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## The Neural Representation of 3-Dimensional Objects in Rodent Memory Circuits

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### Abstract

Three-dimensional objects are common stimuli that rodents and other animals encounter in the natural world that contribute to the associations that are the hallmark of explicit memory. Thus, the use of 3-dimensional objects for investigating the circuits that support associative and episodic memories has a long history. In rodents, the neural representation of these types of stimuli is a polymodal process and lesion data suggest that the perirhinal cortex, an area of the medial temporal lobe that receives afferent input from all sensory modalities, is particularly important for integrating sensory information across modalities to support object recognition. Not surprisingly, recent data from *in vivo* electrophysiological recordings have shown that principal cells within the perirhinal cortex are activated at locations of an environment that contain 3-dimensional objects. Interestingly, it appears that neural activity patterns related to object stimuli are ubiquitous across memory circuits and have now been observed in many medial temporal lobe structures as well as in the anterior cingulate cortex. This review summarizes behavioral and neurophysiological data that examine the representation of 3-dimensional objects across brain regions that are involved in memory.

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anterior cingulate cortex; hippocampus; lateral entorhinal cortex; medial prefrontal cortex; object recognition; perirhinal cortex central

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## INTRODUCTION: A Historic Perspective on the use of 3-dimensional objects in animal experimentation

Episodic memory requires the association of “where” an event occurred with “what” stimuli where encountered (Nadel et al., 1985; Nadel and Hardt, 2004; Eichenbaum et al., 2007). Because stimuli in ‘real-world’ situations are typically 3-dimensional, the neurobiological underpinnings of episodic memory cannot be fully appreciated without an understanding of how such objects are represented across brain circuits. For the purpose of this review, a 3-dimensional object refers to any stimulus within an environment that an animal can directly interact with. These types of stimuli are typically smaller in size than the subject and likely have distinct neural representations from barriers or boundaries of the environment that define the borders (Lever et al., 2009), and from landmarks that tend to be large, distally located and aid in spatial orientation (Knierim et al., 1995).

The assessment of animal cognition with 3-dimensional objects has a long history (Young and Harlow, 1943; Harlow, 1944; Harlow, 1945; Harlow and Poch, 1945) that includes the seminal publication describing the design of the Wisconsin General Testing Apparatus (WGTA; Figure 1). The conceptualization and implementation of effective behavioral protocols for the use of this apparatus laid the foundation for the evolving field of behavioral neuroscience, allowing the ways in which non-human primates discriminate (Young and Harlow, 1943) and recognize (Mishkin et al., 1962; Mishkin and Delacour, 1975) different test objects to be systematically explored. In particular, early lesion experiments using monkeys in the WGTA explored the neural structures responsible for the discrimination and memory of 3-dimensional test objects, and provided a strong observational base upon which modern methods have been built (e.g., Mishkin, 1954; Mishkin and Pribram, 1956; Pribram and Mishkin, 1956; Mishkin et al., 1962; Ettlinger et al., 1968; Meunier et al., 1993). For rodents, on the other hand, because of the domination of behaviorist approaches in experimental psychology in the 1950s and 1960s, operant chambers or mazes were typically used to test spatial cognition, and because 3-dimensional stimuli were not useful in these types of tasks, they were considered to be unsuitable for use in this species.

Approaches to investigating rodent behavior in neuroscience and experimental psychology began to shift in the 1960s and 1970s with the seminal observation that principal neurons of the rat hippocampus were active at discrete locations of an environment, termed the cell’s place field (O’Keefe and Dostrovsky, 1971). This finding generated a good deal of excitement and a concerted effort to discover the neural basis of spatial learning and memory in the rat (for review, see Olton, 1979; Barnes, 1988). The influential book that followed, “*The Hippocampus as a Cognitive Map*,” was a treatise of how human and animal cognition could be compared (O’Keefe and Nadel, 1978). Thus in the 1980s, when researchers became interested in developing rodent models for human amnesia, memory

