice phase. Mice preferentially explored the novel category object, taken as an index of category recognition, in this OCR task when a 30-min retention delay was used. Extensive preexposure to category exemplar objects also enhanced subsequent task performance across a longer (1-h) retention delay at which mice without preexposure did not demonstrate evidence for category recognition. Prechoice administration of the acetylcholine muscarinic receptor antagonist, scopolamine, disrupted OCR performance with or without preexposure, implicating acetylcholine in category recognition. The current study presents a valuable new rodent task for the study of the mechanistic basis of categorization-like processes and its potential relevance to common cognitive disorders.

Keywords: acetylcholine, categorization, generalization, mouse, object category recognition

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Visual categorization of objects is thought to rely on the perceptual process of comparing object information from the environment, such as size, shape or color, to already existing representations (Aggelopoulos, 2015). The processing of low-level object features into complex object representations by the ventral visual stream is thought to support object recognition (Bussey & Saksida, 2007) and categorization (Grill-Spector & Weiner, 2014; Konen & Kastner, 2008; Lehky & Tanaka, 2016). Research on categorization in animals has extensively used pigeons because of their highly sophisticated visual system, which is comparable to primates (Brooks et al., 2013; Castro & Wasserman, 2017; Herrnstein & Loveland, 1964; Soto & Wasserman, 2010). Rodents have not typically been used for categorization research due to the assumption that they possess poor visual discrimination abilities; however, recent studies suggest that rats and mice have a complex visual system that can support categorization-like processes (Brooks et al., 2013; Khastkhodaei, Jurjut, Katzner, & Busse, 2016; Vinken, Vermaercke, & Op de Beeck, 2014). Abnormal categorization is a symptom of several human disorders, including schizophrenia and Alzheimer's disease (Baddeley, Bressi, Della Sala, Logie, & Spinnler, 1991; Bizzozero et al., 2013; Grossman et al., 2003); therefore, due to the abundance of transgenic and knockout mouse models of neurological disorders, we sought to develop a relatively simple task for mice to enable investigation of the behavioral and neurobiological bases of object categorization-like abilities.

All previous rodent categorization studies have been performed with two-dimensional visual stimuli. While such studies have been highly informative, they do not necessarily capture the complexity

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of three-dimensional objects. Using three-dimensional real-world objects to test categorization may provide a more ethologically valid and efficient task to study how rodents generalize within object categories. We therefore adapted the standard spontaneous object recognition task (SOR; Ennaceur & Delacour, 1988), which exploits rodents' innate preference for novelty, to create an object category recognition (OCR) task, in which mice discriminate between objects from studied and novel categories. In the OCR task, mice view two different objects from the same category during the sample phase. After a variable retention delay, mice are then presented with a choice between a third object from the studied category and a novel object from a new category. Preference for the object from the unstudied category is suggestive of category recognition because mice and other species tested in this way tend to preferentially explore stimuli they do not recognize (Ennaceur & Delacour, 1988); a preference for the object from the novel category would appear to indicate generalized recognition of the studied category, as both specific objects presented in the choice phase are equally novel.

Previous work from our group suggests that preexposure (PE) to objects changes the nature of their representations in the brain, which can facilitate certain kinds of flexible object recognition across relatively long retention delays (Jacklin, Cloke, Potvin, Garrett, & Winters, 2016; Reid, Jacklin, & Winters, 2012). For example, simultaneous presentation of tactile and visual object features during PE sessions enhances performance on a tactile-visual cross-modal object recognition (CMOR) task, in which rodents recognize an object visually when they have previously only explored tactile object features (Reid et al., 2012). We have proposed that such PE enables the storage of a multimodal object representation, which may enhance CMOR task performance by enabling the activation of a more complete object representation during the tactile-only sample phase. Similarly, PE was also shown to enable view-invariant object recognition (VIOR), where rodents recognize objects that have been rotated 90° or 180° from their original orientation in the sample phase (Mitchnick et al., 2018). We therefore predicted in the present study that PE to category exemplars would also facilitate OCR performance by inducing stronger category representations. Following preexposure to object categories, we evaluated OCR task performance with a longer retention delay to assess the potential beneficial effects of category PE.

The neurotransmitter acetylcholine (ACh) has been strongly implicated in the acquisition and encoding of new information (Hasselmo, 2006). We have previously demonstrated that cholinergic activity at muscarinic ACh receptors, during the choice phase, is required for tactile-visual CMOR (Jacklin et al., 2015), as well as VIOR (Mitchnick et al., 2018). We suggest that ACh at muscarinic receptors is necessary for the binding of object features across modalities and rotational perspectives. Object categorization likely requires analogous binding of common object features across exemplars to form category representations. To begin to probe the neurobiological basis of object categorization, we therefore evaluated the effects of scopolamine, a muscarinic receptor antagonist, on OCR task performance. We predicted that prechoice systemic scopolamine administration would impair performance and that mice would explore objects from studied and novel categories equally.

Method

Subjects

Fifty adult male C57BL/6 mice were obtained from Charles River (Quebec). A minimum of 9 mice per group were used for behavioral experiments; our previous work indicates power of 0.80 can be achieved with a sample size of 6 mice ($\alpha = .05$; Stiver et al., 2015). Mice were group housed, four per cage, in clear polyethylene cages (16 × 12 × 26 cm) with corncob bedding, Crinkl'Nest and cotton nest squares, and food (Tekland Global 16% Protein Rodent Maintenance Diet, Harlan Tekland, U.S.) and water available ad libitum. Mice were tested during the light phase of a 12-h light/dark cycle (0800 lights on; 2000h lights off). All procedures followed the guidelines of the Canadian Council on Animal Care and were approved by the Animal Care Committee at the University of Guelph.

