Establishing the Oddity Task in Mice

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I would like to thank my supervisors for their indispensable contributions to my thesis. To Martha, thank you for providing me with the tools to get my project started, for your valuable knowledge, for you guidance when writing my thesis, for the time you have invested in me, and for your patience and understanding during this project. To Marianne, thank you for your guidance and valuable feedback when writing this thesis.

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Lastly, I want to thank my family for their encouragement and help.

Oslo, July 2020

Maria Moe Almenningen

Abstract

Vision is our primary sense used for evaluating our surroundings and it has an enormous impact on our behavior.

Mice have previously been underestimated as a visual species, but recent evidence suggest that mice have a competent visual system. With the recent explosion in genetic, optogenetic and chemogenetic tools available for studying neural networks, using mice as a model organism for investigating the visual system offers new possibilities to understand how certain visual areas contribute to visual processing.

An approach to studying the perceptual abilities in non-verbal species could be by using a simultaneous oddity task with a varying degree of feature ambiguous objects. Mice have, to the best of our knowledge, not been used as a model organism in a simultaneous oddity task before.

We conducted three experiments using C57BL/6JRj mice to establish parameters needed for successful performance on the oddity task in mice. In experiment 2, we were able to demonstrate a preference for the odd object on a group level. This is to our knowledge, the first demonstration of oddity preference in mice.

The findings presented in this thesis provide a solid basis for continuing the work of developing the oddity task for mice. Once the oddity task is established in mice, it will bring new possibilities for studying circuit contributions to perceptual function.

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List of abbreviations

DREADDs	Designer receptors activated by designer drugs
PRH	Perirhinal Cortex
V1	Primary visual cortex
V2	Secondary visual cortex
V4	Fourth visual area
VVS	Ventral visual stream
TEa	Temporal cortex association area

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1. Introduction

The human visual system is outstanding at extracting geometrical information from our surroundings (Kersten, Mamassian, & Yuille, 2004). Vision is the primary sense used for evaluating our surroundings and guiding our behavior (Huberman & Niell, 2011). Everyday tasks such as navigation, face recognition, and decision-making rely on visual information obtained from our surroundings. Humans with severe lesions to brain areas involved in visual information processing experience impairments in the recognition of faces, objects, textures and colors (Lê et al., 2002). It is therefore important to understand how the brain processes visual information, how this information is transmitted, and how this information is translated in to behavioral action.

1.1 The visual ventral stream

The visual system consists of at least two subsystems that can be said to serve object and spatial vision. These two distinct anatomical streams, the ventral stream and the dorsal stream, project from the primary visual cortex (V1) (Creem & Proffitt, 2001). There are species differences in the organization of the visual system. As there is not much literature on the organization and function of the higher visual area in mice, we will refer to human, non-human primates, and rat literature. In humans, the ventral visual stream (VVS) consists of the areas V1, secondary visual cortex (V2), fourth visual area (V4) and the inferior temporal cortical areas TEO and TE (Ungerleider, Courtney, & Haxby, 1998). In mice, the visual ventral stream consists of area V1, V2 and temporal cortex association area (TeA) (Wang, Sporns, & Burkhalter, 2012). Leading theories have suggested that the two visual streams have distinctive tasks. The dorsal stream is thought to be responsible for spatial visual information ("where" pathway) while the ventral stream is responsible for object visual information ("what" pathway) (Goodale & Milner, 1992). However, recent studies suggest that this distinction between the dorsal and ventral visual stream might not be clear cut, and that there is coupling between the two visual pathways (Deubel, Schneider, & Paprotta, 1998).

The ventral visual stream is organized as a hierarchy, where the receptive fields of neurons increase as you progress from early to higher level visual areas. Neurons in V1, the earliest

ventral visual area, can detect edges but cannot identify object categorization (Hong, Yamins, Majaj, & DiCarlo, 2016). In humans, the visual information received by TE goes from V1-V2-V4-TEO (Tanaka, 1996), and originally, the VVS was thought to be a series of projections from one area to another in a sequential fashion. A more recent view sees the VVS as a complex recurrent network, where low level visual areas project to multiple areas of the stream (Kravitz, Saleem, Baker, Ungerleider, & Mishkin, 2013). For example, there are direct projections from V2 to TEO and from V4 to TE (Tanaka, 1996), as well as feedback projections from perirhinal cortex to V1 in the monkey (Clavagnier, Falchier, & Kennedy, 2004), and V2 in both the rat (Agster & Burwell, 2009) and the mouse (Schlegel, 2018).

Within the limited mouse visual literature, the emerging pattern is one of similarities in connectivity, anatomy and function to that of higher order species.

1.2 Mice as a model organism

Mice have over the recent years become a widely used animal in neuroscience research. There has been a recent explosion in genetic tools available for the manipulation of specific neuronal populations which provides novel and unique opportunities for causal examination of how the activity of specific neurons contribute to cognitive function. As these genetic tools were developed in the mouse, there are technical challenges with utilizing them in rats and non-human primates. There is therefore a need to expand the behavioral tasks that we use to examine cognitive function in animals to include mice.

In recent years there has been more effort put into understanding the structure and function of the visual pathways in mice (Huberman & Niell, 2011). Studies have shown that mice can perform complex visual discriminations (Bussey, Saksida, & Rothblat, 2001), mice can use visual cues when deciding whether to fight or flight (De Franceschi, Vivattanasarn, Saleem, & Solomon, 2016). So even if mice have not been as commonly used as other species in visual research, there is much potential in using mice as a model organism when studying visual processes (Huberman & Niell, 2011).

What are the genetic tools that have made the mouse the central model organism in neuroscience research? Firstly, optogenetic tools have over the past few years become an important tools and have made a significant impact on neuroscience (Fenno, Yizhar, & Deisseroth, 2011). Genes encoding photoreceptor transmembrane proteins, opsins, can be delivered directly to target

neurons or be incorporated into the genome of transgenic organisms (Bernstein & Boyden, 2011). When these proteins are expressed in neurons they allow us to control the electrical potentials of target neurons, by using brief pulses of light (Bernstein & Boyden). Controlling the electrical activity of a specific neural population can provide information on whether this specific neural population contributes to a certain behavior or cognitive functions (Bernstein & Boyden).

Chemogenetic tools are used in a process were engineered macromolecules interact with small molecules (Sternson & Roth, 2014). The most widely used technology, designer receptors activated by designer drugs (DREADDs), has become a helpful tool in neuroscience. DREADDs will allow us to manipulate neuronal activity in a noninvasive and reversible manner (Zhu & Roth, 2015).

Transgenic mice have had foreign DNA experimentally integrated in their genome (Palmiter & Brinster, 1985). Transgenic mice can e.g. be used to label live neurons of interest for imaging, and electrophysiology (Yang & Gong, 2005). Transgenic mice are hugely valuable for functional neuroanatomy studies trying to dissect how a neural circuitry supports cognitive function.

Previous studies have used rats (Bartko, Winters, Cowell, Saksida, & Bussey, 2007b) or non-human primates (Buckley, Booth, Rolls, & Gaffan, 2001) as model organisms in simultaneous oddity tasks. These species have been successful at identifying the odd object, and previous studies using these species have provided a helpful guide for establishing the conditions used in this study. To our knowledge, mice have not been used in simultaneous oddity tasks previously. The work in this thesis aims to establish a test protocol for simultaneous oddity preference in mice, in order to open the possibilities for later use of genetic tools for neuronal circuit manipulation during perceptual performance on the task

Several of the tools described above were developed in mice, and to this day works best in mice. Therefore; if we can get the simultaneous oddity task working well in mice, we have a whole new tool set available to use for manipulating the neural circuitry. This can give us further insight to the function of the visual ventral stream, and so getting this work done in mice is important.

