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Development of novel tasks for studying view-invariant object recognition in rodents: Sensitivity to scopolamine

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ABSTRACT

The capacity to recognize objects from different view-points or angles, referred to as view-invariance, is an essential process that humans engage in daily. Currently, the ability to investigate the neurobiological underpinnings of this phenomenon is limited, as few ethologically valid view-invariant object recognition tasks exist for rodents. Here, we report two complementary, novel view-invariant object recognition tasks in which rodents physically interact with three-dimensional objects. Prior to experimentation, rats and mice were given extensive experience with a set of 'pre-exposure' objects. In a variant of the spontaneous object recognition task, novelty preference for pre-exposed or new objects was assessed at various angles of rotation (45°, 90° or 180°); unlike control rodents, for whom the objects were novel, rats and mice tested with pre-exposed objects did not discriminate between rotated and un-rotated objects in the choice phase, indicating substantial view-invariant object recognition. Secondly, using automated operant touchscreen chambers, rats were tested on pre-exposed or novel objects in a pairwise discrimination task, where the rewarded stimulus (S+) was rotated (180°) once rats had reached acquisition criterion; rats tested with pre-exposed objects re-acquired the pairwise discrimination following S+ rotation more effectively than those tested with new objects. Systemic scopolamine impaired performance on both tasks, suggesting involvement of acetylcholine at muscarinic receptors in view-invariant object processing. These tasks present novel means of studying the behavioral and neural bases of view-invariant object recognition in rodents.

1. Introduction

Recognition or classification of objects is thought to begin in the ventral visual stream (VVS), a series of brain structures organized hierarchically, both anatomically and functionally [34,1]. Propagating downstream through successive regions of the VVS, neurons not only become increasingly selective to complex features, but a relative increase in tolerance to stimulus changes such as rotation ("view-invariance") also occurs, as demonstrated in human and non-human primate models ([1,2,37]. It was previously believed that rats lacked a complex visual processing system that would justify their use to study processes such as view-invariant object recognition [3]. However, Zoccolan et al. demonstrated that following extensive training, rats were able to recognize familiar images on LCD monitors despite changes in size, lighting, and orientation [4]. More recent studies provide further evidence for the complexity of the rat visual processing system and point towards the presence of cortical machinery that supports view-invariant recognition [5–7].

To date, the behavioural tests that have been used to study view-invariant abilities in rodents have required the recognition of computer generated visual objects [4–7]. For the current study, we were interested initially in developing a complementary "view-invariant" object recognition task for rats that would perhaps be more ethologically relevant (i.e., by using not just visual information) and would not require extensive operant training prior to testing. In rodents' naturalistic settings, object recognition likely involves integration of information from various sensory modalities, and previous findings from our lab suggest that a short multimodal (i.e., visual plus tactile) pre-exposure session to an object prior to crossmodal object recognition testing, changes the nature of the object representation in the brain and how rats perform on

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a crossmodal object recognition task [8]. Here we sought first to develop an analogue of the one-trial crossmodal object recognition task used in our previous studies, in order to investigate similar questions in the context of view-invariance and ultimately facilitate studies on the neurobiological underpinnings of this cognitive function.

Prior to object recognition testing in the current study, rats received pre-exposure to a set of visually and tactilely distinct and complex objects in open field arenas. We first developed a variation of the spontaneous object recognition (SOR) task, which exploits rodents' innate preference to investigate novel objects. Specifically, during a learning or sample phase a rodent explores two identical novel objects. Following a variable retention delay, the rat is presented with one of the now familiar objects and a novel object. Preferential investigation of the novel object suggests recognition of the familiar object. In the view-invariant object recognition (VIOR) task presented in the current study, rats and mice viewed two identical objects in a Y-shaped apparatus, restricting exploration to the 'front' of the object. Following a 1-h retention delay, both of these objects were again presented in a choice phase, but one of the objects was rotated 45°, 90° or 180°. We predicted that rodents pre-exposed to these objects would explore the choice objects equally (i.e., no preference), demonstrating view-invariant object recognition. Conversely, rodents tested with novel objects were predicted to view the rotated copy of the object as novel (i.e., object preference), indicating view-specific recognition.

'Spontaneous' recognition tasks, like the VIOR test described above, infer recognition from a lack of responding towards the objects (i.e., no exploratory preference for the rotated object). In order to obtain a direct behavioural indication of view-invariant recognition, we also developed a complementary view-invariant pairwise discrimination (VIPD) touch-screen task using the same objects (rats only). Rats were initially trained to discriminate between pictures of two objects presented on LCD monitors, by rewarding response to one of the objects. During probe tests, after achievement of acquisition criteria, the rewarded object was rotated 180°. We predicted that rats pre-exposed to the objects (i.e., physical pre-exposure in the open field arenas) would continue to respond to the rewarded object significantly above chance despite its rotation, whereas performance would drop substantially in the first few probe sessions for rats trained and tested with novel objects.

Previously, we reported that acetylcholine (ACh) activity at muscarinic receptors is necessary during the test phase of the tactile-to-visual crossmodal object recognition task, despite no apparent effects on memory retrieval or test phase performance in a variety of non-crossmodal object recognition tasks [9]. We hypothesized that muscarinic receptor activation plays a unique role in the binding of object features from across sensory modalities to facilitate crossmodal recognition [9]. View-invariant object representations involve similar feature integration (i.e., binding information from all sides of an object; [10,36]). Therefore, as a first foray into studying the neural bases of view-invariant object recognition, we also assessed the involvement of muscarinic receptors (rats only) in the VIOR and VIPD tasks, predicting that antagonism with scopolamine would disrupt any view-invariant object recognition displayed.

2. Materials and methods

2.1. Subjects

In Experiment 1a, Experiment 2, and Experiment 3, one set of 20 male Long Evans rats (Charles River, Quebec) weighing between 250–300 g at the start of testing, was used. For Experiment 1b, the subjects were 16 male C57 BL/6J mice (Jackson Laboratories, Maine USA), approximately 5 months of age. Rats were housed in pairs, whereas mice were housed in groups of four. Rats and mice were housed in opaque cages in separate colony rooms, on a 12-h reverse light:dark cycle (8:30 A.M. lights off, 8:30 P.M. lights on). All behavioural testing was completed during the dark phase of the cycle. Rodents were on restricted feed (85-90% of free feed body weight) in order to maintain exploratory behaviour, and water was available *ad-libitum*. On testing days, rodents were fed after the experiment was completed. All procedures adhered to the guidelines of the Canadian Council on Animal Care and were approved by the Animal Care Committee at the University of Guelph.