Drugs

Scopolamine hydrobromide (0.1 mg/kg; Sigma Aldrich, Oakville, Canada) was dissolved in 0.9% physiological saline and administered intraperitoneally (IP). This dose was based on previously work from our group in which scopolamine produced CMOR impairments in mice (data unpublished). Physiological saline (0.9%) was used as a vehicle. Drug condition was a within-subjects factor in both scopolamine experiments, and administration of vehicle or scopolamine was counterbalanced across trials. All injections were delivered 20 min prechoice, so that scopolamine was active during the choice phase of the OCR task.

Object Oddity Apparatus and Procedure

Object oddity was performed in an open-field arena constructed from white corrugated plastic (60 × 60 × 30 cm). Three objects from the same category, two identical (e.g., two green hair clips), one unique (e.g., brown hair clip), were placed on one side of the open field, 5 cm away from the wall. Mice experienced a single 10-min sample phase in which they were allowed to freely explore the three objects. To ensure object preference was due to one object being unique rather than a bias for an object, the order of the three objects and the selection of the unique object were switched after each mouse.

Object Category Recognition Apparatus and Procedure

Object category recognition was run in a modified Y-apparatus with walls 30.5 cm high, and arms 15 cm long and 7 cm wide constructed from white Plexiglas (Bartko et al., 2011). One arm of the Y-apparatus, which has a guillotine door 11 cm from the back of the arm, serves as a start box for the mice. During testing, one object was placed at the end of each of the two exploratory arms. All objects were fixed to the floor of the apparatus using odorless, reusable putty (instant tac), to prevent them from being displaced. The apparatus was cleaned after all behavioral trials with dry paper towel. A video camera was mounted on a tripod above the apparatus to record all testing for subsequent analysis.

Objects had no apparent relevance to the biological fitness of mice and were distinct in size (5–15 cm tall), material (glass, metal, and plastic), and color. Twenty-seven object categories (3 objects per category) were selected based on shared physical features but differing in some facets, such as size or color. Object categories were organized into sets of three for OCR testing to ensure categories within a set were sufficiently different (see Figure 1a in the supplemental materials). Between trials, objects were wiped with 50% ethanol (to eliminate olfactory cues).

Prior to OCR testing, mice were handled and given two 10-min habituation sessions to an empty Y-apparatus on two consecutive days. Testing started at least 24 h after the last habituation session. Immediately before testing, mice were transported in their home cage to the testing room. The OCR task comprised a sample phase, a retention delay, and a choice phase. In the sample phase, two different exemplars of objects from the same category (e.g., two different cars) were placed in the exploratory arms of the Y-apparatus. A mouse was then placed in the starting arm and the guillotine door was lifted to allow access to the objects, with the sample phase timed from the moment the mouse had fully exited the start arm. Once the mouse had fully exited the start arm, the guillotine door was closed to prevent reentry. The sample phase ended after 10min. After the sample phase, the mouse was taken out of the apparatus and returned to its home cage for a retention delay (30 min or 1 h, depending on specific experiment). In the choice phase, two different objects were placed in the exploratory arms of the apparatus. In the control condition, mice were presented two novel objects from two different, and previously unseen, categories (e.g., a ball and a lock). In the experimental condition, mice were presented with two novel objects, one of which was a novel object from a studied category (shown in the sample phase) and the other from a novel category (e.g., a car and a lock). This task takes advantage of rodents' innate preference to explore novelty; if mice in the experimental group are able to generalize features from the category studied in the sample phase to a novel exemplar from the same category in the choice phase, they should preferentially explore the object from the novel category. In the control condition, both objects presented in the choice phase are from novel categories and should be explored equally.

Following testing, an experimenter blind to the experimental groups viewed recordings of the mice and used custom software to score the duration (s) of exploratory bouts for each object in the sample and choice phases (active sniffing within 1 cm of the object and/or touching the object with the nose).

Pre-Exposure Apparatus and Procedure

Object PE sessions were conducted using open-field arenas constructed from white corrugated plastic (60 × 60 × 120 cm). Five mice were run simultaneously in 5 adjacent open fields. Mice were habituated to open fields during two 30-min sessions on two consecutive days. A video camera mounted above the arenas recorded all sessions for subsequent analysis.

In experiments with object PE, all mice were exposed to all objects within each category (6 categories per experiment) for a total of 2h of exploration prior to the start of the OCR testing phase. Each mouse had four 30-min PE sessions per day. During each PE session, for any given mouse, all three objects from two different categories (e.g., 3 cars and 3 locks) were presented simultaneously, and combinations of categories in each session were pseudorandomized over the three days. Mice were tested on the OCR task five days following the end of PE sessions.

Following PE experiments, a researcher blind to experimental groups used EthoVision Software (Noldus, The Netherlands) to analyze videos and record durations of object exploration (s); this automated procedure was used for PE phase scoring because of the significant duration of these sessions. Average exploration for each object category from these PE sessions is reported in supplementary Tables S1 and S2. Differential exploration of objects from different categories was observed in all PE experiments. This finding may indicate a preference for some categories over others. To minimize the potentially confounding effects on subsequent OCR testing, all PE objects were fully counterbalanced.

Experiment 1

Experiment 1 was designed to determine whether mice are capable of discriminating between object exemplars within a category using a perceptual "oddity" task, in which rodents preferentially explore an odd object when presented with two or more identical objects (Bartko, Winters, Cowell, Saksida, & Bussey, 2007; Bartko, Winters, Saksida, & Bussey, 2014). In a single 10-min session, mice were presented with three objects from the same category, two identical and one unique. We tested discrimination using two different object categories used in subsequent experiments (hairclips and tape).