1.3 Measuring perceptual ability in mice

There are opposing theories within the field concerning the involvement of structures in the medial temporal lobe in perception. The representational hierarchy theory predicts that perirhinal cortex is critical in perceptual tasks depending on what stimuli are to be discriminated (Bussey & Saksida, 2002), in contrast to e.g. the medial temporal lobe declarative memory system view fronted by Squire and colleagues, which argues for a purely mnemonic role for medial temporal lobe structures (Squire, Stark, & Clark, 2004). Specifically, Bussey & Saksida (2002) propose that the perirhinal cortex is specifically critical when stimuli with high feature ambiguity are to be discriminated. Feature ambiguity refers to situations where the presence or absence of one particular feature or element within an object cannot be used to discriminate between that and other objects; in other words, the combination of features is critical, rather than single features alone (Norman & Eacott, 2004). In order to test the predictions of the representational hierarchical theory and the medial temporal lobe declarative theory in combination with sophisticated genetic tools, we need a perceptual task for mice in which we can manipulate the level of feature ambiguity.

Oddity preference is the preference for an object/stimulus that stands out or doesn't match the other objects/stimuli in a group (Wright & Delius, 2005). A preference for the odd stimuli has been found in several species, including rats (Bartko, Winters, Cowell, Saksida, & Bussey, 2007a), pigeons (Wright & Delius, 2005) and monkeys (Brush, Mishkin, & Rosvold, 1961). The reason why these species show an oddity preference is yet not understood (Wright & Delius, 2005), but this phenomenon is very useful in the exploration of perceptual ability in non-verbal species.

There are a number of ways to design an oddity task in order to test perception in animals. In a typical oddity task, an animal explores a number of objects where most of them are identical but one of the objects, the "odd one", stands out. The task can be set up in different ways, with and without a delay. In the simultaneous oddity task there is no delay, meaning, the animal encounters the objects for the very first time during the test session, and the mnemonic demand is then reduced or eliminated (Bartko et al., 2007b).

The oddity task can be designed using feature ambiguous objects. In this this type of oddity task, the discrimination of the odd object cannot be done by using one single visual feature. The odd object shares one or more features with the non-odd/identical objects. To successfully identify the odd object, the configuration of the features in the object is critical. An oddity task

object set consisting of five objects can for example contain a first set of two identical objects, a second set of two identical objects, and one odd object (**Feil! Fant ikke referansekilden.**). The identical objects in set 1 will have the features A (boot) and B (bear). The identical objects in set 2 will have the features C (cylinder) and D (lantern). The odd object will have feature B and C, hence sharing one feature with the identical 1 and the identical 2 objects, making the conjunction of the features the identifier of the odd object.

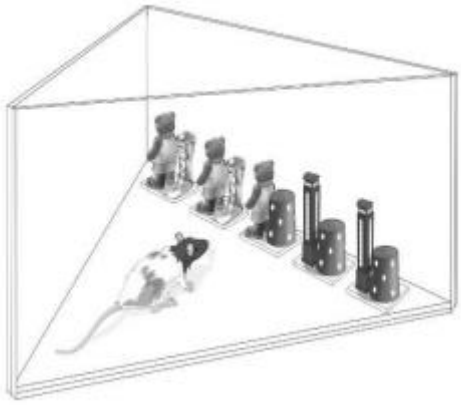

Figure 1: Simultaneous oddity discrimination set up(Bartko et al., 2007b).The rat encounters the five objects for the first time during the test trial. The odd object (middle object) is identified by the conjunction of features.

The degree of feature ambiguity in the objects used in the oddity task can be varied. The value in being able to manipulate the level of feature ambiguity within the oddity task lies in the possibility it opens for testing specific hypothesis about differential involvement of areas of the visual ventral stream and medial temporal lobe system based on the level of feature ambiguity of the stimuli. In order to define an object-set as either low or high feature ambiguous, the number of overlapping features between objects are critical, as well as the complexity of these features.

1.4 Aims of this study

The main objective of this study is establish the task parameters for successful performance in the oddity task, defined as the demonstration of an oddity preference at group level. The secondary aim of this study is to establish object-sets for use in the oddity task that differ in

their level of feature ambiguity, defined as obtaining a stronger preference for the odd object on trials where the feature ambiguity of objects is low compared to trials where the feature ambiguity is high. The work done in this study will lead to future studies where the predictions of the representational hierarchical theory and medial temporal lobe declarative memory system theory will be tested.

2. General material and methods

2.1 Approvals and research animals

The laboratory work was done at the Department of Comparative Medicine, Institute of Basic Medical Sciences, Faculty of Medicine, University of Oslo, Norway. All animal experiments were approved by the Norwegian Food Safety Authority, Mattilsynet, and were performed in accordance with the Norwegian Animal Welfare Act and the European Union's Directive on the Protection of Animals used for Scientific Purposes.

The personnel involved in the experiments hold an animal research certificate (corresponding to a CAREiN function categories A and B).

Twenty-four C57BL/6Jrj male mice approximately 4 months old at the time of the first experiment were used. The same group of mice was used in all three experiments. Food and water were available ad libitum. The animals were housed in groups of four. The animal cages were transparent polycarbonate (42.5 x 26.6 x 15.5 cm) with woodchip bedding and different types of enrichments in their cages. The animals had a 12-hour light and dark cycle (light off from 10 AM to 10 PM) and room temperature was 21 ± 0.1°C. All experiments were performed during the dark phase (11 AM – 8 PM).

2.2 Equipment

The testing arena was a square (60 cm x 60 cm) box covered with sawdust bedding (Figure 2), and was used throughout the experiments presented. The arena had high black walls (50 cm) and dark curtains surrounding the arena to limit extramaze cues. Lighting conditions were kept low during the experiments to reduce anxiety responses in the mice.

Habituating sessions and experimental trials were filmed using a PointGrey FLIR USB3 vision camera. Flycapture (v 2.5) was used with the third-party application Bonsai to track animal behavior.

All objects used in the three experiments were constructed in house using LEGO® manufactured by the LEGO group. The LEGO® objects were clicked onto a LEGO® baseplate which was secured to the floor of the testing arena using adhesive Tack-it. All of the odd objects used in this study share a minimum of 50 % of the features with both the identical 1 and identical 2 objects in their respective object-sets. In this study, the low feature ambiguity object features are considered less complex than the high feature ambiguity object features.

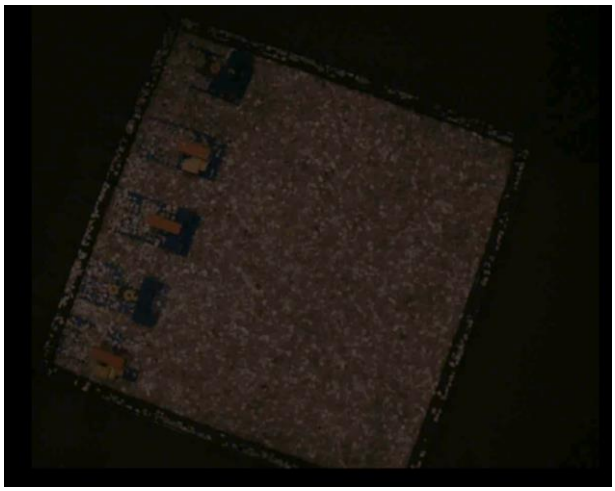

Figure 2: Testing arena and set-up used in experiment 1 under low light condtions

2.3 Testing method

Before testing begun, the animals were handled by the experimenter for five minutes a day over five days. This was done in the room were the testing would take place in order to reduce the

stress of being handled by humans, and in order habituate the animals to both the testing room and the person that was going to test them.