tests for rats using 3-dimensional objects as test stimuli began to be developed (Mishkin and Delacour, 1975; Ennaceur and Delacour, 1988). Initially rat memory for objects was tested by changing the proximal features of a test apparatus (Olton and Feustle, 1981; Aggleton et al., 1986). Aggleton and colleagues (1986) were among the first to incorporate 3-dimensional objects as test stimuli in a rodent variant of the delayed nonmatching-to-sample task (Figure 2A). In this task, 3-dimensional objects were among several features, including different textures and contrasts, used to differentiate pairs of start and goal boxes. Specifically, the rats were released from one start box on a Y-maze, with a unique set of features that included an object. The other two arms of the maze contained goal boxes, one that was identical to the start box and another that was unique. Animals were rewarded for locomoting to the nonmatching goal box (Aggleton et al., 1986). The use of the start/goal boxes as stimuli, however, posed difficulties for acquiring large numbers of unique stimulus sets and in this experiment only 50 different pairs were used. This is considerably less than the several hundred to over a thousand that had been used in primate studies. A version of the delayed nonmatching-to-sample task more comparable to that used in primates was described for rodents in 1987 when Rothblat and Hayes used 250 different 3-dimensional 'junk' objects as test stimuli (Figure 2B). In addition to the larger stimulus set, the sample and test phase structure of each trail was directly analogous to the version used with monkeys. Unlike the performance of monkeys on this task, however, the rats required 12-24 days of pre-training in order to achieve 75% correct, and then did not significantly improve over the next 10 testing sessions (Rothblat and Hayes, 1987). Rats that receive more extensive shaping before testing, which includes two-choice object discrimination testing, however, do reach 90% correct on the trial-unique delayed nonmatching-to-sample task after approximately 16 test sessions (Mumby et al., 1990). Monkeys can perform near 80% correct on the first day, and reach 90% correct within ten testing sessions (Mishkin and Delacour, 1975). These data suggest that, relative to nonhuman primates, rats take considerably longer to acquire the delayed nonmatching-to-sample rule.

Noting the problems of not being able to dissociate object recognition memory from the learning or recall of the nonmatch rule, Ennaceur and Delacour (1988) introduced a task for testing 3-dimensional object recognition that capitalized on the natural tendency of rodents, and many other animals, to explore a stimulus that is novel. In a series of beautifully controlled behavioral experiments, they showed that recognition memory could be assessed by exposing a rat to a novel object for several minutes, imposing a delay (ranging from less than a minute to 24 hours), and reintroducing the rat to a copy of the original sample object and a novel object. They made two particularly important observations in normal young rats using this procedure: first, the animals show habituation to exploration of the sample object on the second exposure, and second, the rats actively explore the novel object more than the familiar object (Ennaceur and Delacour, 1988). Since its original description, this spontaneous object recognition task has been instrumental for investigating the neurobiology of object recognition in lesion (e.g., Ennaceur et al., 1996; Mumby et al., 2002b; Mumby et al., 2002a; Winters et al., 2004; Forwood et al., 2005; Winters and Bussey, 2005c; Barker et al., 2007; McTighe et al., 2010), behavioral pharmacology (e.g., Massey et al., 2003; Hammond et al., 2004; Warburton et al., 2005; Winters and Bussey, 2005b, a; Winters et al., 2006; Brown et al., 2012; Cohen et al., 2013), and aging studies (Cavoy and Delacour, 1993;

Bartolini et al., 1996; Vannucchi et al., 1997; de Lima et al., 2005; Pitsikas et al., 2005; Pieta Dias et al., 2007; Burke et al., 2010; Burke et al., 2011b; Hopkins et al., 2011; Kumar et al., 2012). Furthermore, multiple variants of this task have been developed to test the recognition of novel spatial locations of objects (Cavoy and Delacour, 1993), novel pairings of objects and contexts (Dix and Aggleton, 1999; Eacott and Norman, 2004; Norman and Eacott, 2005), and the temporal order of object presentation (Gilbert et al., 2001; Barker et al., 2007).

A critical factor that should be considered when evaluating data obtained from the spontaneous object recognition tasks, is that in the original publication the authors noted two measures of recognition: habituation (the decrease in exploration related to repeated object exposure) and discrimination (the bias to explore the novel object over the familiar one). These measures are not independent, but are also not comparable (Ennaceur and Delacour, 1988). Unfortunately, many subsequent experiments did not report measures of habituation and summarized the data as a single discrimination ratio measure (Dix and Aggleton, 1999), which only captures the relative preference for novelty. The use of this measure became problematic for lesion, pharmacology and aging studies, when deficits arising from a failure to habituate (i.e., forgetting) could not be dissociated from falsely recognizing the novel object as familiar (Burke et al., 2010; McTighe et al., 2010). Figure 3 shows the two potential sources that could explain a reduced discrimination index based on the exploration times of novel and familiar objects during the test phase of the spontaneous object recognition task. The critical point to understand is that a decline in the discrimination index can arise either from exploring the familiar object more or from exploring the novel object less. Either of these behavior patterns can result in the same discrimination index, even though the behavioral output is different. Thus, studies that only report the discrimination index may be incorrectly drawing a conclusion about the basis of the deficits described. For example, although it has been believed that perirhinal cortical (PRC) lesions or advanced age leads to increased forgetting, more recent data have shown that damage to the PRC (McTighe et al., 2010) or old age (Burke et al., 2010) are associated with reduced exploration of novel objects during test phases that follow long delays and not from a failure to habituate (Figure 3C). This has led to the interpretation that with age or PRC damage, animals are less able to deal with extraneous stimuli that may share common features with the novel test object. Because of this, these animals are less able to discriminate between stimuli with overlapping features (Bartko et al., 2007a, b), resulting in behavior consistent with the animals falsely recognizing novel objects as being familiar.