Experiment 2

Experiment 2 was designed to determine if mice are capable of performing the OCR task. In the sample phase, two different exemplars of objects from the same category were presented. After a 30-min retention delay, in the choice phase, mice were presented with two novel objects. In the control condition, mice were presented two objects from two different, and previously unseen, categories. In the experimental condition, mice were presented with two novel objects, one of which was a novel object from a studied category (shown in the sample phase) and the other from a novel category.

Experiment 3

Experiment 3 was conducted to test the hypothesis that muscarinic acetylcholine receptors are involved in OCR task performance. Experiment 3 was run the same as Experiment 2, except mice were injected with either vehicle or scopolamine 20 min prior to the choice phase.

Experiment 4

Experiment 4 was designed to develop a difficult version of the OCR task that mice could not perform spontaneously. Experiment 4 was run the same as Experiment 2, except the retention delay was 1 h.

Experiment 5

Experiment 5 was conducted to test the hypothesis that PE would enhance performance on the difficult OCR task. Mice were

extensively familiarized with object categories during PE sessions as described above. All mice were pre-exposed to the same object sets, but 10 mice were tested on OCR with a different set of six categories to which they had not been pre-exposed (non-PE group) and the other 10 mice were tested with categories studied in the PE sessions (PE group).

Experiment 6

Experiment 6 was conducted to address the possibility that pre-exposure sessions facilitate OCR by increasing familiarity with the PE objects. Five days after pre-exposure sessions (the same as the delay between PE sessions and OCR testing in earlier experiments), we presented mice with a PE object and a novel object (from a completely novel, unexplored category) for 10 min to assess relative object exploration. If mice perceive the PE object as more familiar, they should preferentially explore this object.

Experiment 7

Experiment 7 was designed to determine if PE could facilitate OCR performance even when novel object exemplars from the pre-exposed categories were used. Experiment 7 was run the same as Experiment 5, except OCR testing was conducted with novel object exemplars from the pre-exposed categories. For example, mice were pre-exposed to a red, teal, and yellow car and were tested on OCR using black and green cars.

Experiment 8

Experiment 8 was conducted to test the hypothesis that muscarinic receptors are necessary for performance on the difficult OCR task following PE. Experiment 8 was run like Experiment 5, except mice were injected with either vehicle or scopolamine 20 min prior to the choice phase.

Data Analysis

For category object oddity, the oddity preference was calculated as [unique object exploration]/[total exploration]. An oddity preference ≥ 0.33 indicated a greater preference for the unique object and intact perception. With three objects, an oddity preference significantly greater than 0.33 ("chance") indicates an oddity preference, from which we infer that mice can perceptually discriminate between objects exemplars from the same category; this analysis was performed using one sample *t* tests. No outliers were removed from this experiment.

For OCR, the novelty preference in the choice phase was quantified by calculating a discrimination ratio (choice DR = (novel category object exploration—studied category object exploration)/(total object exploration)). Data were analyzed using either independent samples *t* tests or split-plot ANOVAs. Where appropriate, post hoc *t* tests were used to analyze group differences. A discrimination ratio was also calculated for the sample phase (sample DR = (object in the location where the novel category object will be placed in during the choice phase−object in the location where the studied category object will be placed in the choice phase)/(total object exploration). In the sample phase, objects should be equally novel and a DR of approximately zero is expected. In the choice phase, a DR significantly greater than the sample DR indicates novelty preference in the experimental condition, from which we infer object category recognition.

Outliers (>2 SD ± mean) were excluded from analyses. Three outliers were excluded from Experiment 1, one mouse in the control condition and two mice in the experimental condition. Three outliers were excluded from Experiment 3, two mice in the control condition and one mouse in the experimental condition. Two outliers were excluded from Experiment 4, one mouse in the group tested with non-PE objects and one mouse in the group tested with PE objects. Two mice were excluded from Experiment 8, one mouse from the control condition treated with scopolamine and one mouse from the experimental condition treated with vehicle.

General exploratory measures (i.e., overall object exploration means from the sample and choice phases) are reported in supplementary tables S3–S11; only significant results from these analyses are reported in the main text.

For object PE sessions, a 2 × 6 repeated-measures ANOVA was used to analyze exploration times between experimental groups as well as object categories.

All statistical analyses were performed with $\alpha = .05$ using SPSS. Where appropriate, the Bonferroni correction was applied.

Results

Mice Demonstrate Performance Consistent With Object Category Recognition

We first show that mice can discriminate between object exemplars in the same category using an oddity task (Experiment 1; Figure 1A). One-sample *t* tests suggest mice can discriminate

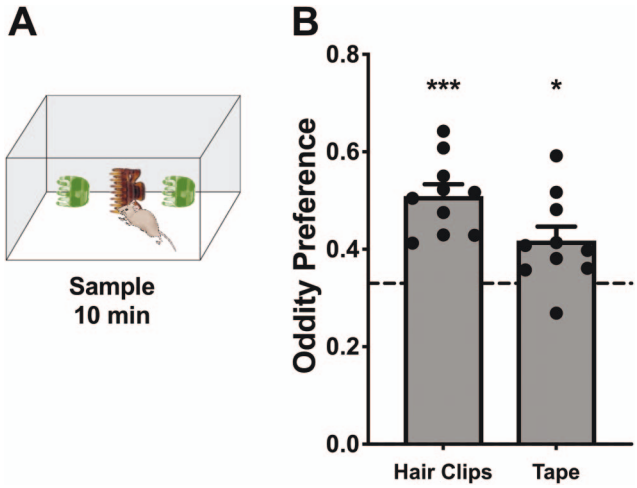

Figure 1. (A) Schematic representation of the oddity task. During a 10-min sample phase, mice were presented with three objects from the same category, two identical (e.g., two green hair clips), one unique (e.g., brown hair clip). We evaluated oddity using two different categories: hair clips and tape. (B) Mice were able to discriminate between different object exemplars within a category, as they explored the odd object significantly above chance (0.33). Data are mean ±SEM. * $p < .05$ indicate a significant one-sample *t* test suggesting intact discrimination. *** $p < .001$. See the online article for the color version of this figure.

between object exemplars from the same category, as mice explored the odd object significantly above chance (hair clips: $t(9) = 7.386$, $p < .001$; tape: $t(9) = 3.038$, $p = .014$; Figure 1B).