The animals were habituated in the empty test arena for 2 x 5 minutes. Two sets of objects were used, one set with low feature ambiguity (identical 1, identical 1, odd object, identical 2, identical 2), and one set with higher feature ambiguity (identical 1, identical 1, odd object, identical 2, identical 2). Each animal explored both sets of objects once, on two different days, with a minimum of 24 hours between trials (maximum 72 hours). An overview of the experiments is found in table 1, and an example of how the testing was organized is found in table 2. The objects were placed in the test arena before the mouse entered. The objects were lined against the wall of the testing arena, 2.5 cm apart. The objects were placed 5 cm from the wall. All four walls of the testing arena were used in order to prevent any preference for one of the walls. The order of the placement of the objects was counterbalanced. The objects were cleaned with 70% ethanol between trials to prevent any odor being transmitted from previous animals. The animals were allowed five minutes exploring the objects before being removed from the arena.

Table 1: Overview of experiments.

	Number of object-sets	**Number of days testing**	**Number of trials per object-set**
Experiment 1	2	2	24
Experiment 2	1	1	24
Experiment 3	2	2	24

Table 2: Example of how testing was organized in experiment 1.

Day	**Animal number**	**Object-set**	**Back wall**
1	1	High feature ambiguity	N
1	2	Low feature ambiguity	S
2	1	Low feature ambiguity	E
2	2	High feature ambiguity	W

2.4 Estimating exploration time

Exploration of an object was defined as directing the nose to the object at a distance of <2 cm and/or touching it with the nose (Bartko, Winters, Saksida, & Bussey, 2014). Climbing on an object was not scored as exploring an object. If objects fell during the animals' exploration, the trial was excluded.

Previous oddity task-studies have chosen to use e.g. the second minute of exploration when calculating the oddity preference score, as this was the first time point the animals would show a preference for the odd object (Bartko et al., 2007b). In our data, there were large individual variation to when the animals showed oddity preference. It was therefore decided to use the entire exploration time when calculating the oddity preference score.

Calculation of oddity preference score was measured by the fraction of time spent exploring the odd object compared to the total exploration time. An oddity preference score of 20% would indicate chance performance, therefore an oddity preference score significantly above 20% would be a meaningful score (Bartko et al., 2014).

2.5 Data analysis

Time estimations were based on manually scoring the videos using an in-house stopwatch programmed in Microsoft Excel by Sara Kruge Nossen. Experimental data were manually logged and sorted in Microsoft Excel.

Statistical analysis was done using IBM SPSS statistics (v.26, IMB corp., Armonk, NY, USA). All data were assessed for normal distribution using P-plots. If the normality assumption was not violated, exploration scores were analyzed with repeated measures ANOVA, with identical and odd object identity, as well as object position, as within subjects variables. If the assumption of sphericity was violated, the Greenhouse-Geisser and/or Huyn-Feldt corrected results are reported. Post hoc analysis of significant within-subject main effects were done using paired samples T-test. Non-parametric tests were corrected for multiple comparisons using the Bonferroni-method. All graphical representations were created using Graphpad Prism 8 (Graphpad Software). Error bars are not shown when the error bars are smaller than the size of the symbol.

3. Experiments

3.1 Experiment 1

The main objective in this experiment was to establish suitable objects to be used in a simultaneous oddity discrimination task, and to test an initial set of chosen parameters for suitability in mice. Two sets of objects were used; one set with low feature ambiguity and one set with high feature ambiguity (Figure 3, Figure 4). Establishing both a high and a low feature ambiguous object set is important because it allows for manipulations of the perceptual difficulty of the task, which is important given literature suggesting that different areas of the VVS are differentially involved in task performance based on the level of feature ambiguity of the stimuli (Peterson, Cacciamani, Barense, & Scalf, 2012). As there is no literature to suggest what may be deemed high vs low feature ambiguity in mice, the objects were built to differ in their degree of feature ambiguity, but with no prediction as to whether one or the other (or both) may prove too ambiguous or not sufficiently ambiguous. By using two sets of objects with a difference in feature ambiguity we could try to establish a limit of how feature ambiguous an object set could be to be considered high feature ambiguous and low feature ambiguous for the mice. We chose parameters based on the rat literature, as they are the closest species to the mouse that is represented in the literature. Based on Bartko et al. 2007b, we selected to use five objects which differed in feature ambiguity. We chose to use LEGO® manufactured by the LEGO group as a way to easily manipulate feature ambiguity. We chose to line the objects 2.5 cm apart based on Bartko et al. 2007b. We decided to let the animals explore the objects for 5 minute long trials.

Stimuli

The low feature ambiguity object set (Figure 3) consisted of five LEGO® objects. This object set was inspired by the Duplo objects used by Norman & Eacott (2004). The identical 1 objects consist of features A (yellow branch) and B (orange base and top with yellow and brown towers). The identical 2 objects consist of features C (blue branches) and D (grey base with yellow circles). The odd object shares one feature with the identical 1 objects (feature B) and

one feature with the identical 2 objects (feature C). The identical 1 objects were 6 cm tall, 5.5 cm long, the widest part was 4 cm and the narrowest part was 1.5 cm. The identical 2 objects were 6 cm tall, 8 cm long, the widest part was 6 cm and the narrowest part was 1.5 cm. The odd object was 6 cm tall, 6.5 cm long, the widest part was 6.5 cm and the narrowest part was 1.5 cm. Since all of the objects were the same height, this feature could not be used to discriminate the odd object from the identical 1 and identical 2 objects.

The high feature ambiguity object set (Figure 4) consisted of five LEGO® objects. The features in this object set are more complex than the features used for the low feature ambiguity object set, as they contain both more individual LEGO® pieces within a feature, and more variety in the shape of the individual features (e.g. orange base with multicolored tower and LEGO® apple as feature A in the high feature ambiguity object identical 1, contrasted with grey base only as feature D in low feature ambiguity object identical 2).The identical 1 objects were 10 cm tall, 4.5 cm long, and 6.5 cm wide. The identical 2 objects were 10.5 cm tall, 3 cm long and 4 cm wide. The odd object was 11.5 cm tall, 4.5 cm long and 6.5 cm wide.

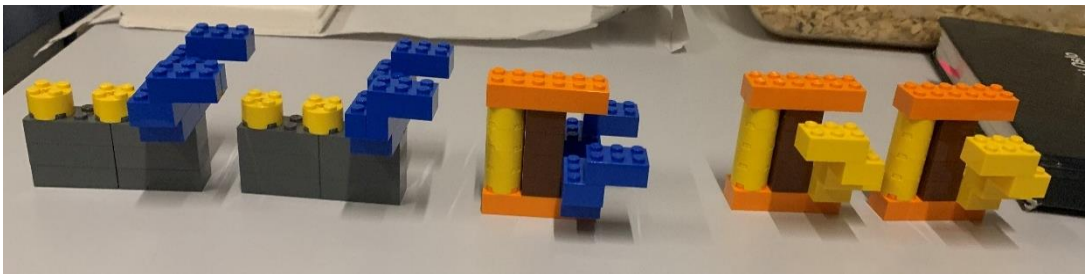

Figure 3: Low feature ambiguity objects used in experiment 1 (identical 2, identical 2, odd object, identical 1, identical 1)

Figure 4: High feature ambiguity objects used in experiment 1 (identical 1, identical 1, odd object, identical 2, identical 2)

Results

On average the mice spent 40.4 seconds exploring the low feature ambiguity objects (SEM = 2.76), and 38.7 seconds exploring the high feature ambiguity objects (SEM = 2.83). Analysis of the preference for the odd objects showed no oddity preference for either of the odd objects, with average oddity preference scores of 16 % and 18 % for the low and high feature ambiguity objects respectively (Figure 5). Statistical analysis confirmed that the time spent exploring the low feature objects was not affected by oddity preference, ($F(2, 46) = 2.194$, $p = 0.123$). Similarly, the time spent exploring the high feature ambiguity objects was not affected by oddity preference ($F(2, 46) = 3.11$, $p = 0.054$) .

