The Reconfigurable Maze Provides Flexible, Scalable, Reproducible, and Repeatable Tests

Satoshi Hoshino, Riku Takahashi, Kana Mieno, Yuta Tamatsu, Hirotsugu Azechi, Kaoru Ide, Susumu Takahashi

stakahas@mail.doshisha.ac.jp

HIGHLIGHTS
The reconfigurable maze enables flexibly in building a variety of maze paths
The maze ensures reproducibility, repeatability, and scalability
The maze does not impair spatial navigational behavior or neuronal activity
Various tests of learning and memory can be conducted in a single environment

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The Reconfigurable Maze Provides Flexible, Scalable, Reproducible, and Repeatable Tests

Satoshi Hoshino,1 Riku Takahashi,1 Kana Mieno,1 Yuta Tamatsu,1 Hirotugu Azechi,1 Kaoru Ide,1 and Susumu Takahashi1,2,*

SUMMARY
Multiple mazes are routinely used to test the performance of animals because each has disadvantages inherent to its shape. However, the maze shape cannot be flexibly and rapidly reproduced in a repeatable and scalable way in a single environment. Here, to overcome the lack of flexibility, scalability, reproducibility, and repeatability, we develop a reconfigurable maze system that consists of interlocking runways and an array of accompanying parts. It allows experimenters to rapidly and flexibly configure a variety of maze structures along the grid pattern in a repeatable and scalable manner. Spatial navigational behavior and hippocampal place coding were not impaired by the interlocking mechanism. As a proof-of-principle demonstration, we demonstrate that the maze morphing induces location remapping of the spatial receptive field. The reconfigurable maze thus provides flexibility, scalability, repeatability, and reproducibility, therefore facilitating consistent investigation into the neuronal substrates for learning and memory and allowing screening for behavioral phenotypes.

INTRODUCTION
Several shapes of mazes such as the T-maze (Small, 1901; Dudchenko, 2001), plus maze (Olton and Feustle, 1981), radial arm maze (Olton and Samuelson, 1976; Olton et al., 1978), and figure-8 maze (Wood et al., 2000) have been designed as behavioral tests to assess the performance of working (Dudchenko, 2004), reference (Olton and Paras, 1979; Xu et al., 2019) and episodic-like memory (Babb and Crystal, 2005), and spatial navigation (O’Keefe and Nadel, 1978), as well as for studying anxiety (Walf and Frye, 2007). As individual tests of learning and memory, all have pros and cons (Andersen et al., 2009). To compensate for the disadvantages, evidence from a battery of maze tests is usually accumulated to understand specific learning and memory (Tecott and Nestler, 2004). However, each test is often conducted in a different real or virtual experimental room because conventional mazes cannot be easily rebuilt in a systematically arranged manner. The details of the maze structure, including shape, coordination, and dimensions, are crucial aspects of the test results, whereas the structures themselves cannot be precisely reproduced in different laboratories in a repeatable way. Thus, conventional maze tests lack reproducibility and repeatability.

Place cells found in the hippocampus encode an animal’s location and are deeply involved in spatial navigation ability (O’Keefe and Nadel, 1978). Place coding responds dramatically to changes in the surrounding environment (Muller and Kubie, 1987), suggesting that even if an animal experiences a single maze set inside different rooms, the hippocampus may generate different cognitive maps for each room, despite the mazes having identical shapes. On the other hand, conventional visual-based virtual reality (VR) systems can realize unlimited shapes of mazes in visual space. However, the lack of non-visual sensory feedback in passive VR results in altered neuronal responses. Furthermore, it forces animals to be partially or fully immobilized because one of the best applications of VR is to monitor neuronal activity from two-photon calcium imaging during the spatial navigation of head-fixed animals. For instance, a fraction of place cells reduced their firings in the spatial receptive field apparently because of the loss of vestibular feedback in the VR (Aronov and Tank, 2014). These altered neuronal responses impede detailed interpretations of the learning and memory mechanisms involved in maze tests.

Here we develop a maze system that overcomes most of these limitations by allowing the reconfiguration of the shape of the maze in a single physical environment. We demonstrate the use of the reconfigurable maze system to replicate existing mazes, including the T-maze, plus maze, W-maze, figure-8 maze, and radial arm maze. We also investigate the spatial navigational behavior and coding of the hippocampal

1Laboratory of Cognitive and Behavioral Neuroscience, Graduate School of Brain Science, Doshisha University, Kyotanabe City, Kyoto 610-0394, Japan
2Lead Contact
*Correspondence: stakahas@mail.doshisha.ac.jp
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place cells of rats to address concerns over possible distortions caused by the interlocking mechanisms and perform a novel experiment that morphs the shape of the maze. Together, these contribute to deciphering the neuronal underpinning of spatial navigation.