In order to determine whether mice are able to recognize and discriminate between objects from different categories, we evaluated OCR with a 30-min retention delay (Experiment 2; Figure 2A). An independent samples t test revealed a significant difference between the control and experimental conditions, $t(18) = -3.64$, $p = .002$ (Figure 2B). Paired samples t tests between sample and choice DR were consistent with category recognition in the experimental condition, $t(9) = -5.46$, $p < .001$, as mice explored the object from the novel category significantly more than an object from the studied category; mice in the control condition explored objects from two novel categories equally, $t(9) = -1.40$, $p = .194$.

Scopolamine Impairs OCR Task Performance

To evaluate the role of muscarinic acetylcholine receptors in OCR task performance, systemic scopolamine was administered prechoice (Experiment 3). A 2 × 2 split-plot ANOVA demonstrated a significant condition (control, experimental) by drug (vehicle, scopolamine) interaction, $F(1, 17) = 8.55$, $p = .010$, as well as main effects of condition, $F(1, 17) = 5.31$, $p = .034$, and drug, $F(1, 17) = 10.94$, $p = .004$ (Figure 2C). Paired samples t tests between sample and choice DR indicated that scopolamine impaired OCR performance in the experimental condition, as vehicle-treated mice preferentially explored the object from the novel category, $t(8) = -3.75$, $p = .006$, but scopolamine-treated mice did not, $t(8) = .36$, $p = .726$, suggesting that the object from the studied category was treated as novel. Both vehicle- and scopolamine-treated mice in the control condition explored objects from novel categories equally, $t(9) = 1.91$, $p = .089$, and $t(9) = 1.36$, $p = .207$, respectively. Post hoc analyses indicated significant group differences between vehicle- and scopolamine-treated mice in the experimental condition, $t(8) = 2.43$, $p = .041$, and between vehicle-treated mice in the control and experimental conditions, $t(17) = -3.28$, $p = .004$.

Object Pre-Exposure Facilitates OCR Task Performance With an Increased Retention Delay

To evaluate the effects of PE to object categories, we first developed a "difficult" version of the OCR task by extending the retention delay to 1 h (Experiment 4). An independent samples t test demonstrated no difference between control ($M = .01$, $SEM = .03$) and experimental conditions ($M = .02$, $SEM = .08$), $t(17) = -.20$, $p = .852$. Paired samples t tests between sample and choice DR indicated that mice in the experimental condition could not perform OCR with a 1-h delay, $t(9) = -1.00$, $p = .344$, as they explored objects from studied and novel categories equally. Mice in the control condition explored objects from two novel categories equally, $t(9) -.50$, $p = .632$.

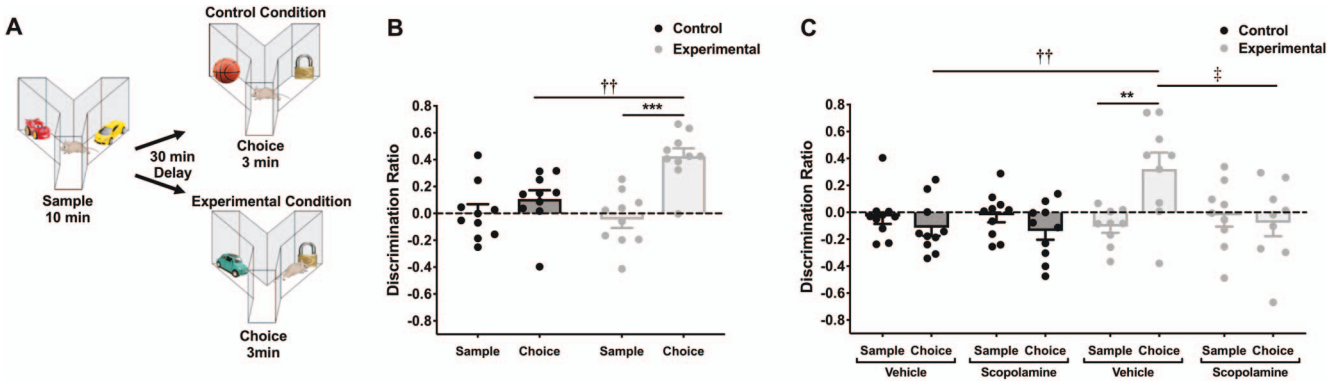

Figure 2. (A) Schematic representation of the OCR task used in this study. During the sample phase, mice were presented with two different objects from the same category (e.g., two cars). Following the sample phase, there was a 30-min or 1-h retention delay. In the control condition, during the choice phase, mice were presented with two novel objects from two different novel categories (e.g., a ball and a lock). In the experimental condition, during the choice phase, mice were presented with two novel objects, one from the same ("studied") category as the objects in the same phase and the other from a novel category (e.g., a car and a lock). (B) OCR task performance with a 30-min retention delay. Mice in the experimental group demonstrated evidence for category recognition, as they were able to discriminate between objects from studied and novel categories. Mice in the control condition explored objects from two novel categories equally. Data are mean ±SEM. †† $p < .01$ indicates a significant difference between control and experimental groups. *** $p < .001$ indicates significant difference between sample and choice DR, suggesting effective category recognition. (C) Effect of scopolamine on OCR task performance with a 30-min retention delay. Vehicle-treated mice in the experimental group showed evidence for category recognition, replicating the findings from the previous experiment. Scopolamine impaired OCR in the experimental condition, as mice explored novel objects from studied and novel categories equally. Vehicle- and scopolamine-treated mice in the control condition did not preferentially explore either object. Data are mean ±SEM. $p < .01$ indicates significant differences between sample and choice DR, suggesting intact category recognition. ‡ $p < .05$ indicates a significant difference between vehicle and scopolamine treatment. †† $p < .01$ indicates a significant difference between vehicle-treated control and experimental groups. See the online article for the color version of this figure.