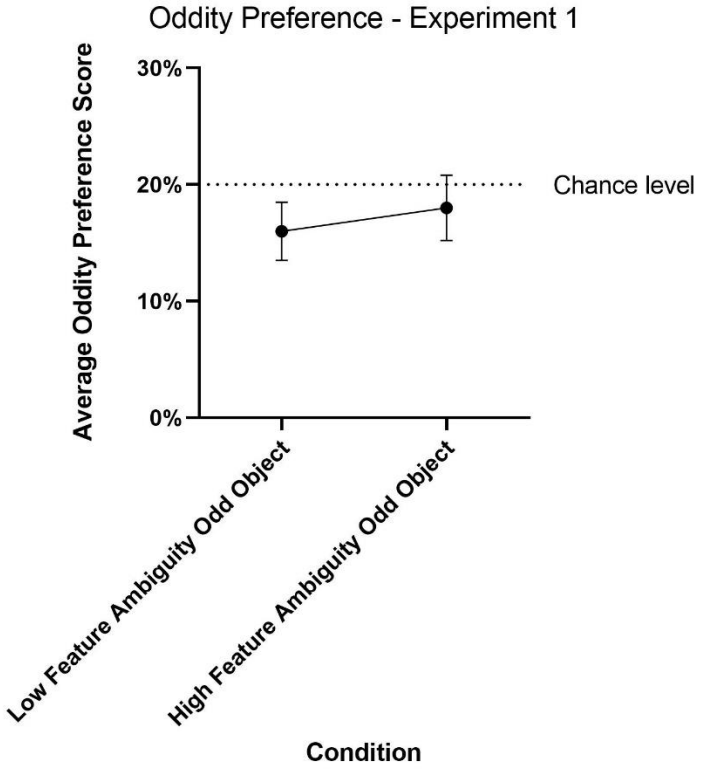

Figure 5: Oddity Preference – Experiment 1. The average oddity preference score for the odd objects in the Low Feature Ambiguity trial and the High Feature Ambiguity trial. Chancel level is 20 % of exploration time. n = 24, 24 trials in each condition. Error bars ±SEM

In order to assess whether exploration of objects was affected by object position, rather than object characteristics, a repeated measures ANOVA with position as within subject variable (2 levels; middle vs corner) was carried out. There was a significant effect of position on performance ($F(1, 47) = 67.4$, $p = 0.0001$), where objects placed in corner positions were explored more than objects placed in mid positions ($t(47) = 8.2$, $p = 0.0001$) (Figure 6).

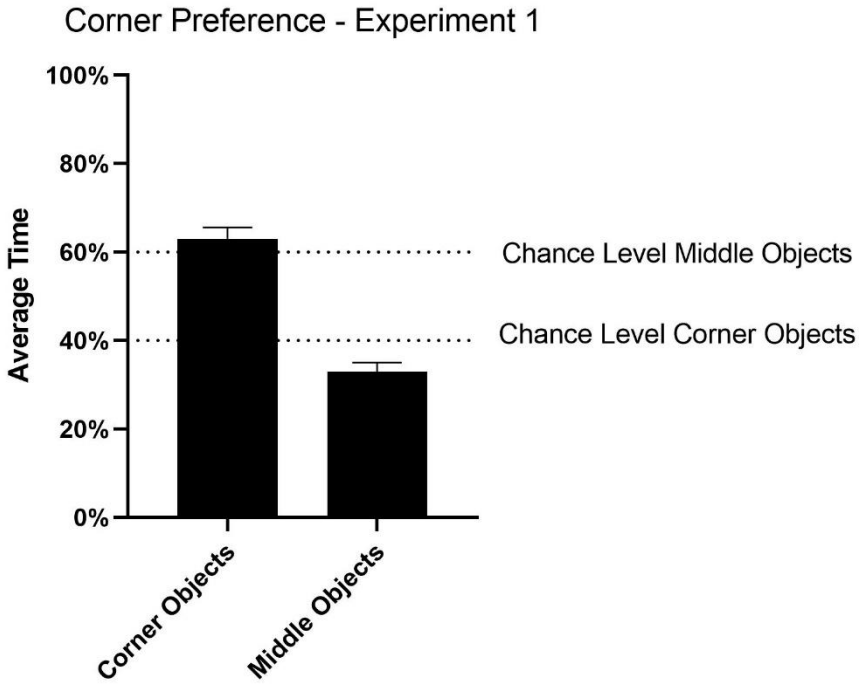

Figure 6: Corner Preference – Experiment 1. The average percentage exploration time spent exploring the objects in the corners and in the middle in both low and high feature ambiguity trials. Chance level for corner objects is 40 % of exploration time, chance level for middle objects is 60 % of exploration time. n = 24, 48 trials. Error bars +SEM.

Discussion

The results from experiment 1 did not show any preference for either the low feature ambiguity odd object or the high feature ambiguity odd object (Figure 5). The lack of oddity preference for the odd objects used in experiment 1 might be due to the animals not being able to perceive the difference in the objects, because the features were too ambiguous. If this is the case, then this issue may be solved by making objects with more distinctive features in the next experiment. We did however discover a significant effect of object position on performance.

The choice to line the objects against the wall was based on methods in the literature, but in the present experiment, analysis of object position on performance showed an unexpected significant effect, whereby objects in the corners were preferentially explored (Figure 6). In previous studies, rats have been used as model organism, and no reports of corner preference have previously been reported. If object position did not have any effect on exploration, we would expect that approximately 60 % of the exploration time would be spent exploring the objects in the middle as there was three of them, and approximately 40 % of the exploration time would be spent exploring the objects in the corners as there was two of them. In this

experiment, the animals spent 63 % of their time exploring the objects in the corners and 33 % of their time exploring the objects in the middle (Figure 6). Rodents show a reluctance to enter exposed spaces, as tested in the open field task, and thus typically spend more time along the wall compared to the center of an open arena (Carola, D'Olimpio, Brunamonti, Mangia, & Renzi, 2002). There may well be species differences between mice and rats that make mice more reluctant than rats to explore the arena beyond the corners, as there are reported strain differences in rats (Harrington, 1972), and mice (Trullas & Skolnick, 1993) in this regard. If indeed mice are more reluctant to leave the corners than rats, it is of greater importance to ensure the objects are equally distant from the corner locations in order to minimize the effect of mice spending a disproportionate amount of time in the corners.

3.2 Experiment 2

The main objective in this experiment was to establish which objects that could be used in a simultaneous oddity discrimination task, and whether adjusting the placement of the objects could reduce the preference for objects based on their location rather than object identity. In experiment 1, two sets of objects were used, one with high feature ambiguity and one with low feature ambiguity, but results from analysis of the data in experiment 1 did not indicate that there was an oddity preference for the low feature ambiguity odd object nor the high feature odd object. It was therefore decided to use only one set of objects with low feature ambiguity in experiment 2 in order to investigate if it was at all possible to establish objects that the animals would show an oddity preference for.

In experiment 1 we discovered that the animals had a preference for the objects placed in the corners, and that this preference had an effect on their performance. It was therefore decided to place the objects in a circle instead of in a straight line, ensuring that all objects were located in a “central position” away from the corners.

Stimuli

The objects used in experiment 2 were placed in a circle (Figure 7). Object 1, 4, and 5 were placed 12 cm from the walls. The corners of baseplate 5 were 23 cm from the corners. Baseplate 2 and 3 were 21 cm from the closest corner. There was 14 cm between the middle of each baseplate.

