

# Neural encoding of the concept of nest in the mouse brain

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**As important as memory is to our daily functions, the ability to extract fundamental features and commonalities from various episodic experiences and to then generalize them into abstract concepts is even more crucial for both humans and animals to adapt to novel and complex situations. Here, we report the neural correlates of the abstract concept of nests or beds in mice. Specifically, we find hippocampal neurons that selectively fire or cease to fire when the mouse perceives nests or beds, regardless of their locations and environments. Parametric analyses show that responses of nest cells remain invariant over changes in the nests' physical shape, style, color, odor, or construction materials; rather, their responses are driven by conscious awareness and physical determination of the categorical features that would functionally define nests. Such functionality-based abstraction and generalization of conceptual knowledge, emerging from episodic experiences, suggests that the hippocampus is an intrinsic part of the hierarchical structure for generating concepts and knowledge in the brain.**

bed cell | episodic memory | nest cell | semantic memory | hippocampus

Formation of concepts and abstract knowledge has been traditionally considered to be exclusive hallmarks that define humans and possibly other highly intelligent nonhuman primates (1, 2). Such high levels of abstract cognition play an essential role in guiding our adaptive behaviors in everyday life (3–8). For example, when we check into a hotel, the concept of “bed” in our brains can help us identify the bed effortlessly among various furniture in the room despite the fact that the bed could be drastically distinct in terms of its physical shape or style from the ones that we have used in our homes. Currently, it is not clear how our brain actually generates and encodes such abstract concepts from daily experiences.

Just like the human's ability to recognize beds, we hypothesize that nonprimate animals such as rodents and birds should also possess the ability to recognize a refuge or object that can serve as their nests. Because a nest or bed can vary widely in its physical appearances (e.g., distinct physical shapes and styles, color, and construction materials, etc.), we further hypothesize that internal representations of those objects are likely to require the brain to encode abstract knowledge and concepts into categories beyond the specific shape or form of each item.

To search for the underlying neural correlates, we focused on the mouse hippocampus, because this structure is crucial for the formation of not only memories of events, people, and places (often known as episodic memory) (9–14) but also memories of knowledge, facts, and concepts (also known as semantic memory), as indicated by neurological studies of human patients (3, 5, 15, 16). Indeed, our recent large-scale recording suggests that some of the memory-coding units in the hippocampus seem to be intrinsically involved in extracting commonality and abstract general features from various episodic events (17, 18). Therefore, we set out to investigate the neural encoding of conceptual knowledge of nests or beds in the mouse hippocampus and studied the correlation between the neural activity and behav-

ioral encounters of various types of nests. Our experimental analyses have revealed the existence of hippocampal cells whose firing exhibited dynamic changes when the mice encountered nests or beds.

## Results

**Nest-Responsive Cells and Their Firing Characteristics.** Although there are many excellent paradigms for the measurement of memory function (14, 15, 19–24), there is no paradigm that would readily permit the molecular and neural analyses of abstract knowledge and concepts in classic genetic models such as mice in which various sophisticated genetic technologies have been developed (21, 25–27). In this study, we propose to use the mouse's natural interactions with various nests as the behavioral paradigm for investigating the neural correlates underlying the representations of conceptual knowledge of objects in the brain.

We have used large-scale ensemble recording techniques (18, 28, 29) and monitored single-unit activities in the CA1 region of the mouse hippocampus when the animals were physically interacting with their nests. We have recorded from seven mice and identified eight single units that responded significantly to nests. Based on their spike-discharge patterns [for waveforms and basic firing properties, see [supporting information \(SI\) Fig. 6](#)], these eight cells can be classified into three groups, namely, the transient-on type (Cells #1, #4, and #5), persistent-on type (Cells #2 and #6), and persistent-off type (Cells #3, #7, and #8). For example, Cell #1 from mouse A exhibited transient-on-type responses, that is, it would transiently, but drastically, increase its firing whenever the animal encountered the nest (Fig. 1A). If the animal faced away from the nest, this cell would remain in near silence. Top video snapshots in Fig. 1A illustrate four consecutive encounters of the home nest within a period of 35 s during which the mouse approached the nest from four different angles (the first encounter from the 1 o'clock direction, the second encounter from the 4 o'clock direction, the third encounter from 7 o'clock, and the fourth encounter from 5 o'clock). The recorded spike-discharge pattern (below the video snapshots) shows the time-locked, robust firing every time the mouse encountered the nest. A total of 10 encounters took place during the 10-min-period, and the cell drastically increased its firing at each encounter (Fig. 1A *Middle*). By marking the time point at which the mouse's nose tip is 1 cm away from the edge of the nest before crossing as time 0, we plotted perievent spike raster and perievent spike histograms (Fig. 1A *Bottom*). Both analyses show that this cell significantly increased its firing at the