We next evaluated the facilitatory effects of PE on OCR with a 1-h delay by using objects from either PE or non-PE categories (Experiment 5; Figure 3A). A 2 × 2 split-plot ANOVA revealed a significant condition (control, experimental) by group (non-PE category, PE category) interaction, $F(1, 17) = 14.62$, $p = .001$, as well as main effects of condition, $F(1, 17) = 15.10$, $p = .001$, and group, $F(1, 17) = 15.674$, $p = .001$ (Figure 3B). Mice in the control condition that were tested with either PE- or non-PE objects explored objects equally during the choice phase, $t(8) = 1.11$, $p = .340$, and $t(9) = 1.91$, $p = .089$, respectively. Post hoc analyses revealed significant group differences between mice in the experimental condition tested with non-PE and PE objects, $t(17) = -4.36$, $p < .001$, as well as mice tested with PE objects in the control and experimental conditions, $t(8) = 4.85$, $p = .001$. A 2 × 6 ANOVA revealed a significant main effect of category (balls, cars, hair clips, locks, nail polish, and tape), $F(11, 1331) = 31.408$, $p < .001$ (Table S1), but no effect of experimental group (on-PE or PE objects) or interaction, on exploration during PE sessions. However, all categories were counterbalanced throughout OCR testing, so the influence of these differences on overall interpretation should be minimal.

It is possible that preexposure sessions facilitate performance on the OCR task merely by increasing the familiarity of PE objects, rather than enhancing category generalization. To rule out this possibility, we conducted two additional experiments with a separate cohort of mice.