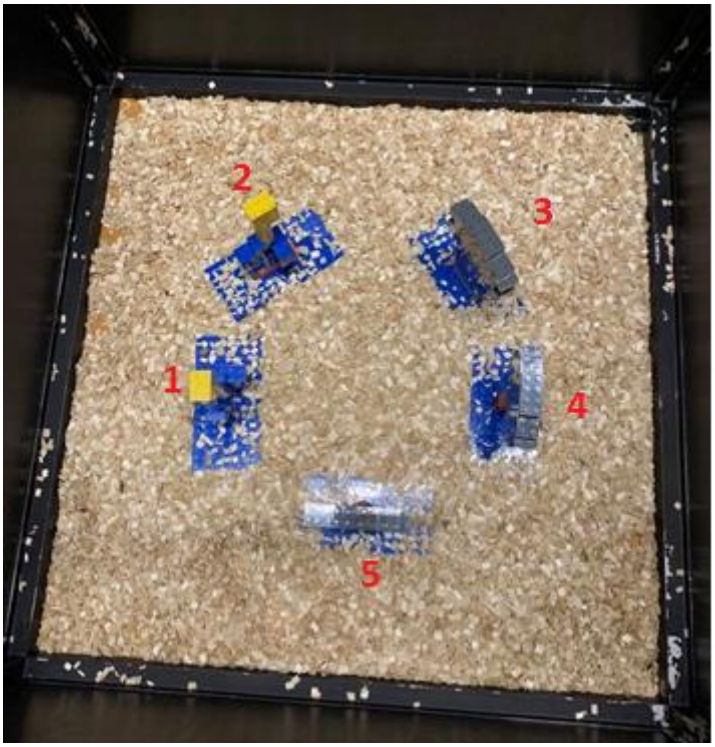

Figure 7: Object set-up used in experiment 2

The identical 1 objects consist of features A (red and blue tower) and B (grey oval). The identical 2 objects consist of features C (orange base with blue branching) and D (yellow tower). The odd object shares one feature with the identical 1 objects (feature B) and one feature with the identical 2 objects (feature C).The object set (Figure 8) used in experiment 2 consisted of five LEGO® objects. The identical 1 objects were 15.4 cm tall, 2 cm long and 8 cm wide. The identical 2 objects were 14 cm tall, 6 cm long, and 6 cm wide. The odd object was 16 cm tall, 8 cm long and 6 cm wide. The objects used in this experiment were built taller than the objects used in experiment 1 to try to make it more difficult for the animals to climb the objects. The objects did not have any form of branches at the bottom, this was also done to make climbing of the objects more difficult.

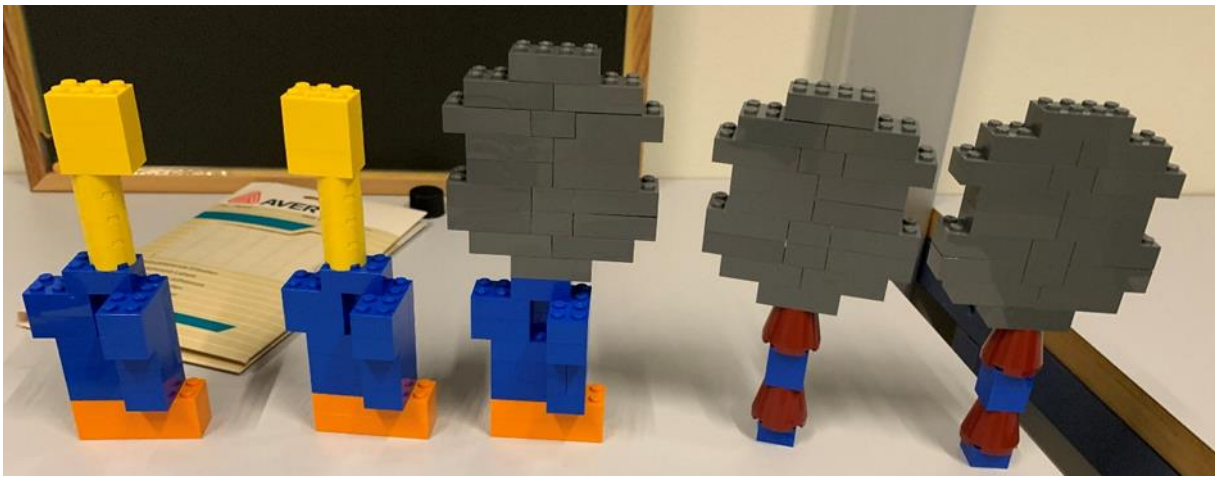

Figure 8: Objects used in experiment 2 (identical 2, identical 2, odd object, identical 1, identical 1)

Results

Two of the animals (animal 7 and 12) were excluded from the analysis because the objects fell during their exploration.

On average the mice spent 18.8 seconds exploring the objects. Analysis of the preference for the odd object showed an oddity preference for the odd object, with an average oddity preference score of 28 % (SEM = 0.01). The results show that the time spent exploring the different objects was affected by object identity, ($F(2, 42) = 41.5$ $p = 0.001$).

Post hoc analysis showed that on average the mice spent 18.2 % of their total exploring time exploring the identical 1 objects (SEM = 0.0064) and 17.7 % of their total exploring time exploring the identical 2 objects (SEM = 0.0048). This difference was not significant ($t(21) = 0.539$, $p = 0.596$). In contrast, the difference in amount of time used exploring the identical 1 objects and the odd object, was significant ($t(21) = -6.34$, $p = 0.0001$), as was the difference in the amount of time used exploring the identical 2 objects and the odd object, ($t(21) = -8.34$, $p = 0.0001$) (Figure 9).

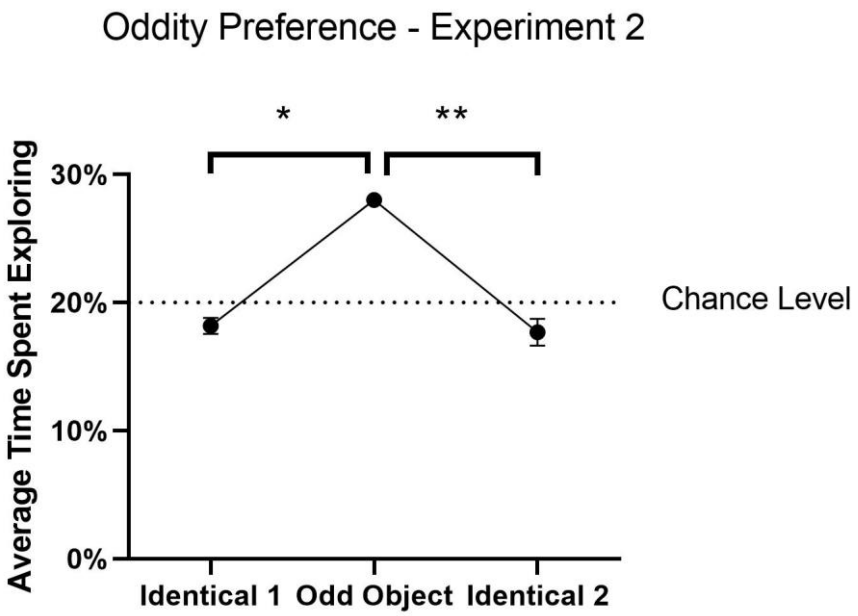

*Figure 9: Oddity Preference – Experiment 2. The average percentage exploration time spent exploring the identical 1, odd, and identical 2 objects in experiment 2. *p=0.0001, **p=0.0001. Chance level is 20 % of exploration time. n =24, 24 trials. Error bars ± SEM*

In order to assess whether exploration of objects was affected by object position, rather than object identity, a repeated measures ANOVA with position as within subject variable (2 levels; middle vs corner) was carried out. Object position did not have a significant effect on object exploration ($F(3.4, 71.2) = 1.01$ $p = 0.4$).