2.2. Drugs and injections

Scopolamine hydrobromide (0.1 mg/kg, 0.2 mg/kg, 0.5 mg/kg; Sigma Aldrich, Oakville, Canada) and scopolamine methylbromide (0.5 mg/kg; Sigma Aldrich, Oakville, Canada), which does not cross the blood-brain-barrier, were dissolved in 0.9% physiological saline and administered to rats through intraperitoneal (i.p.) injections. These doses were chosen from previous studies that demonstrate cognitive impairments in integrating object features in the CMOR task, while sparing motor ability and apparent motivation [9]. Physiological saline (vehicle) was used as a control solution and was administered in equivalent volumes. Injections were given 20min prior to the choice phase or probe trials for the VIOR (Experiment 1a) and VIPD (Experiment 3, drug phase) experiments, respectively. Injections were made on rats only.

2.3. Object pre-exposure

2.3.1. Apparatus and objects (rats)

All rats, regardless of group (pre-exposure or novel), explored the same 10 objects during pre-exposure sessions. Pre-exposure sessions took place in open field arenas constructed of white, corrugated plastic (L:60 cm, W:60 cm, H:45 cm). Five rats were run simultaneously in five adjacent open field arenas for each session. The room was illuminated by a ceiling-mounted white light. Pre-exposure sessions were recorded by a camera mounted above the open fields. Objects were of variable height (5–20 cm), width, color, and texture, and were selected based on feature variability of each side (i.e. no two sides were identical; Fig. 1c,d). Each object was adhered to a clear, circular, plastic base with markers for every 45° (Fig. 1a). Ten objects were used in the pre-exposure sessions to allow five objects in each open field during each session, with equal exposure to all objects. However, only nine of these objects were subsequently used in the experiments as the 'pre-exposure objects'.

2.3.2. Apparatus and objects (mice)

All mice regardless of group (pre-exposure, PE, or novel, NOV), experienced three objects during pre-exposure sessions. Pre-exposure sessions took place in open field arenas constructed of white, corrugated plastic (L:45 cm, W:45 cm, H:45 cm). Eight mice were run simultaneously in eight adjacent open field arenas. The room was illuminated by a ceiling-mounted white light. Objects were of variable height (5–10 cm), width, color, and texture, and were selected based on feature variability of two sides (i.e. the back and front were different; Fig. S1).

2.3.3. Procedure (rats)

Rats were given two habituation sessions on successive days, in each of which they explored a different empty open field arena for 30 min. During the pre-exposure phase, each rat interacted with the 10 objects (i.e., all nine PE objects + one extra object) over the course of six days. On day one, rats were pre-exposed to five objects in four successive 30-min sessions. On day two, rats were shown the other five objects in the same manner. This two-day procedure occurred three times, such that each rat was given 6 h total to explore each object. The specific apparatus, object subset (i.e. five objects in the arena), and object arrangement was counter-balanced to limit any spatial or object-object associations. All objects were washed with 50% ethanol between sessions

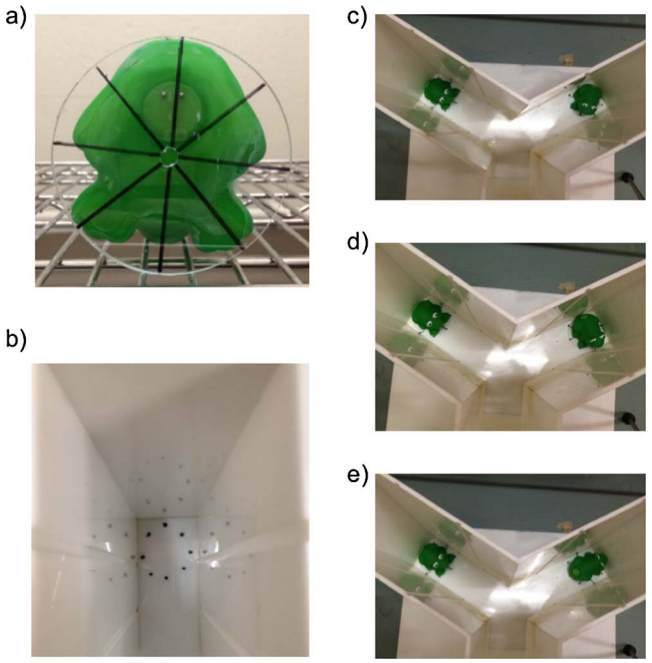

Fig. 1. Object rotation used in the VIOR task. a) Example of a pre-exposure (PE) object adhered to a circular, plastic base with 45° indications. b) Dots aligning with the 45° indications on the plastic object bases. c) Example of a choice phase with a 45° rotation, seen on right side. d) Example of a choice phase with a 90° rotation, seen on right side. e) Example of a choice phase with a 180° rotation, seen on right side. This was the rotation condition that was additionally used in Experiment 2.

and the open fields wiped with paper towel as needed. Rats underwent a 1-week delay before Experiment 1a (see Fig. S2 for experimental timeline).

2.3.4. Procedure (mice)

In a similar manner, mice interacted with the same three pre-exposure objects (see Fig.S1) in different open field arenas, for four successive 30-min bins. This procedure occurred 3 days in a row, for a total of 6 h of object pre-exposure. Mice underwent a 2-day delay before testing began. All other details were the same as performed in rats.

2.4. View-invariant object recognition (VIOR)

2.4.1. Apparatus and objects (rats)

The pre-exposure object set described above was used, in addition to entirely novel objects with similar dimensions (also adhered to plastic bases). Experiments were conducted in a Y-shaped apparatus. As previously described [8], this apparatus consisted of three arms (L:27 cm, W:10 cm, H:40 cm) constructed of white, opaque Plexiglas. One of the arms included a guillotine style door set 18 cm from the end of the arm, providing a start box for the rats. A video camera was mounted on a tripod above the apparatus to record all trials. Object pairs were presented during the sample and choice phases of this study, one at the end of each exploratory arm. Within each exploratory arm, the floor was marked in a corresponding manner to that of the angle markings on the object bases (Fig. 1b), allowing for precise object rotations during choice phases. Object bases were adhered to the floor with reusable putty in order to avoid displacement. The apparatus itself was not cleaned between trials, only wiped down with paper towel as needed.