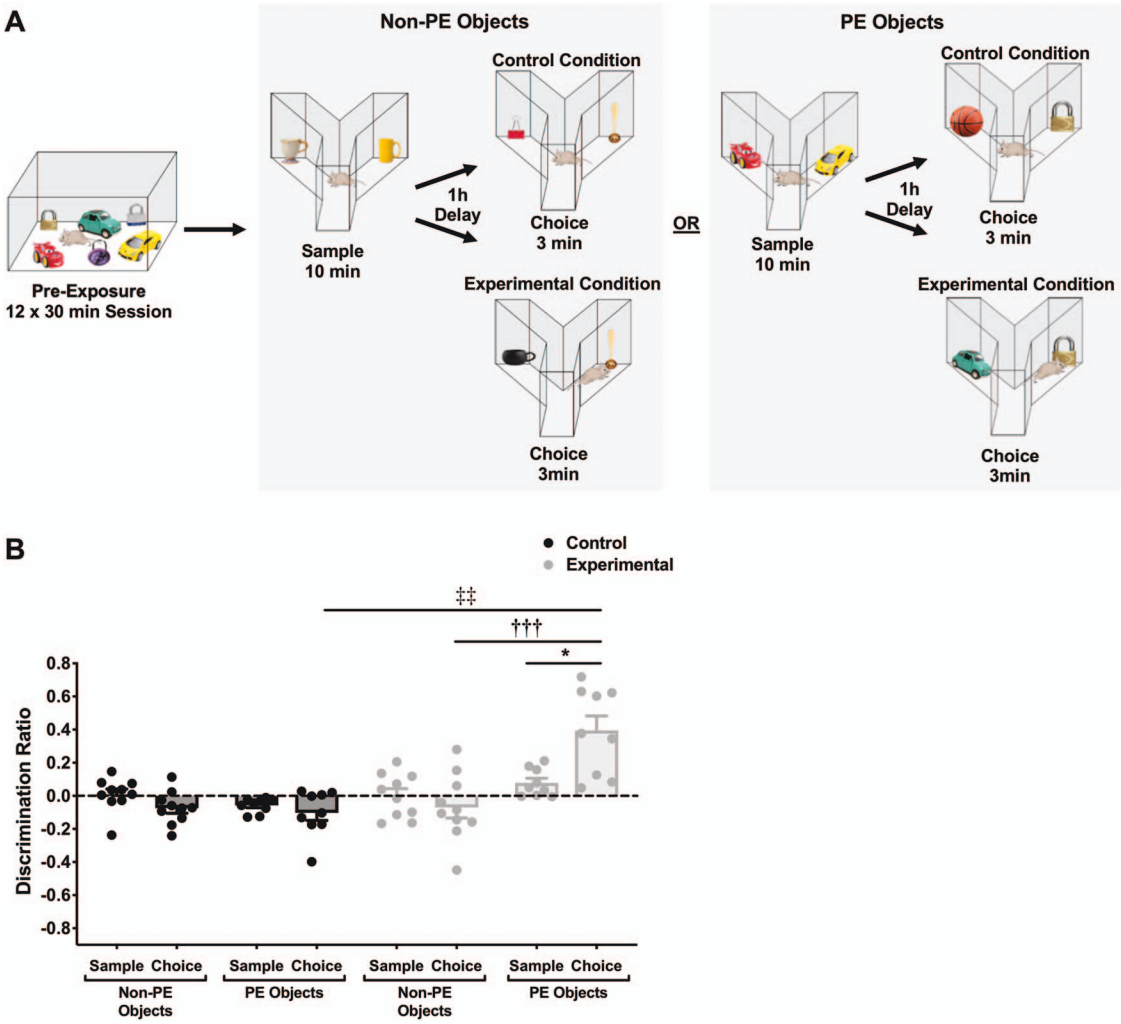

Figure 3. (A) Schematic representation of PE sessions and the "difficult" (long-delay) OCR task using either non-PE or PE objects. (B) After PE, mice in the experimental condition that were tested with PE objects demonstrated enhanced OCR, as they were able to discriminate between objects from studied and novel categories, whereas mice tested with novel (non-PE) objects did not. Mice without PE (control condition) did not demonstrate category recognition, as they explored objects from both categories equally. Data are mean ±SEM. ††† $p < .001$ indicates a significant difference between mice tested with novel and PE objects. ‡‡ $p < .01$ indicates a significant difference between control and experimental conditions. * $p < .05$ indicates significant difference between sample and choice DR, suggesting intact category recognition. See the online article for the color version of this figure.

To determine if mice treat PE objects as familiar because the preexposure sessions served as sample phases, we presented mice simultaneously with a PE object and a novel object to explore for 10 min (i.e., like a typical SOR choice phase; Experiment 6; Figure 4A). A one-sample t test suggested that mice do not treat PE objects as familiar with this procedure, $t(9) = -.198$, $p = .847$ (Figure 4B), as they explored an object from a PE category and a novel object equally. This suggests that the PE sessions, with the parameters we used, do not merely serve as sample phases to enhance memory for the specific PE objects.

We subsequently determined whether PE had a facilitatory effect on the "difficult" version of OCR even when novel object exemplars from the pre-exposed categories were used (Experiment 7; Figure 5A). A paired samples t test revealed a significant difference between control and experimental conditions, $t(9) = -2.920$, $p = .017$ (Figure 5B). Paired samples t tests between sample and choice DR were consistent with category recognition in the experimental condition, $t(9) = -2.58$, $p = .030$, as mice explored the object from the novel category significantly more than a novel exemplar from a pre-exposed category; mice in the control condition explored the two novel category objects equally, $t(9) = -.68$, $p = .515$. A one-way ANOVA revealed a significant main effect of category (balls, cars, hairclips, locks, nail polish, and tape), $F(5, 690) = 3.255$, $p = .007$; Table S3) on exploration during PE sessions. However, all categories were counterbalanced throughout OCR testing, so the influence of these differences on overall interpretation should be minimal.

Scopolamine Blocks the Beneficial Effect of Object Pre-Exposure on OCR Task Performance

To determine if muscarinic acetylcholine receptors remain necessary for OCR task performance following object PE, we evaluated OCR with a 1-h delay (using only PE objects) and administered prechoice scopolamine or vehicle (Experiment 8; Figure 6A). A 2 × 2 split-plot ANOVA revealed a significant interaction between condition (control, experimental) and drug (vehicle, scopolamine), $F(1, 17) = 91.81$, $p < .001$, as well as main effects of condition, $F(1, 17) = 57.82$, $p < .001$, and drug $F(1, 17) = 60.64$, $p < .001$ (Figure 6B). Paired samples t tests between sample and choice DR were consistent with category recognition by mice in the experimental group treated with vehicle, $t(8) = -11.03$, $p < .001$, as they preferentially explored the novel category object in the choice phase, whereas mice from the experimental condition treated with scopolamine did not, $t(8) = -2.05$, $p = .075$, indicating that they treated the objects from the novel and studied categories as equally novel. Mice in the control condition treated with either vehicle or scopolamine explored objects equally, $t(9) = .56$, $p = .587$, and $t(9) = -.60$, $p = .566$, respectively. Post hoc analyses demonstrated significant group differences between vehicle- and scopolamine-treated mice in the experimental condition, $t(8) = 10.94$, $p < .001$, as well as mice in the control and experimental conditions treated with vehicle, $t(17) = -10.78$, $p < .001$. A 2 × 6 ANOVA revealed a significant interaction between experimental condition (control, experimental) and category (cups, hooks, mascara, scissors, shaving cream, and sponges), $F(11, 1331) = 3.06$, $p = .010$, as well as a main effect of category, $F(11, 1331) = 103.166$, $p < .001$ (Table S2), but no main effect of experimental group (control or experimental), on exploration during PE sessions. However, all categories were counterbalanced throughout OCR testing, so the influence of these differences on overall interpretation should be minimal.

Discussion

We present a novel variation of the spontaneous object recognition (SOR) task for evaluating spontaneous categorization-like behavior in mice. The object category recognition (OCR) task uses three-dimensional objects and does not require explicit training or other methods designed to manipulate task motivation; therefore, the OCR task provides a relatively simple and efficient method for studying the behavioral and neurobiological bases of categorization-like behavior in rodents. Our results are the first to suggest that mice are capable of generalizing object features within

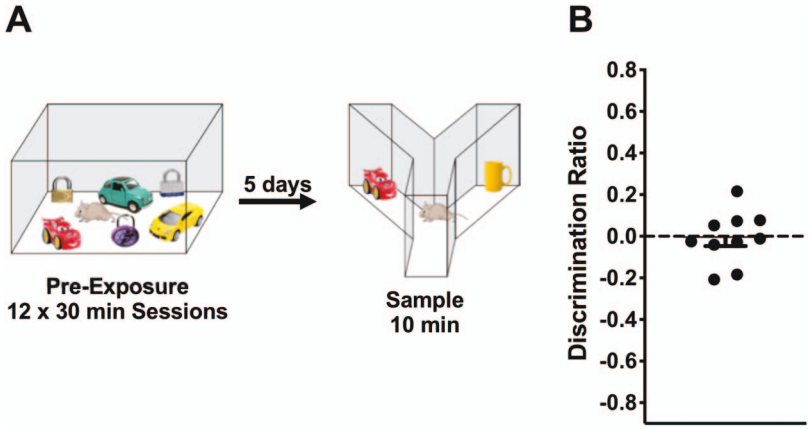

Figure 4. (A) Schematic representation of the control experiment used to rule out the possibility that preexposure sessions facilitate OCR by increasing familiarity with PE objects. Five days after preexposure sessions, we presented mice with a PE object and a novel object (from a completely novel, unexplored category) for 10 min. (B) Mice did not treat the PE object as familiar, as they explored an object from a PE category and a novel object equally. Data are mean ±SEM. See the online article for the color version of this figure.