Discussion

The results from experiment 2 indicate that oddity preference did influence the animals' performance in this task. Mice explored the odd object significantly more than the average exploration time of identical objects 1 or 2. This is the first demonstration of an oddity preference in mice, and opens the possibility of using mice in oddity task-based perceptual research.

When deciding to use this object set it was with the intention of this object set being a low feature ambiguity object set. The oddity preference score does not indicate that it was very easily distinguishable for the animals, and a low feature ambiguity object set should preferably

have had a higher oddity preference score. It would therefore be more useful to consider this a high feature ambiguity object set when making new object sets.

Analysis of object position showed no effect on object exploration in experiment 2. The configuration appears to be successful in avoiding excessive exploration of objects based on their position close to corners, and was therefore maintained for the third experiment.

3.3 Experiment 3

The main objective of this experiment was to establish new sets of objects that could be used in a simultaneous oddity task, as a main goal of this study is to establish two sets of objects where feature ambiguity is manipulated across the object sets. Therefore, two new sets of objects were used in experiment 3; one set which aimed at high feature ambiguity, and one set which aimed at low feature ambiguity. In experiment 2, the animals showed a preference for the odd object. The average oddity preference score in experiment 2 of 28 % indicated that this could represent a limit as to how feature ambiguous the objects could be for the animals to still be able to distinguish them and show a preference for the odd object, making the object set from experiment 2 a candidate for high feature ambiguity objects. We could not use the same object set as we used in experiment 2 because the animals had already explored these objects, and exposing them further to this object set would no longer make it a simultaneous oddity task. It was therefore decided to aim to create low feature ambiguity objects with even more distinctive features.

Stimuli

The low feature ambiguity object set (Figure 10) consisted of five LEGO® objects. The identical 1 objects consist of features A (multicolored “oval” with branching) and B (brown base with orange and blue branches). The identical 2 objects consist of features C (yellow and blue tower) and D (multicolored tower with diamond). The odd object shares one feature with the identical 1 objects (feature B) and one feature with the identical 2 objects (feature C). The identical 1 objects were 14 cm tall, 5.5 cm long, the widest part was 8 cm and the narrowest part was 1.5 cm. The identical 2 objects were 14 cm tall, 1.5 cm long, the widest part was 2.4

cm and the narrowest part was 1.5 cm. The odd object was 14 cm tall, 1.5 cm long, the widest part was 6 cm and the narrowest part was 1.5 cm.

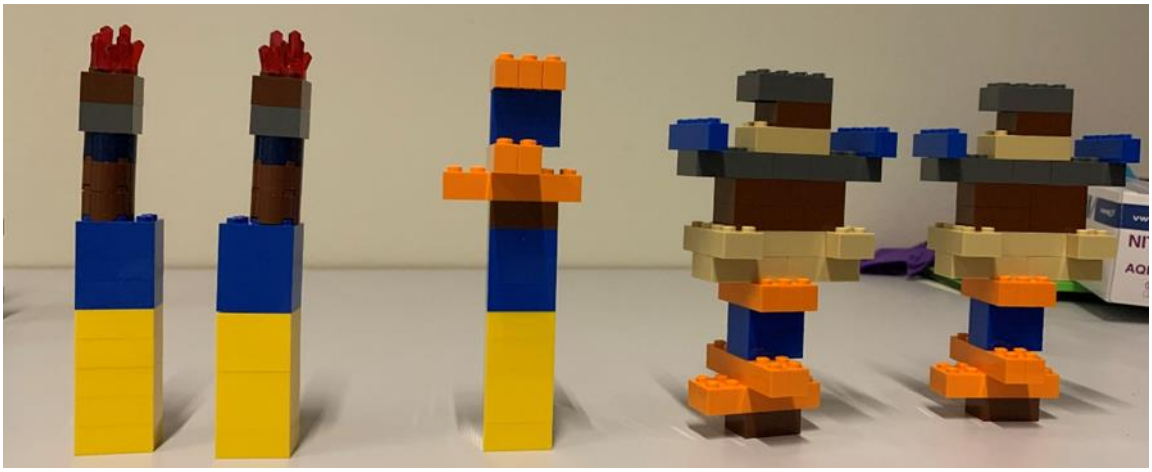

Figure 10: Low feature ambiguity object set used in experiment 3 (identical 2, identical 2, odd object, identical 1, identical 1)

The high feature odd object in experiment 1 was a combination of one feature/part from each of the pairs of identical objects. In experiment 3 all of the high feature ambiguity objects (Figure 11) shared one feature, feature C. Identical objects 1 consisted of features A (red and blue tower), B (grey branches), and C (multicolored stack with blue branches). The identical objects 2 consisted of features C, D (brown and orange base with yellow and blue towers), and E (multicolored stack). The odd object consisted of features BCD, therefore sharing two features with each of the identical objects. The high feature ambiguity object set consisted of five LEGO® objects. The identical 1 objects were 20 cm tall, 5 cm long, the widest part was 6.5 cm. The identical 2 objects were 20 cm tall, 3.2 cm long, the widest part was 4.7 cm. The odd object was 20 cm tall, 5 cm long, the widest part was 6.5 cm and the narrowest part was 1.5 cm.

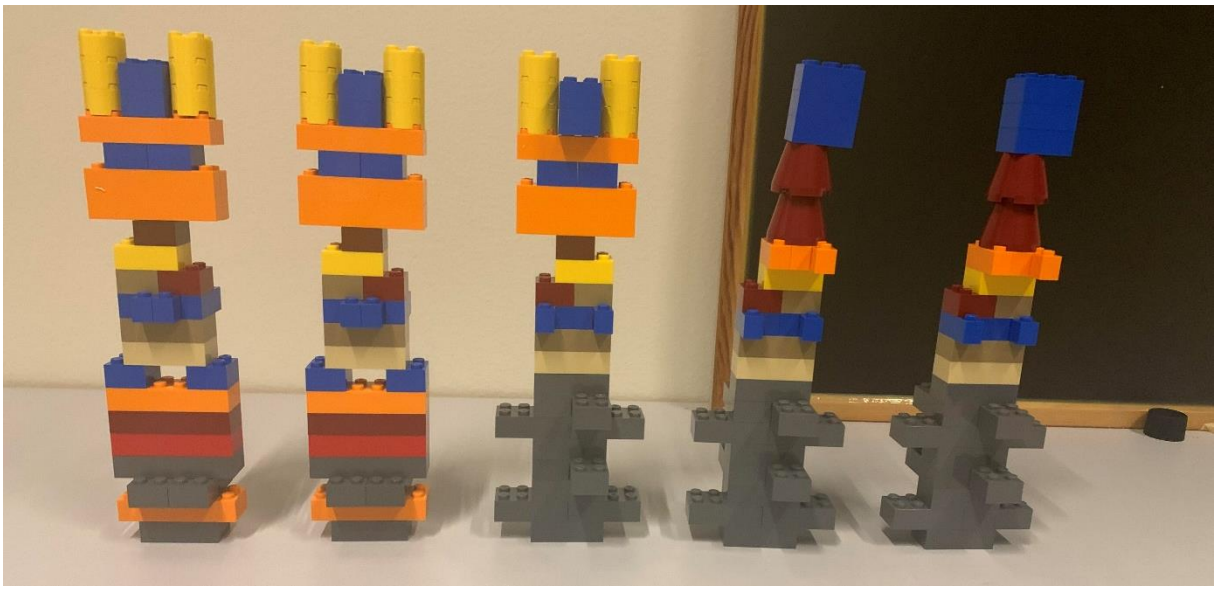

Figure 11: High feature ambiguity object set used in experiment 3 (identical 2, identical 2, odd object, identical 1, identical 1)

Results

Two of the animals (animal 2 and 20) were excluded from the analysis because the objects fell during their exploration of the low feature ambiguity object set.