Another procedural issue that has arisen in experiments utilizing the spontaneous object recognition task, is that object exploration is defined as the animal orienting towards the object within 2 cm. Thus, object recognition defined in this manner is, in fact, a multi-modal process incorporating olfactory, tactile and visual information. This needs to be considered when comparing data across species as well as considering the use of 2-dimensional test stimuli.

## Integration of multi-modal information for object representation

As discussed above, the identification and recognition of 3-dimensional objects in rodents requires the assimilation of polymodal sensory information. This is likely to be particularly important for rodents that have lower visual acuity than do humans and other primates. Thus, the neural substrates that promote the object representations used for recognition memory must receive afferent input from visual, olfactory and somatosensory cortices. The integration of sensory information in the support of recognition memory was first examined in primates. In these tasks, monkeys were exposed to stimuli in the dark and allowed to touch them so that the sample phase allowed the animal to have access to tactile information. During the test phase of this variant of the delayed nonmatching-to-sample task, monkeys were allowed to view the sample object along with a novel object but could not touch them prior to making the choice. Initially, it was believed that the amygdala was critical for integrating somatosensory with visual input for cross-modal recognition (Murray and Mishkin, 1985). Later it became evident that full aspiration lesions of the amygdala lead to damage in the anterior rhinal cortices, including the PRC, and later investigations demonstrated that the PRC in particular was critical for cross-modal recognition (Goulet and Murray, 2001) in addition to its role in uni-modal recognition (Suzuki et al., 1993; Buffalo et al., 1999; Winters and Bussey, 2005c; McTighe et al., 2010). These findings may not be surprising since anatomically the PRC is well poised to support cross-modal recognition, as it receives direct afferent input from all sensory cortices (Suzuki and Amaral, 1994; Burwell and Amaral, 1998). Consistent with the lesion monkey data, functional MRI in humans has also shown that the BOLD signal increases in the PRC during poly-modal recognition tasks (Holdstock et al., 2009).

A paradigm for testing cross-modal object recognition in rats has recently been developed that uses spontaneous object recognition procedures. Rats are allowed to explore identical objects during a sample phase in a dark room illuminated by a red light, eliminating visual input. Sixty minutes later, the test phase is conducted under lighted conditions but the familiar and the novel object are placed behind a Plexiglas screen so that the animals can only use visual information for recognition (Winters and Reid, 2010). Normal rats show reduced levels of exploration for the familiar relative to novel object even when distinct sensory information was available during the sample and test phases of the task. Consistent with the primate studies (Goulet and Murray, 2001), rats with the PRC inactivated did not show a significant preference for the novel relative to the familiar object in the cross-modal task. Unlike the data with monkeys, however, PRC inactivation did not impair a uni-modal tactile recognition task. In rats, the posterior parietal cortex appeared to be critical for tactile recognition as well as cross-modal recognition. Moreover, disconnection of the posterior parietal cortex and PRC produced cross-modal object recognition deficits, suggesting that in rodents this cognitive process may be distributed across several cortical structures (Winters and Reid, 2010). This conclusion is supported by recent data showing that multiple cortical regions in rodents show neural activity tuned to objects (see below).

## Neural activity related to 3-dimensional object representation

In rats, one of the first regions that experimenters recorded neuron activity from during the presentation of 3-dimensional objects was the PRC and adjacent cortical structures (area TE and the entorhinal cortex), as well as occipital cortex and hippocampus. In this study, the objects were always behind a Plexiglas screen so that they could be viewed, but the animals had limited access to other sensory information. Nonetheless, 30-50% of the neurons in each area showed firing rate changes during the presentation of an object relative to baseline (Zhu et al., 1995a). In this study no differences were reported between the five different regions from which neurons were sampled. Furthermore, a large proportion of the stimulus-selective neurons showed decreased firing rates during the object presentation relative to baseline, rather than stimulus-related increases. The computational significance of this finding is unclear and could have been due to limitations in data analysis procedures for isolating principal neurons from interneurons during the time that this investigation was conducted (Bartho et al., 2004).