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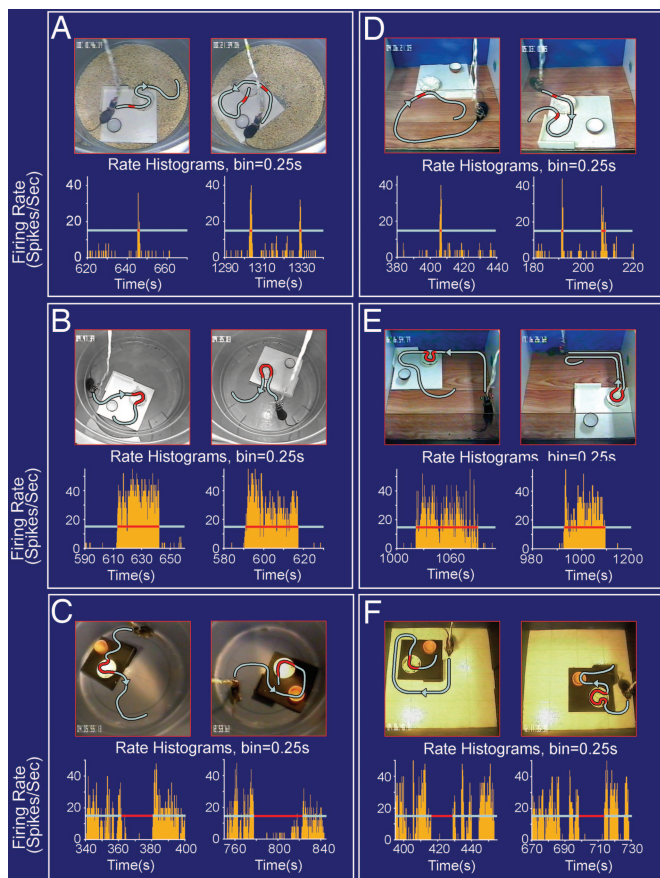
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**Fig. 2.** Firing changes of nest-responsive cells are independent of the nest's spatial locations and environments. (A) Cell #1 of mouse A fired robustly upon encountering the nest regardless of the nest's locations as indicated by the firing-rate histograms beneath each video snapshot. The gray lines illustrate the animal's movement path; the red segments show the near contact points where the mouse crossed the edge of the nests. (B) Cell #2 from mouse B showed a persistent increase in its firing upon entry into the nest regardless of the fact that the nest was placed at different locations in the home environment. (C) Cell #3 of mouse C ceased to fire once the mouse entered the nest regardless of the fact that the nest was placed at different locations. (D) Cell #1 preserved its robust responsiveness to the nest after it was relocated to two different locations in this new environment. The gray lines illustrate the animal's movement paths, and the red segments show the near contact points right before the mouse's nose crossed the edge of the nests. (E) Cell #2 continued to exhibit persistent-on-type firing even after the nest was relocated to two different locations in this new environment. (F) Cell #3 ceased to fire once the mouse entered the nest, which was placed in two different locations in a new environment. For B, C, E, and F, the gray lines illustrate the animal's movement paths, and the red segments indicate the time duration in which the mouse was inside the nest. The time bin used in the firing-rate histograms is 250 ms.

Moreover, we have found three cells (Cells #3, #7, and #8) that exhibited the “persistent-off”-type firing patterns when the mice entered and stayed inside the nests. For example, Fig. 2C Top shows the video snapshots of five epochs during a 5-min period during which the mouse encountered its nest. The spike-discharge patterns of Cell #3 (from mouse C) show that it had a very high basal firing level ( $16.08 \pm 3.49$  Hz) when the mouse was outside of its home nest but ceased to fire once the animal's head got inside the nest (with an average firing rate of  $0.56 \pm 0.27$  Hz) and continued to stay in near silence as long as the mouse remained inside the nest (Fig. 1C Middle). We also noted that the cell tended to fire at a higher level (up to 40 Hz) immediately upon exiting the nest and then tapered off some-

what to  $\approx 10$ –20 Hz range. Similarly, Cells #7 and #8 from two different mice also exhibited persistent reductions in their firings once the animals entered their nests ( $1.08 \pm 0.59$  Hz inside the nest, and  $10.66 \pm 3.15$  Hz outside the nest for Cell #7;  $0.22 \pm 0.15$  Hz inside the nest, and  $9.98 \pm 2.59$  Hz outside the nest for Cell #8) (also see SI Fig. 7).