Low feature ambiguity objects

Analysis of the preference of the low feature ambiguity odd object showed that there was an effect of object identity on object exploration ($F(1.4, 28.4) = 28.9$, $p = 0.001$).

On average the animals spent 26 % of the time exploring the identical 1 objects (SEM= 0,008), 12 % of the time exploring the identical 2 (SEM = 0.006), and 23 % of the time exploring the odd object (SEM = 0.008). The difference in time spent exploring identical 1 objects and the identical 2 objects, was significant ($t(21) = 11.9$, $p = 0.001$). The difference in time spent exploring the identical 1 objects and the odd object, was not significant ($t(21) = 1.447$ $p = 0.163$). The difference in time spent exploring the odd object and the identical 2 objects, was significant ($t(21) = -5.328$, $p = 0.001$) (Figure 12)

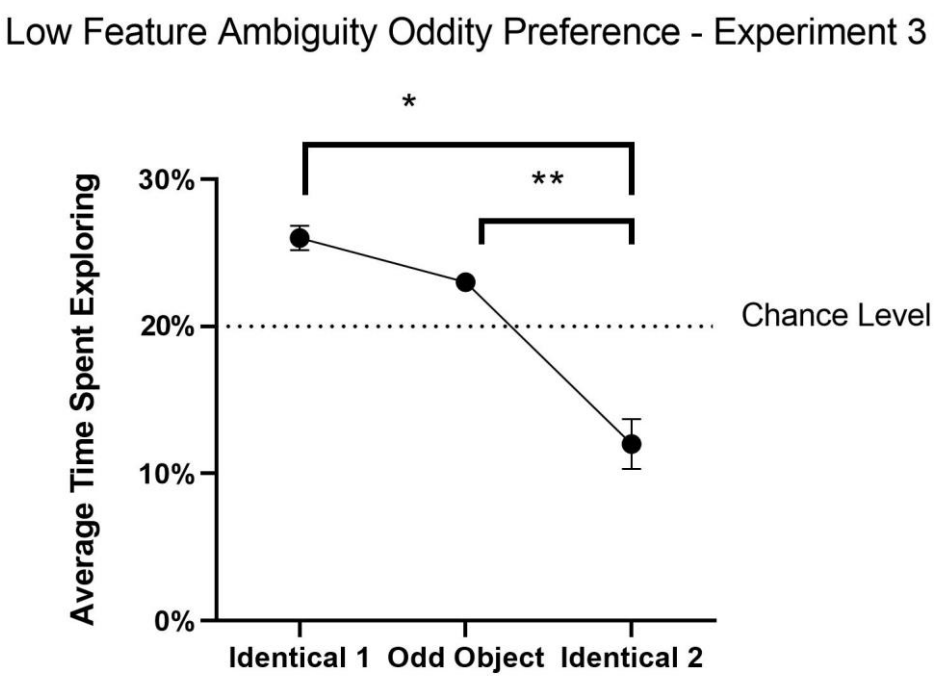

*Figure 12: Oddity Preference Low Feature Ambiguity – Experiment 3. The average percentage exploration time spent exploring the low feature ambiguity objects; identical 1, odd, and identical 2 objects in experiment 3. *p=0.001 **p=0.001. Chance level is 20 % of exploration time. n = 24, 24 trials. Error bars ± SEM*

High feature ambiguity objects

Analysis of the preference of the high feature ambiguity odd object showed limited evidence of an effect of object identity on object exploration. The Greenhouse-Geisser corrected values indicated that there was not a significant effect of object identity on object exploration ($F(1.5, 34.3) = 3.541$, $p = 0.052$), whereas the Huyn-Feldt corrected values indicated that there was a significant effect of object identity on object exploration ($F(1.6, 36.1) = 3.541$, $p = 0.050$).

On average the animals spent 20.4 % of the time exploring the identical 1 objects (SEM= 0,005), 18.5 % of the time exploring the identical 2 objects (SEM = 0.011), and 22 % of the time exploring the odd object (SEM = 0.005). The difference in time spent exploring the identical 1 objects and the identical 2, was significant ($t(23) = 2.18$, $p = 0.001$). The difference in time spent exploring objects the identical 1 objects and the odd object, was not significant ($t(23) = 1.447$ $p = 0.272$). The difference in time spent exploring the odd object and the identical 2 objects, was significant ($t(23) = -2.3$, $p = 0.03$) (Figure 13).

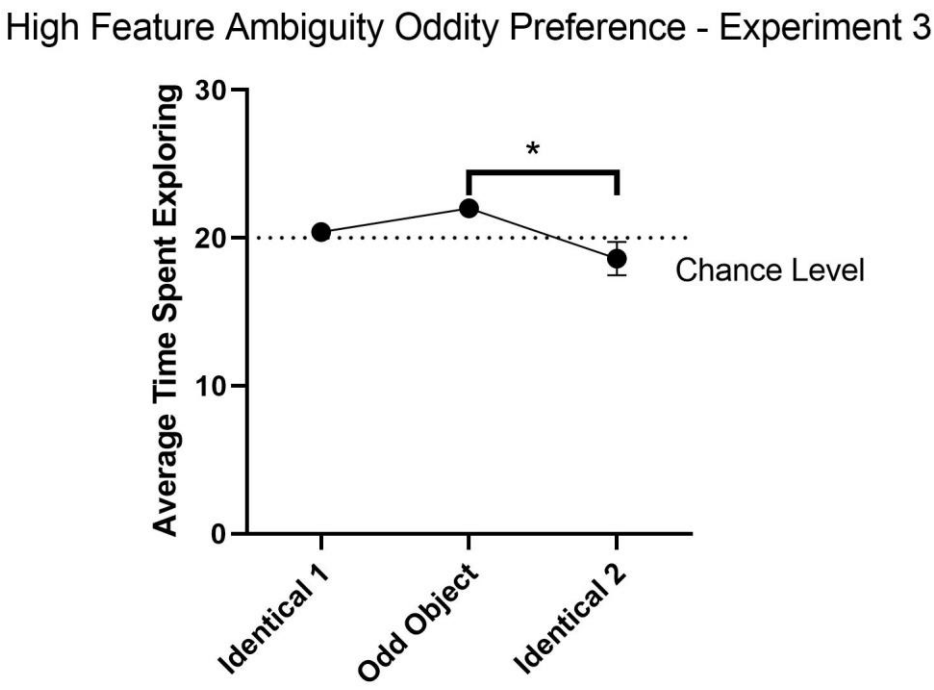

*Figure 13: Oddity Preference High Feature Ambiguity Objects – Experiment 3. The average percentage exploration time spent exploring the high feature ambiguity objects identical 1, odd, and identical 2 objects in experiment 3. *p=0.03. Chance level is 20 % of exploration time. n = 24, 24 trials. Error bars ± SEM*

Discussion

Low feature ambiguity objects

The results from experiment 3 did not show that the low feature ambiguity odd object was the preferred object to explore (Figure 12). The oddity preference score for the low feature ambiguity objects was not much above chance level. This could indicate that that the low feature ambiguity objects were in fact not low feature ambiguous objects for the animals. There was however a preference for the identical 1 objects. Approximately half of the time spent exploring was spent exploring the identical 1 objects. Since the odd object was not the preferred object to explore, and with an oddity preference score not much above chance level, this object set is not well suited for a simultaneous oddity task with mice as a model organism.

This could be a case of stimulus bias, where there is a preference for one object (identical 1 objects) over another that it is paired with (Bussey et al., 2008). Stimulus bias is a common problem in recognition paradigms, as well as touchscreen work with visual images on screen (Zeleznikow-Johnston, Burrows, Renoir, & Hannan, 2017). Stimulus bias is very hard to

predict, and when seen on a group level like this, it is very disruptive, as it will compete with the innate novelty preference, thus making exploration patterns uninterpretable.