RESULTS
Implementation of the Reconfigurable Maze
We developed a maze system that enables the reconfiguration of the shape of the maze in a single physical environment using interlocking runways. Figure 1 demonstrates the existing standard mazes configured by our maze system in an enclosure, namely, the T-maze, W-maze, figure-8 maze, plus maze, and radial arm maze. The runways are each placed atop towers with baseplates (Table S1); these baseplates have protrusions that connect to a grid of holes in a floor-based breadboard (Figure 1F). The insertion of the protrusions into the breadboard connection enables coordination with the grid pattern and minimizes the swinging of the runways resulting from the movement of the animal (Figure 1G). Similarly, an array of accompanying parts, including feeders, movable walls, and treadmills placed atop towers of their own, can also be attached on the breadboards. Thus, the feeder can be placed by the side of any runway to change the reward location (Figure 1H, arrows). The movable wall can be placed at any of the interlocking gaps between runways as a dead end to dynamically control possible running paths (Figure 1H, arrowheads), and any runway can be replaced by the treadmill (Figure 1H, double arrowheads). Moreover, the shut-off sensor can be attached alongside any runway to signal a triggering event to the feeders and treadmills (Figure 1H, dashed arrows). Placing these interlocking parts onto the grid pattern enables the experimenters, even if they are not familiar with the maze, to precisely reproduce a variety of coordinated patterns of mazes for a brief period in a repeatable manner in a single physical environment. The maze also provides a scalable experimental setup because the complexity and the area of the maze is incrementally expandable by adding extra parts. When the morphing of a maze from square to cruciform was timed the time it took beginners to assemble a maze was not significantly different from the assembly time of experts who had used the reconfigurable maze daily over 3 months (3 experts: 131 ± 9 s; 3 beginners: 158 ± 12 s [mean ± SEM]; Figure 1J, Video S1, S2, S3, S4, S5, and S6). Runway sections with tall sidewalls (35 cm height) for reducing fear can be placed to test anxiety (Figure S1). Instead of runways, open platforms can also be embedded into the maze, which enables the plus maze and radial arm maze to be configured (Figures 1D and 1E). Furthermore, the scheduler for the timing of sensors and actuators installed in the accompanying parts allows the experimenter to design several behavioral tasks, even within a single maze shape. Such flexible maze design also allows experimenters to rapidly select the tasks best matched to the needs of a particular experiment during preliminary studies. In accordance with this flexible design principle, we also developed a small version of the reconfigurable maze system for mice (Figure 1I, Table S2). Using the mouse version, we could also configure existing standard mazes, namely, the T-maze, W-maze, figure-8 maze, and plus maze (Figure S2). The assembly time for morphing a maze shape from square to T between experts and beginners was not significantly different (3 experts: 204 ± 15 s; 3 beginners: 186 ± 11 s [mean ± SEM]; Figure 1K), further demonstrating the usability of our system for screening behavioral phenotypes in mice.

Interlocking Gaps Do Not Alter Navigational Behavior
To manufacture the interlocking parts at a reasonable cost or by hand, a short gap between runways (~1 cm) (Figure 2A) is a prerequisite margin for the parts to interlock. Ideally, perfectly manufactured parts with a precision under 1 mm would not require these gaps as margins; however, such high-precision manufacturing is expensive and does not permit the use of low-precision handcrafted parts. When the gaps are not included as a margin, runways manufactured with lower precision (>1 cm) will overlap if the location of the runway is only slightly shifted, as shown in Figure S3.

We next answered the question of whether this gap affects animal behavior. Four rats were trained to smoothly run along a square-shaped maze in a clockwise direction. The running speed at the gaps did not abruptly change from the running speed between gaps (Figure 2B). Incidences of abrupt changes in the head direction were significantly lower at the gaps than between gaps (Figure 2C). These results suggest that the gaps did not distort normal rats’ behaviors. To corroborate the result, we prepared two gapless runways the total lengths (149 cm long) of which were the same as those of the three interlocking runways, including the gaps between the sections. We then replaced three runway sections at the top or bottom of the square-shaped maze with a single runway without gaps (Figure S4A). Five rats were trained to run on the mazes, with or without gaps, over a 20-min interval. Both running speed and head direction on
Figure 1. The Reconfigurable Maze

For a Figure360 author presentation of this figure, see https://doi.org/10.1016/j.isci.2019.100787.