The observation that visual association cortices are active during the presentation of an object is not surprising (Zhu and Brown, 1995; Zhu et al., 1995a). Object-selective activity in areas of the brain that have traditionally been credited with mnemonic functions, however, has interesting implications regarding mechanisms for how these types of stimuli are incorporated into a memory trace. A current prevailing theory is that object representation in the PRC-lateral entorhinal cortical (LEC) circuit provides the hippocampus with non-spatial sensory information that is integrated with spatial representations to support episodic memory (Aggleton and Brown, 1999; Knierim et al., 2006; Komorowski et al., 2009; Manns and Eichenbaum, 2009; Burke et al., 2011a; Deshmukh and Knierim, 2011; Burke et al., 2012b). In support of this idea is the observation that object-related activity patterns have been reported for the PRC (Burke et al., 2012b; Deshmukh et al., 2012), LEC (Deshmukh and Knierim, 2011; Tsao et al., 2013), and hippocampus (Manns and Eichenbaum, 2009; Burke et al., 2011a; Deshmukh and Knierim, 2013).

Given the overwhelming amount of data illustrating that the PRC is critical for object recognition (e.g., Mumby and Pinel, 1994; Ennaceur and Aggleton, 1997; Buffalo et al., 1999; Baxter and Murray, 2001; Malkova et al., 2001; Winters et al., 2004; Norman and Eacott, 2005), including a rodent's natural preference for exploring a novel object over one that is familiar (Winters and Bussey, 2005c; McTighe et al., 2010), it is somewhat surprising that it was only very recently that activity patterns of neurons during epochs of object exploration have been recorded. Principal cells of the PRC show increases in spiking at the locations of 3-dimensional objects when rats navigate around a track (Burke et al., 2012b; Burke et al., 2014), and when they randomly forage in an open arena containing 4-5 different objects (Figure 4A; Deshmukh et al., 2012). One interesting aspect of these data is that under conditions in which rats repeatedly move past 3-dimensional objects, and during the time period over which the objects go from initially being novel to being more familiar, there is no significant change observed in PRC neuron firing rate. This occurs even though the rats express behavior indicative of recognition of the individual objects (Burke et al., 2012b). These electrophysiological data are also in agreement with an experiment that has used the expression of the immediate-early gene *Arc* to label neurons that were active during

two epochs of object exploration. *Arc* transcription is tightly coupled to principal cell spiking during behavior. The proportion of neurons that express *Arc* did not differ when animals explored novel objects versus familiar ones, providing additional support for the idea that novelty may not modulate PRC activity during object recognition (Burke et al., 2012a).

A long standing view is that the PRC encodes information about the relative familiarity of a stimulus with a simple rate code. Specifically, neuron firing rates have been reported to be higher if a stimulus is novel or if a stimulus has not been experienced recently (for review, see Brown and Aggleton, 2001). On the other hand, several newer studies conducted in rats (Burke et al., 2012b; Burke et al., 2014) and monkeys (Thome et al., 2012; Woloszyn and Sheinberg, 2012) have not confirmed the firing rate decreases that would support the hypothesis of such a rate code. Notably, many of the experiments that reported significant PRC neuron activity changes in response to stimulus repetition were conducted under conditions in which the animal views two-dimensional images while it was fixed in place (e.g., Miller et al., 1991; Zhu et al., 1995b; Xiang and Brown, 1998). Even in humans, there are robust differences in how two-dimensional images are processed relative to 3-dimensional objects. In fact, humans given multiple presentations of real-world 3-dimensional objects during fMRI do not show the repetition effects in the BOLD signal that are commonly reported to occur in flat screen images (Snow et al., 2011). Thus, it is likely that rodents, which under natural conditions have little need to represent 2-dimensional computer screen images, have distinct neural mechanisms that support the recognition of 3-dimensional objects.