**Responses of Nest Cells Are Independent of the Nests' Locations and Environments.** Because it is well known that some of the hippocampal cells exhibit place-related firing changes (9, 13), it is necessary to determine whether the observed nest-related firing changes are influenced by the spatial locations of the nest. To address this question, we placed the home nests in various locations in their home environments. We found that all of those nest cells maintained their nest-responses regardless of the spatial locations of the nests. For instance, Cell #1 fired robustly when the mouse encountered the nest which was moved from its original place to two different locations with different orientations (Fig. 2A). Cell #2 also showed a persistent increase in its firing during the period when the mouse was inside the nest whether the nest was placed at one location or another (even with rotated orientation) (Fig. 2B). Similarly, Cell #3 ceased to fire whenever the mouse stayed inside of the nest regardless of its location (Fig. 2C). Therefore, the nest cell responses are independent of the nest's spatial locations and orientations.

Next, we asked whether the overall environmental context has any significant influence on the responses of these nest-responsive cells. We examined this question by placing the home nest in a completely new environment and recording the responses of those cells (Fig. 2D–F). We found that the nest cells continued to exhibit nest encounter-induced responses even when the nests were placed in the drastically different recording environments. Therefore, those experiments show that the responses of the nest cells are not affected by either the nest's spatial locations or the surrounding environments in which the nests are located.

**Selectivity of Nest-Responsive Cells.** To investigate the response selectivity of these cells, we measured their responses to various objects that existed in the nest environments. The first question we asked was whether the nest-responsive cells reacted to a similar shaped object (e.g., the drinking water cup) or to other differently shaped objects (e.g., the corner fence, floor pads, or food pellets) in the home environments. Our perievent spike raster and spike histograms revealed no significant change in the firings of those nest cells during the encounters of nonnest objects, indicating their nest-response selectivity. For example, although Cell #1 exhibited strong responses to the encounter of the home nest (7.5 cm in diameter, with 2-cm low wall) (Fig. 3A, the first image on the left), it did not respond to the encounter of the edge of its water cup, which has a similar geometric shape (Fig. 3A, the second image from the left). Moreover, this cell was not responsive upon encountering other objects in the environment such as the corner fence which had a similar height (2-cm height) to the nest wall (Fig. 3A, the third image from the left) or the edge of a floor pad (Fig. 3A, the image on the right).

To further define the encoding features of the nest cells, we investigated whether the size of the nest plays any role in defining the responses of these cells. We constructed several versions of the circular nests of various sizes, ranging from the normal size (7.5 cm in diameter, 1.5-cm low wall) to medium size (15 cm in diameter, 1.5-cm low wall) and super-size (30 cm in diameter, 1.5-cm low wall) and recorded the nest cell responses. As shown in Fig. 3B, when two new circular nests (which had the identical diameter in comparison with the home nest but made with a 1.5-cm wall by using different cardboard materials) were presented to the mouse, Cell #1 exhibited a robust increase in its firing regardless of whether the nests were placed on a small or

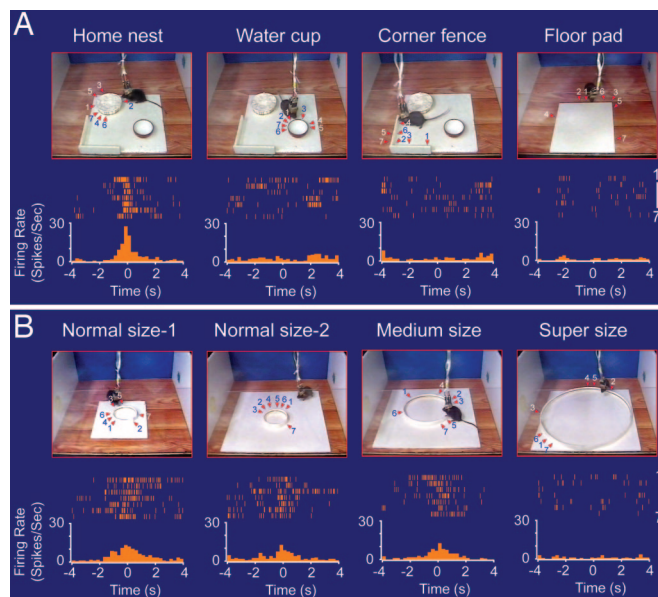
large floor pad (the left two images in Fig. 3*B*). Because the nests were newly constructed, this also allowed us to exclude the possibility that nest encounter-induced responses depended on odors associated with home nests. To further control the odor effects, we introduced these new nests to mice in novel environments, outside of their home environments. The fact that the cell continued to exhibit robust nest encounter-induced firing suggests that odor does not play any significant role. Moreover, this cell also exhibited significant firings when the animal encountered a medium-sized nest that had twice the diameter of the normal nest (the third image from the left in Fig. 3*B*). However, when the diameter of the nest was increased to approximately four times the size of the original, the cell no longer responded to this super-sized circular structure (Fig. 3*B* Right). Our metric analyses suggest that the size of the nest plays an important role in defining the nest cell's response.