2.4.2. Apparatus and objects (mice)

The pre-exposure object set (three objects) described above was used, in addition to three entirely novel objects (Fig. S1). All other details were the same as those described for rats, with the exception of the size of the apparatus; the Y-shaped apparatus measured 15 cm long, 7 cm wide, and 30.5 cm high to accommodate the mice. Additionally, objects were not adhered to bases, as only a 180° rotation was used.

2.4.3. Procedure (rats)

Rats were given two, 3-min habituation sessions in an empty Y-apparatus on two successive days, prior to VIOR testing. On the day of testing, rats were divided into two equal groups. The pre-exposure (PE) group was presented with objects from the pre-exposure sessions, whereas the novel object (NOV) group was presented with novel objects. For each VIOR trial, a sample phase was conducted in which a pair of identical objects from either the pre-exposed object set or novel object set, for the PE and NOV groups, respectively, were placed at the ends of the exploratory arms in an orientation that was designated as frontwards facing for each object. The rat was placed in the start box, and the guillotine door was raised to allow it to explore. Once the rat had fully exited the start box, the door was lowered to prevent it from re-entering, and recording of exploration began. The sample phase ended after 30 s of recorded object exploration or the passage of 3 min, whichever came first. The rat was then removed from the apparatus and returned to its cage and colony room for a 1h retention delay. During the choice phase (2 min), rats were presented with the same pair of identical objects from the sample phase, but one of the objects was rotated either 45°, 90° or 180° clockwise or counter-clockwise, from its designated frontward orientation. All objects were washed with 50% ethanol between phases and the Y-apparatus wiped with paper towel as needed.

In Experiment 1a, nine trials were run on successive days, each trial with a different object for each rat (i.e., all 9 PE objects), counterbalanced. Over these nine trials, each rat saw three objects rotated 45°, three at 90°, and three rotated 180°, and these rotations were counterbalanced between objects, rat, and trial number. Between all trials, the arm containing the rotated object and the direction of rotation was counterbalanced between rats.

In Experiment 2, the same rats received an i.p. injection of scopolamine hydrobromide (0.2 mg/kg) or saline 20 min prior to the choice phase. All rats were tested in one trial each with drug and vehicle, counterbalanced. Two of the nine original PE objects (counterbalanced) were used for all rats in the PE group, and two new novel objects (counterbalanced) were used for rats in the NOV group. The 'front' of the PE objects remained consistent with Experiment 1. During the choice phase, a 180° rotation was used. See Fig. S2 for the experimental timeline.

2.4.4. Procedure (mice)

Experimental procedures were very similar to those used with rats, with the exception of the duration of each phase; specifically, habituation sessions and the sample phase were both 10min, the retention delay was 5 min, and the choice phase was 3 min. These modifications were made to enhance exploration, as mice tend to explore less than rats in such tasks. Because memory in mice tends to be less robust than rats, the retention delay was reduced. Mice only received one trial in which they were tested with a 180° rotation in the choice phase. The three pre-exposure and novel objects were counter-balanced between mice, within the PE and NOV groups.

2.4.5. Data analysis

During rat pre-exposure phases, total exploration of the five objects in each open field was scored and calculated using Ethovision Software (Noldus, The Netherlands). A two-way ANOVA was used to analyze object exploration times of the two groups within each session (6d). Descriptive statistics are reported in Table S1. For both rats and mice, exploration in the VIOR task was scored by an experimenter using a program written in Visual Basic 6.0, and was defined as directing the nose at the object from a distance of < 2 cm or direct contact of the nose with the object. Data from the first minute of the choice phase were used to calculate a discrimination ratio (DR) [1-min novel object

exploration −1-min familiar object exploration/1 min novel object exploration +1-min familiar object exploration], as this measure has been shown to be the most sensitive time point when detecting a novelty preference in rats [35]. DR has a range of −1 − 1. When there were multiple trials per condition, an average DR was taken (Experiment 1a). Data were analyzed using two-way mixed-factor analysis of variance (ANOVA), independent samples *t*-tests, and paired samples *t*-tests, using SPSS software. Between group comparisons for total sample and total choice exploration were also conducted as control measures. Additionally, the number of bouts (i.e., single exploratory periods) made to both the rotated and non-rotated object in the choice phase was analyzed for both groups, using within-subjects *t*-tests. Descriptive statistics can be found in Table S2/3 (Experiment 1a; VIOR rats), Table S4/5 (Experiment 1b; VIOR mice), and Table S6/7 (Experiment 2; VIOR scopolamine rats). All control analyses were non-significant unless otherwise reported. A p-value of 0.05 was used for all analyses. Bonferroni correction for multiple comparisons was applied for all post-hoc analyses.

2.5. View-invariant pairwise discrimination (VIPD)

2.5.1. Apparatus and stimuli

All pre-training and testing phases of the touchscreen PD task were conducted in eight automated touchscreen testing chambers (Fig. 2g). The testing chamber was a standard modular testing chamber placed in a sound-attenuating box (MED Associates, Inc., St. Albans, Vermont). The sound attenuating box was equipped with a 28 V DC fan that provided ventilation and blocked outside sounds. The inner operant chamber (30.5 × 24.1 × 8.25 cm) consisted of clear Perspex walls (one with a door to allow placement of the rat inside the box), a metal frame and a stainless-steel grid floor. A magazine was attached to a 45-mg pellet dispenser. There were two 3 W lights in the chamber, one illuminating the magazine and one acting as a house light. A 15" LCD touchscreen (ELO TouchSystems, Menlo Park, CA) was placed at the front of the chamber. All programs were written in and run by K-Limbic software (Conclusive Marketing Ltd., Herts, UK). Stimuli in the VIPD task consisted of pictures of three of the original nine pre-exposure objects (from the VIOR experiment) and three completely novel objects, taken using a digital camera positioned at a constant distance from the objects (to maintain relative size; Fig. 2). The images were then converted to JPEGs and scaled to fit onto the touchscreens based on the largest image, ensuring that the relative size of the objects was maintained. All images were placed on a white background (340 × 643 pixels; Fig. 2h). Images were not matched for brightness in an effort to keep the images as similar as possible to the physical objects, as some were previously seen (i.e., pre-exposure objects).

2.5.2. Procedure

The basic training procedure followed closely to that described in Winters, Bartko, Saksida and Bussey [11] for visual pairwise discrimination.