Taken together these data suggest that the PRC may not use a rate code to represent object familiarity. In fact, this type of rate code may not be optimal for providing the signal-to-noise ratio that can relay a “recognition signal” for this purpose, since it can be vulnerable to noise and spike failures, and the probability of synaptic transmission between the PRC and its cortical efferents is low (Pelletier et al., 2004). A plausible alternative is that the PRC utilizes a population code, which relies on the joint activities of a number of neurons, each neuron having a different distribution of responses over some set of inputs. To date, however, PRC representations of 3-dimensional objects in rodents have only been tested under spontaneous recognition procedures (Burke et al., 2012b; Deshmukh et al., 2012). Whether or not the PRC represents objects with a population code needs to be tested under additional behavioral paradigms. Of particular importance is how PRC neuron activity patterns in rats and mice are modulated by objects associated with a reward outcome, such as during the DNMS task. Future research should aim to compare PRC object-related representations during spontaneous recognition versus object discrimination and rewarded recognition.

Neural activity related to 3-dimensional objects has also been observed in the LEC (Deshmukh and Knierim, 2011; Deshmukh et al., 2012; Tsao et al., 2013). Although it appears that the LEC inherits non-spatial stimulus representations from afferent PRC input, object-related activity in this region is distinct from the PRC in two important ways. First, although consistent punctate firing fields of LEC neurons are only observed when objects are present in the environment, the location of these fields do not always coincide with

objects (Figure 4B; left panel). Deshmukh and Knierim (2011) have reported that a small portion of LEC neurons fire away from object location and express activity patterns that are reminiscent of hippocampal neuron ‘place fields’ (O’Keefe and Dostrovsky, 1971). Critically, while this activity is not near an object, removing objects from the environment abolishes the place-specific firing patterns of LEC neurons, which suggests that these cells may create landmark-based spatial representations. In contrast, stable firing fields away from object locations are not observed in the PRC (Deshmukh et al., 2012). Another important distinction between PRC and LEC object-related activity is that LEC neurons will fire at a location within an environment from which an object has been removed, showing object-memory trace activity (Figure 4B; Tsao et al., 2013). This is not observed in PRC neurons, in which consistent firing fields after objects have been removed is uncommon (Burke et al., 2014).

Both the PRC and LEC project directly to the hippocampus, where nonspatial sensory information, such as object representation, is presumably integrated with spatial information relayed by the medial entorhinal cortex (Aggleton and Brown, 1999; Knierim et al., 2006; Komorowski et al., 2009; Manns and Eichenbaum, 2009; Burke et al., 2011a; Deshmukh and Knierim, 2011; Burke et al., 2012b). Although the predominant activity correlate of hippocampal principal cells is an animal’s spatial location (O’Keefe and Dostrovsky, 1971), several investigations have demonstrated that these activity patterns, referred to as the neuron’s place field, are updated in the presence of 3-dimensional objects (Cressant et al., 1997, 1999; Renaudineau et al., 2007; Manns and Eichenbaum, 2009; Burke et al., 2011a; Deshmukh and Knierim, 2013). Specifically, principal cells in the CA1 and CA3 subregions of the hippocampus appear to carry information regarding both the identity and spatial location of objects that correlate with an animal’s object recognition performance (Manns and Eichenbaum, 2009). Furthermore, the distal portion of CA1, which receives direct afferent input from both the PRC and LEC (Amaral and Witter, 1995; Naber et al., 1999) and is less spatially tuned relative to proximal CA1 (Henriksen et al., 2010), contains principal cells that express a larger number of firing fields with smaller spatial receptive fields in conditions with objects compared to those without (Figure 4C; Burke et al., 2011a). Finally, a portion of CA1 neurons express spatial receptive fields at a set distance from an object, which may be indicative of the use of a landmark-vector representation to map an animal’s location relative to an object (Figure 4C; Deshmukh and Knierim, 2013). Taken together these data indicate that although the hippocampus may not be required for object discrimination and recognition (e.g., Winters et al., 2004; Forwood et al., 2005; Ainge et al., 2006), 3-dimensional object representations are normally incorporated into hippocampal activity patterns. Thus, the influence of both spatial and non-spatial information on neuron spiking in the hippocampus may account for the involvement of this structure in recollection (Fortin et al., 2004) and episodic memory (Nadel et al., 1985; Nadel and Hardt, 2004; Eichenbaum et al., 2007), both of which require the association of “what” stimulus was encountered with “where” it was experienced.

