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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