2.5.3. Touchscreen pre-training

Touchscreen pre-training consisted of five stages: Habituation, Initial Touch, Must Touch, Must Initiate, and Punish Incorrect. For all testing, only one session per day was completed for each rat, and testing occurred at approximately the same time each day. Rats were habituated to the touchscreen testing chamber on two successive days, by placing them inside the chamber for 15 min with 45-mg sucrose pellets (TestDiet, Richmond, IN) in the magazine. During Initial Touch, rats were presented with 30 black-and-white designs (342 × 341 pixels; pre-installed; K-Limbic software) on one half of the screen (counterbalanced for side) for 20 s/image, with a 20-s inter-trial interval (ITI). At image offset, the magazine light illuminated and a sucrose pellet was released. The rat did not have to touch the screen; however, if the rat did touch the screen prior to stimulus offset, the trial ended early (i.e. stimulus disappeared), followed by the presentation of the light and sucrose pellet. All rats completed two 30-min Initial Touch training sessions. In Must Touch, rats were trained to respond to the touchscreen in order to receive a sucrose pellet. For each trial, a stimulus was shown in one of the two adjacent response windows and remained on the screen until the rat responded to the screen. Upon touching the image on the screen, the rat was rewarded with the pellet, the magazine light, and the tone. A 20-s ITI occurred between reward delivery and the successive stimulus presentation. Rats were required to complete 50 trials in a 30-min session before proceeding to the next training phase. Must Initiate followed a similar protocol, but rats were required to initiate each trial by entering their head into the magazine to initiate the trial (stimulus presentation). Rats were again required to complete 50 trials in 30 min. For the final training phase, Punish Incorrect, rats were trained to respond to the stimulus (correct response) and not the empty half of the screen (incorrect response). A correct response triggered the delivery of a sucrose pellet, followed by the ITI. Incorrect responses were punished by the illumination of the house light for 5 s, with no

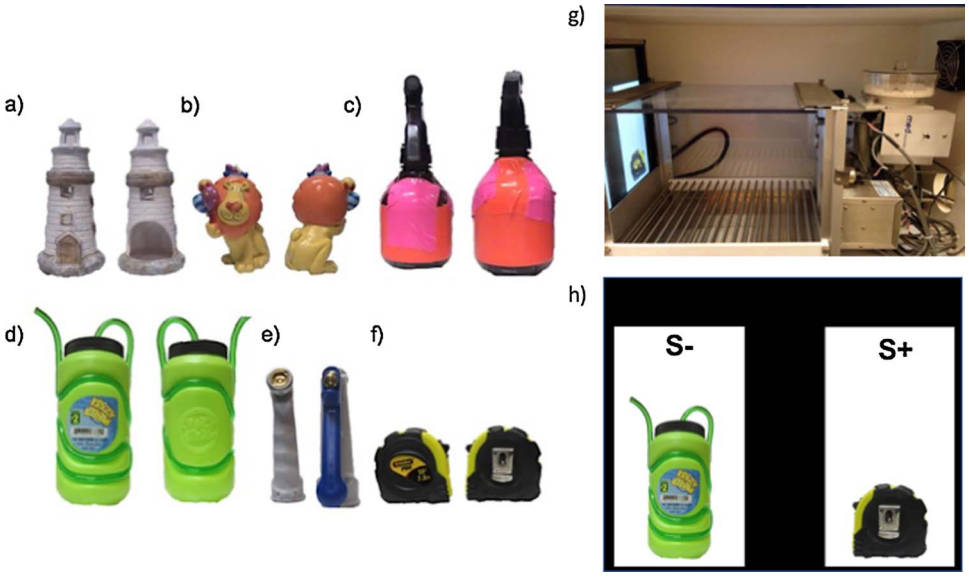

Fig. 2. VIPD touchscreen task. a–f) Objects used in the VIPD task. The left image in each pair represents the default side (0°; S−). The right image in each pair represents the rotation (180°; S+). a–c) Novel objects. d–f) Pre-exposure objects. g) Automated rat touchscreen chamber. h) Image pair as displayed on touchscreens.

reward. A correction procedure was implemented whereby incorrect trials were repeated until the rat made the correct response. Rats were required to complete 60 trials in less than 1h with an accuracy of 90% on two consecutive days. Upon achieving the Punish Incorrect criteria, rats were given two reminder object pre-exposure sessions on two successive days; specifically, rats were allowed to explore the 10 pre-exposure objects in an open field, in two 30-min sessions (five objects per session), on two days (i.e. all objects were viewed for a total of 1 h over 2 d). Two additional Punish Incorrect sessions were given to ensure memory of the task prior to pairwise task acquisition.

2.5.4. Pairwise discrimination, acquisition

Rats were trained to respond to one image (S+) and not the other image (S−) for a reward until they achieved 80% accuracy on two successive days. Upon initiation of the trial (i.e. nose entering the magazine), the S+ and S− images were presented side-by-side; the side of the S+ or S− was counterbalanced throughout the session from trial-to-trial. Rats were rewarded and punished during Training as they were during Punish Incorrect. All rats completed one session per day, and each session consisted of 60 trials plus correction trials, until they achieved 80% accuracy (i.e. not including correction trials; 48–60 correct responses) on two consecutive days. Rats in the PE group were trained to discriminate between two images of objects from the pre-exposure set, whereas rats in the NOV group were trained with images of two novel objects. Upon completion of the Acquisition criteria, rats were transitioned to the Probe phase.

2.5.5. Pairwise discrimination, rotation probe phase

In the PE group, the S− image was replaced with the image of a different object in the PE set, while the S+ object remained the same but was rotated 180°. Similarly, in the NOV group the S+ image was rotated 180°, and the S− image was replaced with an image of a different novel object. The probe sessions consisted of 60 trials/day, with no correction trials, and each rat completed 20 probe sessions to enable analysis of reacquisition of the task.

2.5.6. Pairwise discrimination, drug phase

Rotation probe sessions were followed by drug probe sessions. Rats first underwent three additional sessions with i.p. injections of saline, which acted as a habituation/baseline phase. Performance was then tested following 20-min pre-session infusions of saline, Scopolamine Hydrobromide (0.1 mg/kg, 0.2 mg/kg, and 0.5 mg/kg), and Scopolamine Methylbromide (0.5 mg/kg), which does not cross the blood-brain-barrier, in five additional probe sessions. The order of drug conditions was counter-balanced for each rat, and a drug-free baseline day separated each drug session to allow for wash-out. See Fig. S2 for the experimental timeline.