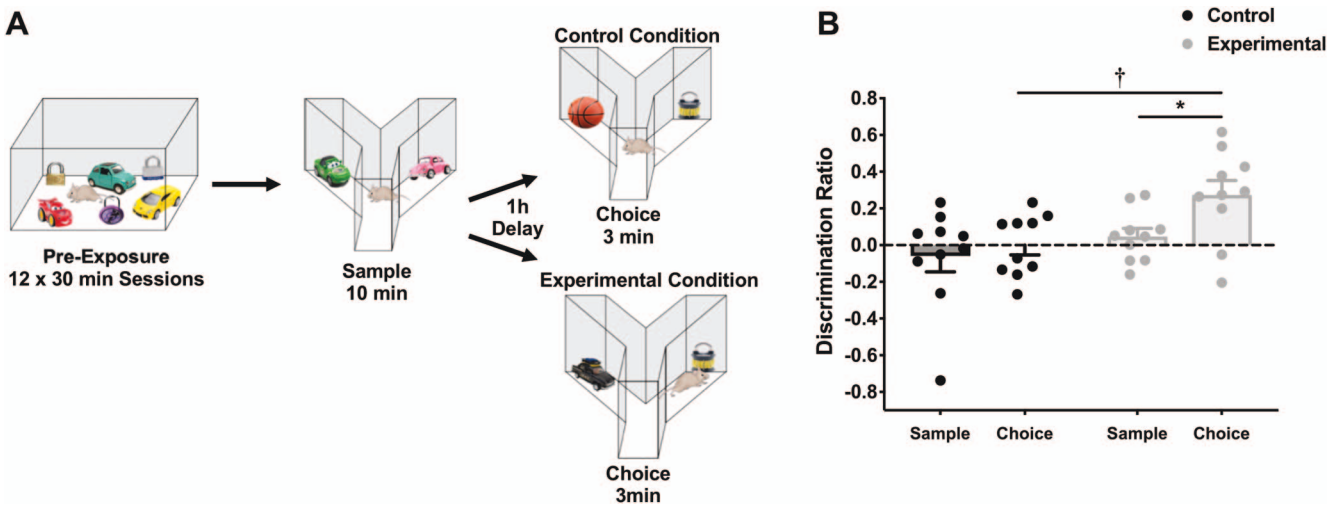

Figure 5. (A) Schematic representation of PE sessions and the "difficult" (long-delay) OCR task using novel object exemplars from PE categories. (B) After PE, mice in the experimental condition that were tested with novel exemplars of PE objects demonstrated OCR when tested with a 24-h retention delay, as they were able to discriminate between objects from studied and novel categories. Mice in the control condition did not demonstrate evidence for category recognition, as they explored objects from both categories equally. Data are mean ±SEM. † $p < .05$ indicates a significant difference between control and experimental groups. * $p < .05$ indicates significant difference between sample and choice DR, suggesting intact category recognition. See the online article for the color version of this figure.

categories, which is likely a precursor to human-like categorization processes. We have also implicated the cholinergic system in the memory retrieval aspect of this process.

Using the object oddity and OCR tasks, we show that mice discriminate between and recognize objects from different categories. Mice were able to perform the OCR task with a retention delay up to 30 min between studying and test. In the experimental group, category recognition was inferred from preferential exploration of objects from a novel category when compared to novel exemplars from a category studied during the sample phase. Categorization-like abilities in mice are consistent with previous studies in rats. Brooks et al. (2013) and Vinken et al. (2014) trained rats to perform categorization tasks based on two dimensional images or videos of various exemplars from a category. Similar to