While a critical role in object and object-place recognition primarily has been attributed to medial temporal lobe structures, more recent lesion data have shown that the rodent medial prefrontal cortex is also involved in object-location associations (Barker et al., 2007; Barker and Warburton, 2013). This suggests that high-order representations of 3-dimensional



objects exist outside of the medial temporal lobe. Although no recordings have been obtained from areas that have been lesioned in the rodent prelimbic or infralimbic cortices, which result in object recognition deficits, neurons in the anterior cingulate cortex show activity that is strongly modulated by the presence of objects (Weible et al., 2009; Weible et al., 2012). These activity patterns show several interesting characteristics that include changing in response to replacing a familiar object with one that is novel and responding to moving an object from a familiar location to one that is novel (Weible et al., 2009). Furthermore, some anterior cingulate neurons show activity at locations that used to contain a highly familiar object. This “absent object”-associated activity has been shown to persist for up to 30 days (Figure 4D), indicating that the anterior cingulate may play a unique role in the remote memory for objects (Weible et al., 2012). Together these data indicate that high-order object representations exist outside of the medial temporal lobe.

## Conclusions

Three-dimensional objects have been used to probe the neurobiology of associative and explicit memory for several decades. An interesting aspect of the neurophysiological data examining the neural representations of these types of stimuli is that activity related to objects is ubiquitous across memory circuits. In fact, it has now been reported that principal cell spiking in the hippocampus (Manns and Eichenbaum, 2009; Burke et al., 2011a; Deshmukh and Knierim, 2013), perirhinal (Burke et al., 2012b; Deshmukh et al., 2012), lateral entorhinal (Deshmukh and Knierim, 2011; Deshmukh et al., 2012; Tsao et al., 2013), and anterior cingulate (Weible et al., 2009; Weible et al., 2012) cortices are all modulated by the presence of objects. This provides strong evidence that object-related information is integrated with spatial input to comprise neural traces that serves associative and episodic memory.

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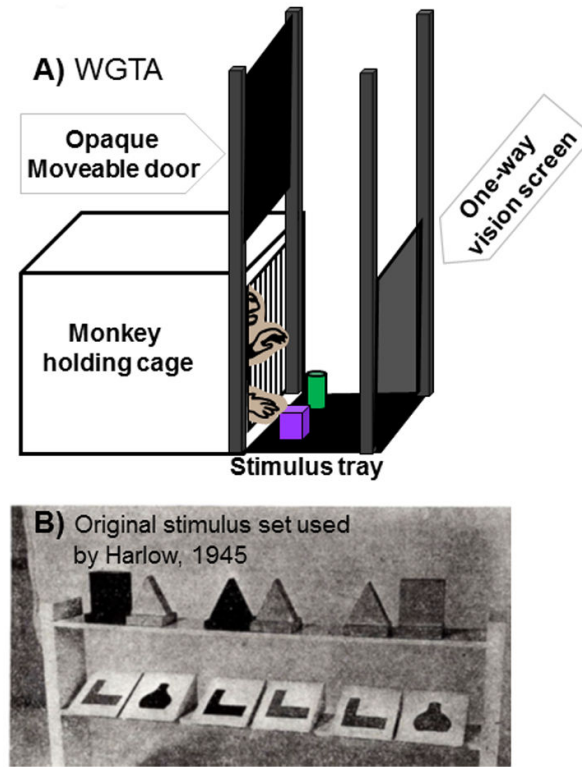
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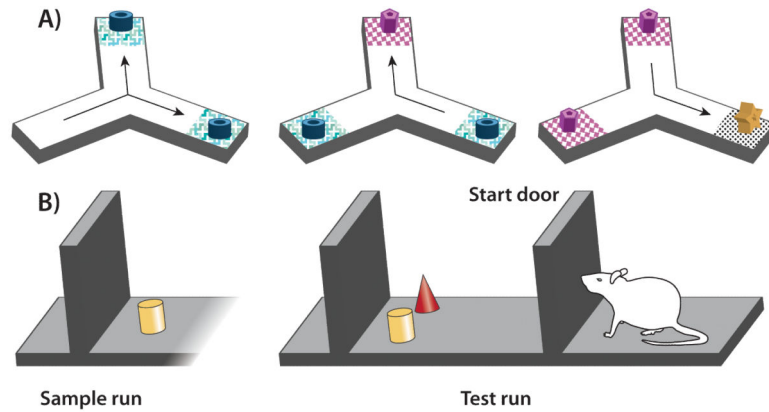
### Highlights

- Three dimensional object stimuli are used extensively to probe memory function.
- Object representations involve polymodal sensory input to the medial temporal lobe.
- The representation of 3-dimensional objects in memory circuits is ubiquitous.