2.5.7. Data analysis

To examine the rates of learning for each stage of Pre-Training as well as Acquisition, the number of sessions to criteria was compared between groups, using independent samples *t*-tests. Accuracy in selecting the S+ image was calculated for each group in each rotation probe session (i.e., reacquisition). Rotation probe session and drug probe session performance were analyzed in separate two-way ANOVAs, using SPSS software. For drug probe session performance, response accuracy was calculated as a percent change relative to the average baseline accuracy (i.e., 3 baseline/habituation sessions with saline injections run just prior to the start of the drug phase; see Table S9), to account for any differences in baseline accuracy. Separate two-way ANOVAs were additionally used to analyze three control measures taken during probe and drug probe sessions: latency to respond to the screen on correct trials, latency to respond to the screen on incorrect trials, and latency to collect the reward from the magazine. Descriptive statistics for control measures can be found in Table S8 (Experiment 3) and Table S10 (Experiment 3, drug phase). Control analyses were non-significant unless otherwise reported. A p-value of 0.05 was used to indicate significance for all analyses.

3. Results

3.1. Experiment 1a: object pre-exposure promotes view-invariant object recognition of three-dimensional objects in rats

Object exploration during the pre-exposure phase was assessed using a 2 × 6 (group × session) mixed-factors ANOVA. Although there was a significant main effect of session (days) on total exploration in the pre-exposure phase ($F_{1, 18} = 8.42$, $p < .001$), which might be expected given the reduced novelty of the objects over sessions, there was no significant effect of group ($F_{1, 18} = 0.042$, $p = .840$) or interaction effect ($F_{1, 18} = 0.38$, $p = .777$). Descriptive statistics are provided in Table S1.

To determine whether prior multi-modal object exposure enabled rats to recognize objects at various rotations, a 2 × 3 mixed-factors ANOVA was run; specifically, the effect of group (NOV, PE; between-subjects factor) and rotation (45°, 90°,180°; within-subjects factor) on object preference (DR), was assessed. Object pre-exposure appears to have enhanced view-invariant object recognition (Fig. 3a). There was a significant interaction ($F_{2, 36} = 5.56$, $p = .008$) and significant main effects of both rotation ($F_{2, 36} = 6.70$, $p = .003$) and group ($F_{1, 18} = 45.30$, $p < .001$). Post-hoc analyses revealed significant group differences when objects were rotated 90° ($t_{18} = 3.91$, $p = .001$) and 180° ($t_{18} = 3.86$, $p = .001$), as the PE group did not discriminate between the rotated and un-rotated objects, but the NOV group did. There was no group difference in the 45° condition ($t_{18} = 0.31$, $p = .764$; Fig. 3), indicating that objects were identifiable by both groups at this slight rotation.

3.2. Experiment 1b: object pre-exposure promotes view-invariant object recognition of three-dimensional objects in mice

As was demonstrated in rats, mice performed in a manner consistent with view-invariant object recognition abilities (Fig. 4). An independent samples t-test indicated a significant difference between the PE and NOV groups ($t_{14} = 4.80$, $p < .001$; $n = 16$); specifically, the NOV group preferred the rotated object significantly more than the PE

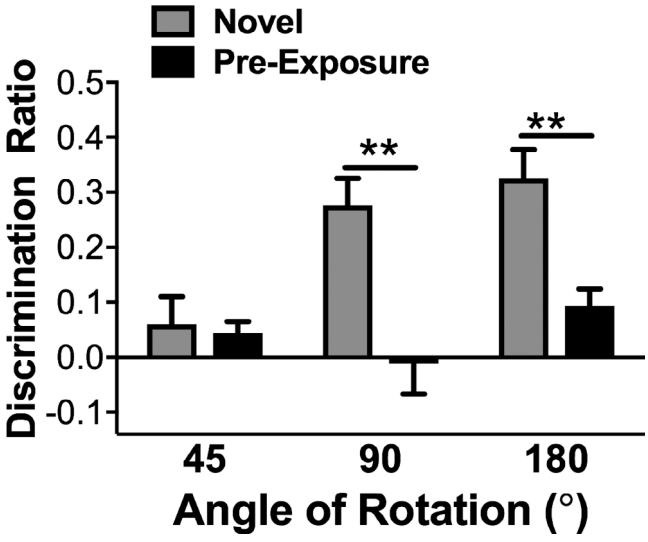

Fig. 3. Experiment 1A – VIOR task performance. Neither group preferred to explore the 45° rotated object, indicating that both PE and NOV rats recognized the rotated object as the same as the sample. Rats in the NOV group explored the rotated object in the 90° and 180° conditions more than the PE rats, suggesting that NOV rats did not recognize the objects at these rotations, but rats that had previous experience with the object in its entirety (PE group) did. Bars are mean DR ± SEM. ** p < .01.

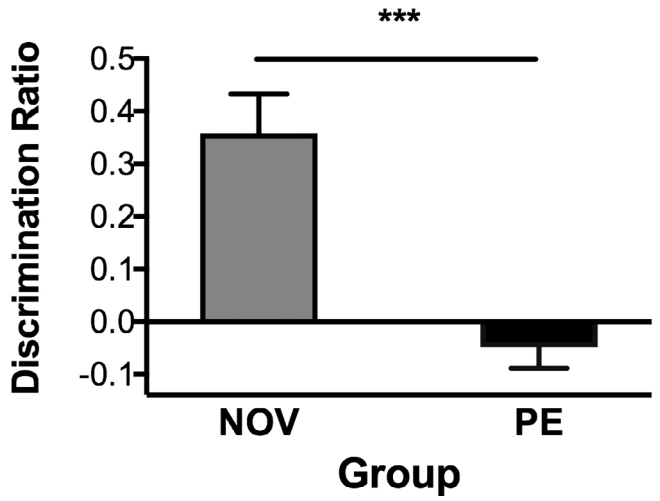

Fig. 4. Experiment 1b – Mice that were introduced to a novel object treated this object as novel 5min later upon rotation. Conversely, mice previously exposed to the test objects treated objects as familiar despite a 180° rotation. Data are mean discrimination ratio ± SEM. *** $p < .001$.

group, suggesting that it was viewed as novel, whereas the PE group treated the rotated object as familiar.

It should be noted that PE mice explored significantly less ($M = 27.67$ s, $SEM = 1.90$ s) during the sample phase than NOV mice ($M = 38.14$ s, $SEM = 1.70$ s) ($t_{14} = 4.10$, $p = .001$). This might be expected given that the PE mice had previously viewed the objects presented to them in the sample phase.