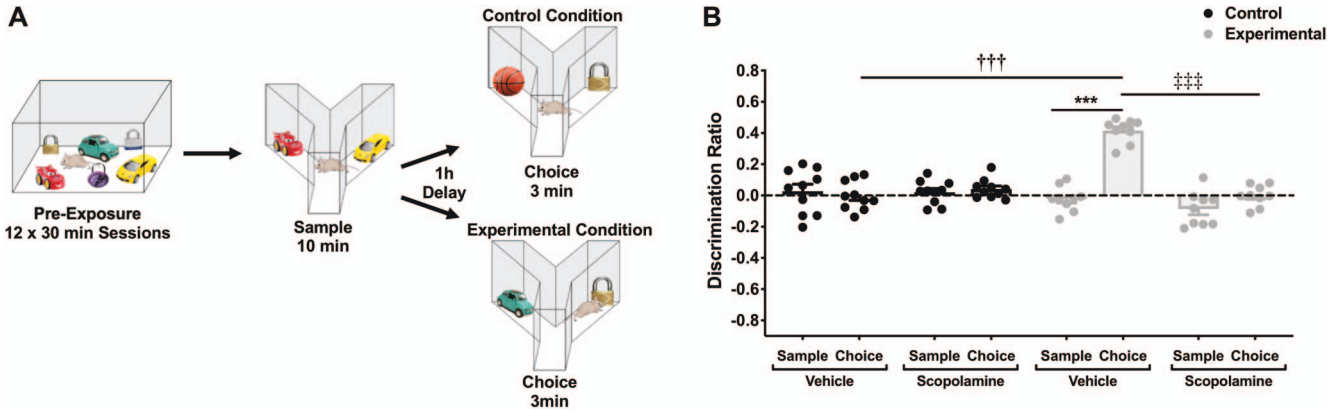

Figure 6. (A) Schematic representation of PE sessions and the "difficult" (long-delay) OCR task using PE objects. (B) Effect of scopolamine on OCR task performance after PE. Scopolamine impaired OCR task performance in the experimental condition, as mice explored objects from studied and novel categories equally, whereas vehicle treated mice preferentially explored the novel category objects. Mice in the control condition did not demonstrate OCR, as they explored objects from both categories equally. Data are mean ±SEM. ††† $p <$ .001 indicates a significant difference between vehicle treated control and experimental groups. ‡‡‡ $p < .001$ indicates a significant difference between vehicle and scopolamine treatments. *** $p < .001$ indicates significant difference between sample and choice DR, suggesting intact category recognition. See the online article for the color version of this figure.

our results, both studies found that rats were able to differentiate between categories and generalize to novel exemplars.

Given the well-established role of ACh receptors in object memory and perception (Barker & Warburton, 2009; Bartko et al., 2014; Jacklin et al., 2015; Mitchnick et al., 2018; Warburton et al., 2003; Winters, Bartko, Saksida, & Bussey, 2007), as well as impaired visual categorization in macaques following scopolamine treatment (Aggelopoulos, Liebe, Logothetis, & Rainer, 2011), we predicted impairing effects of scopolamine on OCR task performance by mice. Indeed, prechoice systemic scopolamine administration significantly impaired OCR in the experimental group. Muscarinic receptors do not appear to be necessary for object memory retrieval, as pretest scopolamine does not typically affect standard SOR performance in rodents (Jacklin et al., 2015; Warburton et al., 2003; Winters, Saksida, & Bussey, 2006) or delayed nonmatching-to-sample (DNMS) performance in monkeys (Aigner & Mishkin, 1986; Aigner, Walker, & Mishkin, 1991; Tang, Mishkin, & Aigner, 1997). The present scopolamine result with OCR is comparable to impairments in CMOR and VIOR induced by prechoice scopolamine (Jacklin et al., 2015; Mitchnick et al., 2018). Unlike the standard SOR task, we have previously proposed that accurately discriminating objects in the choice phase of the CMOR and VIOR tasks requires a significant binding or associative process to link the tactile/visual or various rotational perspectives of the objects, as these features are not simultaneously available during the sample phase of these tasks. We have hypothesized that this putative associative process, across modalities or perspectives, which enables the flexible use of object representations in such tasks, is ACh dependent (Jacklin et al., 2015; Mitchnick et al., 2018); ACh, acting at muscarinic receptors, may play an analogous role in the OCR task to facilitate the association of novel object exemplars within an established category representation. Additional research will be required to determine the specific role of muscarinic receptors and ACh in this process, but the absence of reliable prechoice effects in similar object memory tests like the SOR and DNMS tasks suggests that this is not merely a general memory retrieval function.

Preexposure to object categories facilitated OCR task performance with an extended retention delay (1 h) at which mice could not perform the task without pre-exposure (PE). The results from control conditions suggest that mere enhanced familiarity with the specific pre-exposed objects does not explain these findings. Indeed, mice did not preferentially explore a novel object over a PE object, and the effect of PE on OCR was seen even when novel exemplars from pre-exposed categories were used. Although we have also shown using the oddity task that mice can discriminate between objects within a category prior to PE, it is still possible that extensive PE may lead to the formation of more generalized object representations based on size, shape, and smell that would induce familiarity for any other similar object. Further research is necessary to address this possibility. One other potential counter to this argument, however, is related to the effects of scopolamine in the current study. Previous studies have strongly suggested that rats with lesions of the cholinergic basal forebrain become less likely to pattern separate and more likely to pattern complete (Easton, Douchamps, Eacott, & Lever, 2012; Easton, Fitchett, Eacott, & Baxter, 2011; Seel, Eacott, Langston, & Easton, 2018); these findings would seem to predict, if anything, an enhancement of the likelihood of scopolamine-treated rats to treat similar (or similarly familiar) objects (i.e., exemplars from the same category) as the same. However, the current results demonstrate the opposite pattern, suggestive of an effect on a cognitive process that is distinct from familiarity-related pattern completion across objects with similar features.

During PE sessions, mice were given extended periods of time to explore multiple exemplars within each to-be-tested category. This could have enabled the mice to discern more structural similarities between objects and create stronger categorical representations (McClelland, McNaughton, & O'Reilly, 1995). Again, this finding is consistent with a beneficial effect of PE on CMOR task performance over extended delays (24 h; Reid et al., 2012). Interestingly, scopolamine blocked the enhancing effects of object PE on OCR with a 1-h delay. This finding is consistent with what is reported for the VIOR task, in which scopolamine impaired VIOR performance even after PE, but not the CMOR task (Jacklin et al., 2015; Mitchnick et al., 2018). Thus, the involvement of muscarinic receptors in VIOR, OCR, and CMOR task performance differs slightly. More work is required to elucidate the differential nature of these contributions.

In conclusion, the OCR task presented here should facilitate the study of object categorization/generalization behavior in mice and demonstrates an important role for muscarinic ACh receptors in this cognitive process. The OCR test, along with other categorization-like tasks for rodents (Brooks et al., 2013; Vinken et al., 2014), can be used to probe the anatomical and mechanistic bases of this important form of cognition and could be a valuable addition to the behavioral tools used for the evaluation of cognitive deficits and therapeutic interventions in mouse models of neurological disorders, such as Alzheimer's disease.

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