Because the hippocampal cells are known to respond strongly to the startling episodes such as sudden air blows to the back of the animals or earthquake-like shakes of the mouse cages (17, 18), we also measured the effects of those episodic stimuli on the firing of those nest-responsive cells. Our analyses revealed that those nest-responsive cells did not change their firings in response to those startling events (SI Fig. 8*A*), whereas other simultaneously recorded CA1 cells in the same animals changed their firings (17, 18) (SI Fig. 8*B*).

In addition, we examined the response selectivity of those nest cells by introducing additional novel objects (e.g., toys) to the mice. We found that those cells did not alter their firing patterns when encountering those toys (SI Fig. 8*C*). Moreover, those cells also did not change their firings upon encountering food pellets, during food consumption and/or drinking-water behaviors (data not shown). Together, these experiments show that the nest-responsive cells do not respond to other external events or any other objects (e.g., the floor pads, toys, or corner fence).

**Invariant Responses over Physical Shapes, Appearances, and Construction Materials.** The fundamental feature of abstract concepts and generalized knowledge is that it should no longer ascribe to a specific type of shape and physical appearance of the objects. Therefore, we conducted a parametric analysis by constructing a set of new nests that were made from cardboard in several different geometric shapes. As exemplified by the perievent spike raster and perievent spike histograms shown in Fig. 4*A*, Cell #1 fired robustly during encounters with a circular nest (Fig. 4*A* Left), a square nest (Fig. 4*A* Center), and a triangular nest (Fig. 4*A* Right), suggesting that the cell's response is invariant over the geometric shapes of nests.

Furthermore, we introduced the mice to additional new nests that were made from distinct materials including tin can caps, plastic bottle caps, porcelain caps, and cotton. This cell still exhibited highly significant changes in its firing when the animal encountered a metal nest which has distinct contexture and color (Fig. 4*B* Left). Similarly, encounters with a plastic nest also triggered marked changes in its firing (Fig. 4*B* Center). In addition, the cell further exhibited strong responses to a porcelain nest (Fig. 4*B* Right). Interestingly, two kinds of natural cotton bedding, made from cotton balls by other mice, also triggered robust responses of the cell in this animal (Fig. 4*C* Left and Center). However, five cotton balls that were simply placed together in the similar geometric shape were incapable of eliciting the responses from the nest cell (Fig. 4*C*, Right). Together, the above experiments suggest that the responses of the nest cell remained invariant over the physical appearances, geometric shapes, design styles, colors, odors, and construction materials, thereby encoding highly abstract information about nests. The invariant responses over the shapes, styles, and materials were also observed in other nest cells. Several examples of invariant responses of Cell #2 (persistent-on type) and Cell



**Fig. 3.** The selectivity of the nest-responsive cells. (A) Although Cell #1 responded robustly to the home nest (7.5 cm in diameter and 2 cm wall height) (the first column on the left), it did not respond to other objects such as the water cup (the second column from the left), the corner fence (the third column from the left), and the edges of a floor pad (right column). Seven trials are shown for each spike histogram. (B) The responses of the same nest cell were sensitive to the size of the nest. Two normal-size nests (7.5 cm in diameter and 1.5 cm wall height) placed on two different-sized floor pads, a medium-size nest (15 cm in diameter and 1.5 cm wall height), and a super-sized nest (30 cm in diameter and 1.5 cm wall height) were tested. The arrows indicate the position at which the mouse encountered the nests or other objects. The time “zero” was set to the time point at which the tip of the mouse's nose crossed the edge of the nest. The perievent spike (top of each histogram) and perievent spike histograms (bottom of each histogram) are shown. The bin width in the perievent spike histogram is 250 ms.

#3 (persistent-off type) over shapes, appearances, color, and materials are shown in the SI Fig. 9*A* and *B*, respectively.