3.3. Experiment 2: scopolamine impairs view-invariant object recognition in rats

The effects of pre-choice scopolamine on VIOR performance were tested using a 180° rotation, given the robust performance by rats in this condition in Experiment 1a. Systemic scopolamine disrupted the view-invariant object recognition displayed by the PE group (Fig. 5). A 2 × 2 mixed-factors ANOVA was conducted, assessing the effect of group (PE, NOV; between-subjects factor) and drug condition (saline, scopolamine; within-subjects factor). There was a significant interaction, ($F_{1,18} = 18.63$, $p < .01$), whereas the main effects of drug ($F_{1,18} = 1.26$, $p = .276$) and group ($F_{1,18} = 0.63$, $p = .436$) were non-significant. Post-hoc analyses indicated significant group differences in the saline condition ($t_{18} = -3.70$, $p = .002$), as NOV rats ($M = 0.33$, $SEM = 0.05$) preferred the rotated object more than the PE rats ($M = 0.07$, SE = 0.05), indicating the rotation was treated as a novel object by the NOV group, but not the PE rats. This replicates the finding in Experiment 1. There were no group differences in the scopolamine condition ($t_{18} = 2.00$, $p = .62$), as both sets of rats treated the rotated object as if it were unfamiliar. Within the PE group, performance differed significantly following pre-choice administration of scopolamine in comparison to saline ($t_9 = -3.57$, $p = .006$), suggesting that scopolamine interfered with view-invariant object recognition. Furthermore, Experiment 2 occurred approximately 3 months following the pre-exposure sessions and Experiment 1a (see Fig. S2), demonstrating that rats are capable of a robust and long-lasting view-invariant object memory.

There was a significant group by drug interaction for sample phase object exploration ($F_{(1, 18)} = 7.77$, $p = .012$; Table S6). The main effects of drug ($F_{(1, 18)} = 0.94$, $p = .346$) and group ($F_{(19, 285)} = 1.07$, $p = .383$) were both non-significant. Post-hoc analyses demonstrate no significant differences between groups or conditions after correction for multiple comparisons. Nevertheless, both groups explored well within the typical range for rats in SOR tasks, and the superior performance of the NOV groups with scopolamine compared with saline, along with the replication of the original PE group finding in Experiment 1a, suggests that these relatively minor differences in object exploration do not explain the substantial recognition performance difference between the PE-saline condition and all others in Experiment 2.

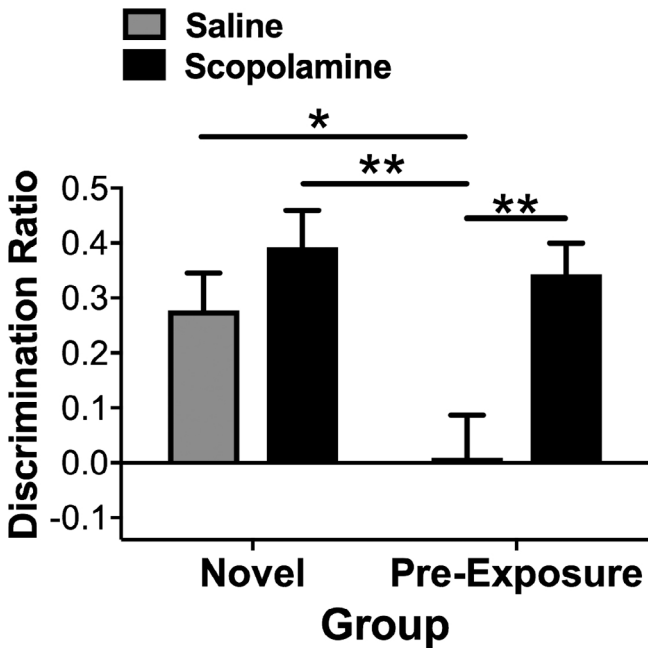

Fig. 5. Experiment 2 – Effects of Scopolamine on VIOR performance. NOV rats preferred the rotated (180°) object in the saline condition (i.e. "novelty" preference) more than the PE rats, replicating the findings from Experiment 1 that PE rats identified the rotation as a familiar object. Scopolamine administered 20min pre-choice impaired PE rats' recognition of the rotated object when compared to saline treatment, as they treated the rotated object as if it was novel. NOV rats treated the rotated object as if it was novel in both the saline and scopolamine conditions. Bars are mean DR ± SEM. * $p < .05$; ** $p < .01$.

3.4. Experiment 3, rotation probe phase: object pre-exposure promotes view-invariant recognition in an automated visual pairwise discrimination touchscreen task

3.4.1. Pre-training & PD acquisition

No group differences on the number of sessions required to reach criteria were found in the various training stages: Must Initiate ($t_{(18)} = 1.22$, $p = .237$), Punish Incorrect ($t_{(17)} = 1.36$, $p = .192$), and PD Acquisition ($t_{(15)} = 1.81$, $p = .091$; Fig. S3).

3.4.2. Rotation probe phase

To determine whether object pre-exposure facilitates view invariant pair-wise discrimination in probe sessions, a 2 × 20 mixed-factors ANOVA was conducted; specifically, the effect of probe session (1–20; within-subjects factor) and group (PE, NOV; between-subjects factor) on accuracy in selecting the S + object (rotated), was assessed. Data suggest a substantial benefit for the PE group in reacquisition following S+ rotation (Fig. 6). The group by session interaction was non-significant ($F_{(19, 285)} = 1.07$, $p = .383$), but the main effects of probe session ($F_{(19, 285)} = 13.54$, $p < .001$) and group were both significant ($F_{(1, 15)} = 6.35$, $p = .024$; Fig. 5). The latter effect suggests that rats tested with objects that they had been previously exposed to in the open fields were significantly better at selecting the rewarded object (S+) despite a 180° visual rotation. Moreover, these results indicate that this view-invariant object representation was quite robust, as the initial object pre-exposure sessions occurred approximately 7–8 months prior (see Fig. S2).