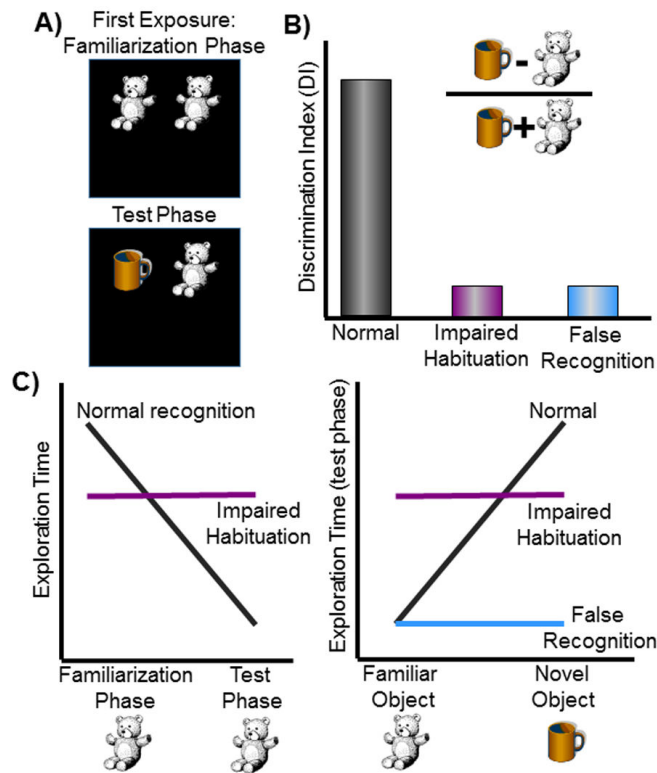
**Figure 1. Schematic of the original Wisconsin General Testing Apparatus (WGTA)**  
**(A)** Harry Harlow implemented the original WGTA in the 1940s, and its simple but elegant design has become a mainstay for evaluating non-human primate cognition across a broad range of modalities. The subject sits in a holding cage with bars at one end that allow the animal to interact with stimuli. A solid guillotine door can be lowered between the animal and the stimuli to impose delay periods. A second guillotine door is constructed from one-way glass and is between the experimenter and the test tray allowing the experimenter to observe the subject without being seen. **(B)** A picture of the some of the early 3-dimensional object test stimuli that Harry Harlow used to test visual discrimination in monkeys (reproduced from Harlow, 1945).





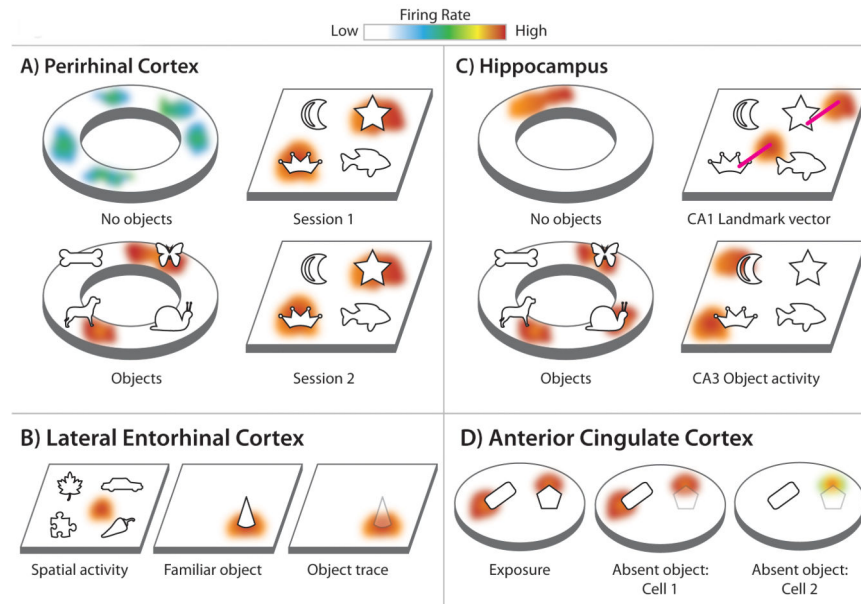
**Figure 2. Delayed nonmatching-to-sample (DNMS) tasks in rodents**