**Functionality-Based Abstraction.** To investigate whether nest cells might tune toward the fundamental features that would categorically define the functionality of nests, we built a nest that was buried in the ground rather than above ground (as in previous experiments). Interestingly, we found that the nest cells also exhibit significant responses to the underground nests. For example, both Cell #1 and Cell #2 increased their firing in response to the underground nests (Fig. 5*A* Top and Middle; also see SI Movie 1).

In addition, we placed a glass floor over a typical circular nest so that the functionality, but not the visual image, of the nest was blocked (Fig. 5*A* Bottom). Interestingly, under this glass floor scenario, the cell no longer changed its firing during the multiple crossings over the nest, as evident from both the perievent spike raster and perievent spike histogram. Thus, those experiments suggest that the cell's responses are tuned to the behaviorally determined nest function rather than viewing the mere visual images of nests through the glass floor. In another word, this form of conscious awareness of the presence of a nest is achieved by episodic, physical explorations.

Functionality-based responsiveness of the nest cells were further analyzed by our additional sets of experiments in which we used the same plastic bottle cap as the nest but presented it to the mice in two different ways: either in an inverted manner (so the cap would function as a small stage) or in the normal nest position (flipped on its back). Because it was the same cap and



Our elucidation of the functionality-based conceptualization of nests at the level of the hippocampus also provides experimental evidence for the existence of network-level encoding units capable of simultaneously extracting commonality and abstract features from various episodic encounters (17, 18). Unlike the face cells in the inferior temporal cortex which continue to respond to faces even under anesthesia (8, 36), the responses of the hippocampal nest cells depend on conscious awareness, because the nest cells would lose their firing selectivity once the mice were anesthetized or in sleep (data not shown). In addition, the fact that the mere visualization of the nest did not trigger nest cell responses, as shown by the glass-floor experiment (Fig. 5A Bottom), further shows that the hippocampal encoding of the abstract concept of nests requires episodic experiences and physical determination of the functionality of the objects. Currently, it is unclear as to the existence of nest-selective cells in higher cortical areas that might be capable of responding to the visual images of nests alone.

In conclusion, we report the identification of nest cells that encode functionality-based conceptual knowledge of nests or beds. Our study suggests that the hippocampus is an intrinsic part of the hierarchical architectures engaged in extraction, processing, and representation of abstract concepts and knowledge from daily behavioral experiences.

## Materials and Methods

**Construction of Recording Microdrives and Animal Surgery.** The 96-channel electrodes, consisting of two independently movable bundles of 48 stereotrodes or 24 tetrodes (48-channel on each side of the hippocampi), were constructed as described (18, 28). Two or 3 d before surgery, wild-type B6BCA/J mice were removed from the standard mouse cage and placed to the customized recording home environment (plastic bucket, 50 cm in diameter) in which a cardboard circular nest, a water cup, and food pellets were provided. On the surgery day, the electrode array was inserted at 2.0 mm lateral to the bregma and 2.3 mm posterior to the bregma on both right and left sides. The electrode bundles were advanced slowly toward the hippocampal

CA1 region at least 2 or 3 d after surgery, in daily increments of  $\approx 0.07$  mm until the tips of the electrodes had reached the CA1, as deduced from an assessment of high-frequency ripples, field potential, and neuronal activity patterns (18, 28). For details, see *SI Methods*.

**On-Line Search for Nest-Responsive Cells and Off-Line Spike Sorting.** Overall off-line spike sorting was carried out by using the MClust 3.0 and KlustaKwik 1.5 programs (36), and the details are the same as previously described in our recent publications (18, 28). We used the Boxes sorting method of Sort Client to achieve initial on-line sorting and classification and then off-line sorting again by using the MClust 3.0 and KlustaKwik 1.5 programs as described (18, 28, 29, 36). Histological staining, with (1% cresyl echt violet) was used to confirm the electrode positions. A total of 255 single units from seven mice were recorded and carefully examined for nest responsiveness. Of them, eight single units were identified to respond significantly to nests. The examples of waveforms and basic firing characteristics of the units are shown in the *SI Fig. 6*.

**Construction of Nests.** A variety of nests in different geometric shapes and sizes were made (see *SI Methods*). Behavioral explorations of nests were completely voluntary. All nests, other than the home nests as shown in the text (*SI Fig. 10*, the top two left images in the photos of nest construction), were new to mice and had not been represented to mice until the recording experiments. The functional categorization of these new objects as a nest was also confirmed by the observations that mice indeed slept inside those objects (data not shown).

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