There was a significant group by session interaction effect for Incorrect Response Latency ($F_{(19, 285)} = 1.655$, $p = .044$). No obvious pattern emerged; the average response latency was higher in the PE

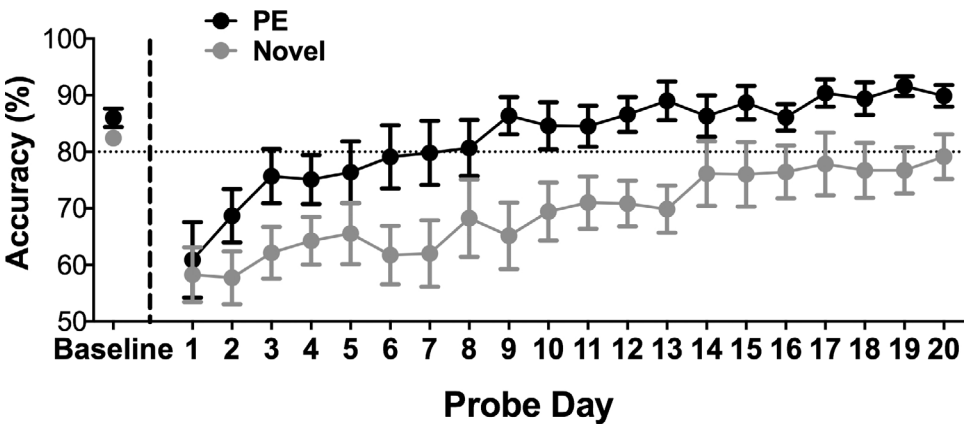

Fig. 6. Experiment 3 – VIPD rotation probe sessions. Group means for baseline performance on the pairwise discrimination task can be seen on the left side of the graph. During VIPD rotation probe sessions, the S+ and S− objects remained the same for each rat, but the image of the S+ object was rotated 180°. Over the 20 probe sessions, there was a main effect of group; specifically, PE rats re-acquired the discrimination significantly faster than the NOV rats, suggesting that they had a more view-invariant representation of the objects.

than NOV group on 12/20 days, in no particular order (Means are presented in Table S8).

3.5. Experiment 3, drug phase: scopolamine impairs visual pairwise discrimination performance after the S+ is rotated

The requirement of muscarinic receptors for VIPD was assessed using a 2 × 5 ANOVA, comparing the effect of drug condition (0.1, 0.2, 0.5 mg/kg ScopHBr, 0.5 mg/kg ScopMeBr, Saline; within-subjects factor) and object group (PE, NOV; between-subjects factor) on response accuracy. To account for the significant difference in baseline response accuracy (i.e., average of the three baseline/habituation sessions; $t_{(15)} = 3.27$, $p = .005$; see Table S9) between the NOV and PE groups following rotation probe trials, performance in each drug condition was calculated as a percentage difference from the corresponding baseline. Scopolamine HBr impaired performance in a dose-dependent fashion in both groups (Fig. 7). The group by drug interaction ($F_{(4, 60)} = 1.39$, $p = .247$) and main effect of group were non-significant ($F_{(1, 15)} = 1.08$, $p = .315$). The main effect of drug, however, was significant ($F_{(4, 60)} = 23.89$, $p < .001$).

A main effect of drug was also found for Correct Response Latency ($F_{(6, 60)} = 6.79$, $p < .001$), Incorrect Response Latency ($F_{(4, 60)} = 2.61$, $p = .044$), and Reward Response Latency ($F_{(4, 60)} = 9.06$, $p = .008$), as latencies increased when scopolamine was on board. Increased latencies with relatively high doses of scopolamine is a common finding with touchscreen-based operant tasks [12].

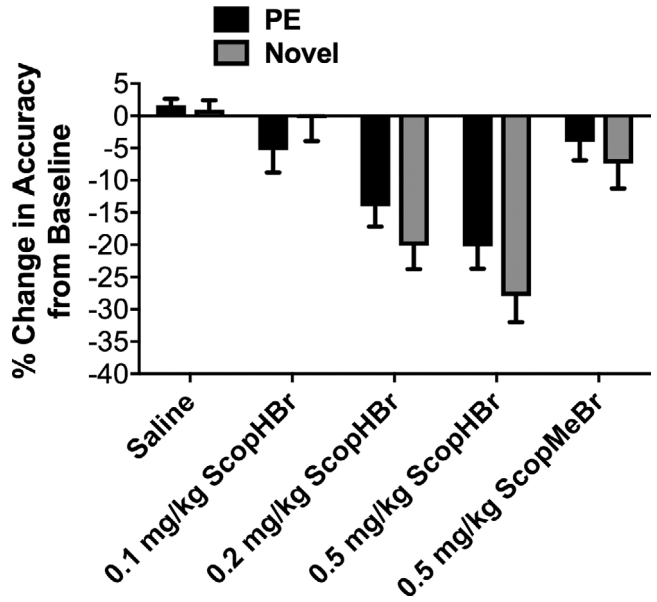

Fig. 7. Experiment 3 – Effects of Scopolamine on VIPD performance. To account for any difference in baseline VIPD performance (accuracy) between the groups, calculations for each drug condition were made as a percent change from baseline performance **(i.e.,** average accuracy over three baseline/habituation sessions with saline injections, occurring immediately preceding the drug trials; see Table S9). Both groups were dose-dependently impaired by scopolamine hydrobromide.

4. Discussion

4.1. Rats display view-invariant object processing capabilities on two novel tasks

The current study investigated the view-invariant object processing capabilities of **rodents** in two novel tasks. Prior to testing, rats and mice were provided with extensive pre-exposure to objects in an open field area, allowing them to form representations of the objects in their entirety. In both tasks, object identification had to be made by viewing only one side or angle of the objects, forcing rats to engage view-invariant processes. In the view-invariant object recognition (VIOR) task, rats and mice underwent an altered version of the SOR paradigm. Specifically, during the sample phase rodents explored two identical objects in the same orientation; these objects had either been pre-exposed (PE group) or were completely novel (NOV group). Use of a Y-apparatus prevented exploration of the back of the object and reduced exploration of the object sides, allowing object exploration to be focussed to the object front. Following a 1h retention delay, rats experienced the same objects during the choice phase, but one object had been rotated 45°, 90°, or 180°. Rats in the NOV group preferentially explored the rotated object in the 90° and 180° conditions, suggesting that they perceived the objects at these rotations to be novel. Conversely, rats in the PE group displayed no preference between the objects in any rotation condition, implying that they perceived no difference between the two objects. Similar effects were found for mice using a 180° rotation. Therefore, in agreement with previous reports [4–7], the current study suggests that rats and mice possess view-invariant object recognition capabilities. Additionally, this is the first demonstration of rodent view-invariant object processing with more ethologically valid stimulus presentations (i.e., real objects that are experienced visually and tactilely compared to images present on screens), and under relatively passive viewing conditions as opposed to reinforced operant conditioning. These features are more akin to human declarative memory [13,14], for example passively recognizing everyday objects that are encoded in entirety, such as your bag or car, from various angles. Therefore, this task extends the translational potential of rodent work examining view-invariant object processing. Moreover, from a pragmatic point of view, the VIOR task requires less equipment, set-up, and time than the operant conditioning tasks that have been used previously [4–7] and therefore represents a highly complementary tool.