(A) An early version of a DNMS task for rats that incorporated 3-dimensional objects as test stimuli (Aggleton et al., 1986). The task is run on a Y-maze and during the first trial the rat is placed in a neutral start box with no stimuli. The two other arms contain identical goal boxes with textured floors and a 3-dimensional object in each. The rat can obtain food reward at either goal box. During the subsequent trial, the rat remains in the goal box from the previous trial, and one arm contains the identical goal box while the third arm has a novel box at the end containing a unique textured floor and object. The animal is allowed to leave the current box and is rewarded for running to the new (nonmatching) box only. This continues for multiple trials. (B) Another variant of a DNMS task with 3-dimensional objects as stimuli used in rats (Rothblat and Hayes, 1987). Each trial consists of a sample phase (left panel) and a test phase (right panel). During the sample phase the rat locomotes down a short track to displace a single object for a food reward located in a small well near the object. After a delay, the test phase is performed in which the rat traverses the track and is presented with two objects near two food wells: one is identical to the sample object and the other is novel. The rat receives a food reward for displacing the novel object.



**Figure 3. Performance measures on spontaneous object recognition tasks**

(A) Schematic of the spontaneous object recognition task. Rodents are initially exposed to two novel objects in an arena during a familiarization phase (top box). Following a delay, subjects are placed in the same arena but with an object that is identical to the ones from the familiarization phase and a novel object (bottom box). Intact adult rats spend more time exploring the novel object relative to the familiar one. (B) The exploration times during the test phase can be used to calculate a discrimination index (DI). The DI (difference in time spent exploring the novel vs familiar object / total object exploration time) is often used to quantify rodent performance on the spontaneous object recognition task. A reduced discrimination index, however, can be obtained from either a failure to habituate to the familiar object (for example, forgetting it was experienced) or falsely recognizing the novel object as familiar, and this measure alone cannot distinguish between these two different behavioral outcomes. (C) Shows the exploration times of sample identical objects during the familiarization and test phase (left panel) and of the distinct individual objects during the test phase (right panel). These figures show how it is possible to obtain the same DI measure from different behavioral output, either a failure to habituate or an inability to identify the novel object as new. These two distinct behavioral causes of a reduced discrimination index (forgetting versus false recognition) could have different neurobiological etiology. Thus, it is critical to examine the raw exploration times of objects during the sample and test phases in order to determine the source of lower discrimination indices.



**Figure 4. Single-unit activity associated with 3-dimensional object representation in perirhinal cortex, lateral entorhinal cortex, hippocampus and anterior cingulate cortex**

(A) A schematic example of PRC principal cell spiking activity recorded when rats traverse an empty track (top left panel) and a track that contains 3-dimensional objects (bottom left panel). When a rat traverses an empty track, a portion of PRC neurons show dispersed low levels of spiking activity around the track (top left panel). When 3-dimensional objects are added to the track (bottom left panel) many of the cells that had previously shown non-selective activity on the empty track begin to fire at the locations near objects (Burke et al., 2014). Similar spiking of PRC principal cells near the locations of objects (top and bottom right panels) is observed when rats randomly forage in an open arena containing objects (Deshmukh et al., 2012). (B) Schematic examples of LEC activity related to 3-dimensional objects. Unlike the PRC, during exploration of an arena containing objects, a portion of neurons in the LEC show punctate firing fields not near the locations of objects (left panel). This spatial selectivity of LEC neurons is not observed in environments without objects (Deshmukh et al., 2012). LEC principal cells also fire at object locations (middle panel; Deshmukh et al., 2012; Tsao et al., 2013), and a portion of these cells show “object trace” activity by continuing to fire even after the object has been removed (right panel; Tsao et al., 2013). (C) Schematic examples of hippocampal CA1 principal cell activity related to 3-dimensional objects. When a rat traverses an empty track hippocampal neurons show spatially-selective receptive fields referred to as the cell’s place field (top left panel). When objects are added to the track principal neurons in distal CA1 have a higher probability of expressing multiple place fields near object locations and the size of the receptive fields is smaller (bottom left panel; Burke et al., 2011a). In an open arena containing objects, CA1 place fields will also show activity at a fixed distance and orientation from objects (indicated by the magenta line), referred to as landmark vector activity (top right panel). In CA3, object-related activity is more likely to be near the location of objects than at a fixed distance away (bottom right panel; Deshmukh and Knierim, 2013). (D) Schematic example of ACC principal cell activity during object exploration in a circular arena. While mice are

being familiarized with objects, ACC spiking can be observed near object locations (Weible et al., 2009, 2012; left panel). After a delay, a portion of ACC neurons will show “absent object” activity either by continuing to fire at both the present object and the removed object locations (middle panel), or by only firing at the location of the removed object (right panel; Weible et al., 2012).

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