High feature ambiguity objects

The results from experiment 3 showed that the high feature ambiguity object was slightly more explored than the identical 1 and identical 2 objects. The Greenhouse-Geisser corrected values indicated that this difference was insignificant whereas the Huynh-Feldt corrected values indicated that this difference was significant. It is clearly a borderline case whether to consider these results significant or not. The oddity preference score is close to chance level (Figure), and it does not indicate that oddity preference had an effect on the exploration of these objects. This was the first object set where all of the objects shared one common feature, and the odd object shared to features with both the identical 1 and identical 2 objects, making this object set more feature ambiguous than any of the previous object sets. This may be a reason for why the animals did not show an oddity preference when exploring the objects. In order to conclude whether the objects were too complex in features, or whether they were too ambiguous given the shared features across all five objects, it would be interesting to try a different object set where all of the objects share one feature like the high feature ambiguity objects in experiment 3 did, whilst simultaneously making the individual features less complex.

This was the last experiment we were able to conduct before the Covid-19 pandemic hit. We used the same group of mice for all three experiments. The same 24 animals were exposed to five different object sets with a total of 25 LEGO® objects. This might have had an impact on the animals' exploration of the objects, as their curiosity for LEGO® objects could have diminished during the five test sessions. It is possible that this is a case of habituation to novelty where a reduction in response to new stimuli occur as a function of the number of exposures to the stimuli (Hughes, 2007).

4. General discussion

The work presented in this thesis demonstrates an oddity preference in mice. To the best of our knowledge, this study is the first that demonstrates an oddity preference in mice. This opens the possibility for using mice in this type of perceptual task, combined with genetic tools to explore the neural circuitry within the VVS and the medial temporal lobe that contribute to perceptual function. The object set used in experiment 2 can serve as guide when designing future object sets for a simultaneous oddity task in mice, as it might represent a limit to how feature ambiguous an object set can be.

We observed a tendency among the mice to explore the corner objects more than would be presumed by chance in experiment 1. We decided to deviate from the set-up used in the rat literature to avoid this preference. By using a circle formation instead of a straight line set-up, we could no longer find an effect of object position on object exploration.

When making the objects for the task, there were no previous objects used for mice, and the objects where therefore to some degree based on objects used on rats. Analysis of the experimental data might indicate that the objects designed for this study were too feature ambiguous, with the exception of the object set used in experiment 2. Even if the object sets designed for experiment 3 are not useful as objects in future oddity tasks for mice, they can still be helpful for designing future objects for oddity task testing. When designing new objects for an oddity task in mice, the objects should be made less feature ambiguous than the objects used in this study.

4.1 Methodical considerations

The arena used for this study (60 cm x 60 cm) was similar size to the arena (60 cm x 50 cm x 30 cm) used in a visual discrimination task for mice by Braida et al. (2013). The arena used in this study might have been too big for mice. In experiment 1, the animals showed a preference for the objects in the corners, likely caused by animals being anxious for open areas. Previous studies such as Bartko et al. (2007b) have used a triangle shaped arena. By using a triangle shaped arena instead of a square shaped arena the number of corners available is reduced, this could maybe contribute to a lower level of anxiety in the animals. If corners prove to be the

preferred locations for the mice, using a cylinder test arena will eliminate the issue, similar to the one used for rats by Hales et al. (2015).

All of the object sets used in this study were made of LEGO®s. We were only able to establish an oddity preference for the object set used in experiment 2. The advantage of using LEGO®s to build the objects is to allow different types of bricks in endless combinations. However, a limitation when using LEGO®s as material for the objects is that all of the surfaces and textures of the objects are non-variable, and could be considered to share a high number of features for that reason alone. The object set used in experiment 2 might represent a limit to how feature ambiguous objects can be in order for mice to perceive a difference in the object and show an oddity preference for the odd object. It would therefore be interesting to use objects of different types of materials. Making objects of different types of materials have previously been done by e.g. Bartko et al. (2007b), in future studies the use of object set consisting of LEGO® objects and object sets consisting of junk objects could prevent the animals from habituating to novelty.

Since we can only get one data point from each animal it is critical to avoid having to exclude any of the trials. In experiment 3, four of the trials were excluded due to objects falling down during exploration. The LEGO® objects were clicked onto LEGO® baseplates that were fastened to the floor of the arena using tack-it. In order to prevent the objects from being unfastened to the LEGO® baseplate and falling down, it could be wise to consider gluing the objects to the LEGO® baseplate or a baseplate of a different material. The baseplates can then be weighted with for example wax (Norman & Eacott, 2004).

4.2 Future considerations

In order to test the predictions of the representational hierarchal view, we need object set with differing feature ambiguities. We were able to establish one set of objects that can be used in a simultaneous oddity task for mice. This object set can be used as a high feature ambiguity object set in future studies. We were not able to test the object set used in experiment 2 on a behaviorally naïve cohort because of the Covid-19 pandemic. This should be done in order to confirm our findings. A set of low feature ambiguity objects should also be established. Once two sets with a difference in feature ambiguity are established, it would be interesting to e.g. inactivate the PRH. This can be done by using e.g. DREADDs. The representational hierarchal view suggests that inactivating the PRH will lead to impairment on the high feature ambiguity

objects, whilst the medial temporal lobe declarative theory suggest no impairment, as it is not a memory task.

There has been some criticism of the task from the standpoint of it involving working memory, and hence not being a task that can be solved based only on perceptual abilities. Working memory refers to a temporary storage and manipulation of information in order to resolve cognitive tasks (Baddeley, 1992). It could therefore be argued that the simultaneous oddity task is not a purely perceptual task, since the animals must retain feature information when exploring the objects. Previous studies have shown that patients with PRH lesions are impaired in high feature ambiguity conditions compared to low feature ambiguity conditions, and argues that this could be caused by a difference in processing conjunctions of object features, and that the working memory demand is the same across the conditions (Barense, Gaffan, & Graham, 2007). However, Jeneson & Squire (2012) argue that this is a result of a difference in demands on memory.

4.3 Conclusion

Three experiments were conducted to establish and optimize testing parameters and object stimuli to be used in an oddity task with mice. This study was, to our knowledge, for the first time able to demonstrate an oddity preference in mice. The object set used in experiment 2 shows potential for future oddity task objects, and it can likely serve as a model for high feature ambiguity objects, when developing new sets of objects for oddity tasks in mice. Even if rats are the closest species to mice that have previously been used in oddity tasks, the objects used for rats may be too ambiguous for mice.

Overall, the presented findings provide a solid basis for continuing the work of developing the oddity task for mice. The information obtained on the importance of avoiding corner locations when testing mice is highly valuable moving forward, and the range of objects tested will provide much needed guidance for object choices in future work. Once established as a task with varying degrees of feature ambiguity in the stimuli set, the oddity task for mice will open up for important work assessing both circuit contributions to perceptual function, and the direct assessment of specific predictions made by competing theoretical accounts of medial temporal lobe function.

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Statement on work contributions and the impact of the Covid-19 pandemic

All experimental work and habituation of the animals was conducted by Maria Moe Almenningen.

All object sets were designed and built by Maria Moe Almenningen.

All videos were scored by Maria Moe Almenningen.

All statistical analysis was done by Maria Moe Almenningen.

The experimental work done for this thesis started in January 2020. The initial project plan was to conduct pilot experiments from January to mid-March, before the main experiments would begin. In the main experiments, we would use a behaviorally naïve cohort of mice. On March 12th the Covid-19 pandemic led to a lockdown in Norway. This led to all future experiments being cancelled. We were therefore not able to use our findings from the pilot experiments to further develop the oddity task for mice. The object set used in experiment 2 has therefore not been tested on a behaviorally naïve cohort of mice. The experimental data presented in this thesis is therefore from what was originally pilot experiments done in the 2.5 first months of 2020.