Although the VIOR task appears to have many strengths, one

potential caveat is that a *lack* of exploration preference is used to infer recognition of the rotated object. In typical object recognition tasks, a lack of novel object preference is seen when memory for the previously viewed object is impaired, following drug administration for example. Additionally, the fact that animals can view and touch the objects during the testing trials means that it is possible they can perform the VIOR task according to some other rotation-invariant, but not strictly view-invariant, representational process. Therefore, we developed a second task that required rats (mice were not used) to make explicit responses toward exclusively visual stimuli to demonstrate converging evidence for view-invariant object recognition abilities in the current study. Specifically, the same rats were trained to discriminate between two different pre-exposed or novel objects in a view-invariant visual pairwise discrimination task (VIPD), run with touchscreen equipped operant chambers. After PE and NOV rats had learned to nose-poke to the rewarded stimulus (S+) of the pair, it was rotated 180°. Although initial performance decreased for both groups upon S+ rotation, the PE rats re-acquired the discrimination significantly faster than the NOV rats, suggesting substantial residual recognition in the PE group despite the rotation. This demonstration of rat view-invariant object processing is somewhat similar to the previous accounts in its use of operant conditioning [4–7] and a visual pairwise discrimination task [4,7]; however, one key difference in the present VIPD task (and VIOR task) is the use of real objects for the intial pre-exposure. Many of the objects that humans and other animals encouter daily have both visual *and* tactile features. Even the myriad stimuli humans experience on screens, billboards, and other flat surfaces, have typically been previously experienced tactilely. Therefore, the current VIPD task, in addition to the VIOR task, could be considered quite ethologically valid. Furthermore, the VIPD introduces a scenario similar to many types of human cognitive tasks, where participants are asked to respond to known, everday stimuli (e.g. cars, animals, faces, buildings) that appear in front of them on computer screens [15–17] enhancing the translational potential of the VIPD task. Finally, the present findings demonstrate that rats are tolerant to changes in the medium of object presentation (i.e., physical interaction to visual experience on screen), an interesting behavioral result and one that can inform future studies given the growing prevalence of touchscreen-based cognitive testing in rodent research [18,19].

Many object-based tasks for rodents use novelty preference as an indicator of memory for a familiar object. To ensure novelty, each object is only used once, as was done in the present experiments for all objects seen by the NOV group of rodents. However, for rodents in the PE group, prior object experience was essential. As such, objects seen in the pre-exposure sessions were then seen by the PE group in the VIOR task. We were additionally interested in the longevity of this view-invariant memory; therefore, a subset of the pre-exposure objects (for rats) were also used in Experiment 2, to test the effects of scopolamine. When rats in the PE group received control injections, they demonstrated intact view-invariant object memory, similar to Experiment 1, approximately 3 months after learning about these objects in the pre-exposure sessions. Furthermore, a different subset of the pre-exposure objects were subsequently used in the VIPD task 7–8 months following the initial pre-exposure sessions, preceded by only a short 'reminder' session. These results demonstrate that the current pre-expsosure session parameters are sufficient to create a robust and long-lasting view-invariant object memory in rats.

These two complementary tasks can now be used to investigate additional behavioural challenges or nuances in view-invariant object processes, the brain regions involved, and the molecular and neurochemical substrates of view-invariant object recognition. Given the role for acetylcholine and muscarinic receptors in object memory and perception [20,38,21,11,33,22,23,9], we began these investigations by assessing the effects of scopolamine on VIOR and VIPD performance.

4.2. Acetylcholine is necessary for some aspects of view-invariant object recognition

Systemic antagonism of muscarinic ACh receptors by scopolamine disrupted pre-exposure facilitated VIOR performance, suggesting an involvement of ACh in view-invariant object retrieval. Typically, ACh has been shown to be necessary during acquisition, but not retrieval in object recognition tasks [20,24–32]. However, we have recently demonstrated that pre-choice scopolamine impairs rats' performance on a tactile-to-visual crossmodal object recognition (CMOR) task [9]. Because rats had never experienced the tactile and visual properties of the objects together, it was hypothesized that ACh helped to 'bind' these features during the choice phase, to facilitate visual recognition based on a tactile memory representation. In the current VIOR task, ACh might be playing a similar role by binding the various perspectives of the objects to facilitate view-invariant recognition by the PE group in the choice phase.

Unlike with VIOR, in the VIPD task the effects of scopolamine were not selective to the PE group. This could be related to a general effect of muscarinic receptor antagonism on visual discrimination performance [23]. Alternatively, it is possible that, after 20 sessions of reacquisition following rotation of the S+, both the PE and NOV groups were performing view-invariant object recognition processes that require muscarinic receptor involvement. Unfortunately, this issue cannot be resolved currently, but represents an easily addressed question for future research in this area. It is evident from the current findings that muscarinic receptors are necessary for some aspect of view-invariant object recognition. Their exact role remains to be determined, as does the location of their effect. Future studies with the VIOR VIPD, and similar tasks should investigate the role that ventral visual stream constituents, such as perirhinal cortex [34,1], play in view-invariant object processing.

5. Conclusion

Until recently, it was believed that rodents lacked the complexity in their visual system to enable invariant object recognition, and initial attempts to demonstrate this phenomenon in rodents were unsuccessful [3]. However, Zoccolan et al. [4] showed that rats indeed possess this ability, and suggested that task demands required in the paradigm employed by Minini and Jeffery [3] were perhaps less complex and did not require the rats to adopt a feature-invariant or view-invariant approach. Consistent with the view-invariant object processing demonstrations by the Zoccolan lab [4–7], we here introduce two novel tasks that appear to confirm rats' abilities to recognize objects in a view-invariant manner. In addition to the enhanced ethological validity of our tasks through the physical interaction with objects, our tasks are highly complementary; specifically, the VIPD task requires an explicit response from the rats to indicate recognition of the rotated version of the object, whereas the VIOR task holds merit in its unreinforced and spontaneous nature, similar to human declarative memory. Using these tasks, we took a first foray into studying the neural underpinnings of view-invariant object recognition, demonstrating a requirement for muscarinic acetylcholine receptors. Future studies might seek to investigate the brain regions and molecular processes subserving this form of cognition.

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Conflict of interest

The authors declare no competing financial interests.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.bbr.2018.01.030.

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