(A–E) The maze can be configured to form T (A), W (B), figure-8 (C), plus (D), and radial arm (E) mazes in a single enclosure.

(F) Runway sections are placed atop towers.

(G) The baseplate of each tower has four protrusions that coordinate the placement of the section on the breadboard in a flexible, repeatable way.

(H) Configured plus maze for rats with two feeders (arrows), two movable walls (arrowheads), one treadmill (double arrowhead), and two shut-off sensors (dashed arrows).

(I) Small version of the reconfigurable maze for mice, configured as a figure-8 maze with two feeders (arrows), two movable walls (arrowheads), one treadmill (double arrowhead), and two shut-off sensors (dashed arrows).

(J) Assembly time of the rat version of the reconfigurable maze for morphing the shape from square to cruciform. Performance improved with consecutive trials in a day (performance versus trial: $F_{2,8} = 7.453, p = 0.0149$), but not with expertise (expert/beginner) (performance versus expertise: $F_{1,4} = 5.654, p = 0.0762$). There was no significant interaction between expertise and experience in a day (expertise versus trial: $F_{2,8} = 0.320, p = 0.735$). Two-way mixed ANOVA was used.

(K) Assembly time of the mouse version of the reconfigurable maze for morphing the shape from a rectangle to a T. There was no significant difference between performance and consecutive trials in a day (performance versus trial: $F_{2,8} = 3.997, p = 0.0625$) or expertise (expert/beginner) (performance versus expertise: $F_{1,4} = 0.351, p = 0.58$). There was no significant interaction between expertise and experience in a day (expertise versus trial: $F_{2,8} = 0.658, p = 0.543$). Two-way mixed ANOVA was used.
the gaps were not significantly different from those on the corresponding portions of the single runway without gaps (Figures S4B and S4C).

As shown from the trajectory (Figure 2A) and abrupt changes in the head direction (Figure 2C), the rats appeared to frequently exhibit exploratory behaviors at preferred locations (Hu and Amsel, 1995). To examine whether such exploration is preferentially observed at gap locations, four rats were trained to run in a clockwise direction on a square-shaped maze configuration. All rats sometimes paused and exhibited
exploratory behaviors around food dispensers or corners. Indeed, the occupancy time when the rats visited the gap location around a food dispenser (gap #7) was significantly longer than the occupancy time on two other gaps (gaps #3 and #10) (Figure 2D), suggesting that unidentified factors, excluding the gap presence, might also affect rats’ behaviors around food dispensers or corners. In addition, it is well known that such exploratory behaviors modulate hippocampal neuronal activity (Gauthier and Tank, 2018). Thus, to exclusively examine the gap effects, we focused on the gaps (#3, #4, #9, and #10) between the three interlocking runways arranged in a straight line on the top and bottom sides of the square-shaped maze in the subsequent analyses.

Interlocking Gaps Do Not Alter Hippocampal Place Coding
To determine whether the gaps distort neuronal activity in terms of spatial navigation, we monitored the activity of 236 neurons from the hippocampal CA1 of both hemispheres of four rats running on the square-shaped maze in a clockwise direction (Figure 3A, Table S3). The average width of place fields of 62 place cells on the gaps settled between the 5th and 95th percentiles of distribution for shuffled data from the entire set of place fields (Figure 3B). Moreover, the number of place fields from the 62 place cells and the firing rate of multiunit activity in the hippocampal CA1 on the gaps did not change when compared with recordings taken between the gaps (Figures 3C and 3D). At the ensemble level, the trajectory decoded from the activity of simultaneously monitored place cells using a memoryless Bayesian decoder (Zhang et al., 1998) depicted a seamless path even on the gap locations (Figures 3E and 3F). Thus, both behavioral and neuronal responses at the gaps support the view that rats behave as if they perceive the interlocking gap and gapless portions on the runways similarly.

Rats and Mice Can Learn Spatial Alternation Tasks in the Reconfigured Maze
After the five rats became familiar with the square-shaped maze, the maze shape was morphed from square to figure-8 using the reconfigurable maze system. The rats were trained to alternate between left and right at the branchpoint of the central stem for 1 h per day over 5 days (Figure 4A). All rats gradually learned the spatial alternation task (Figure 4C). They achieved a mean score of greater than 80% correct choices over 50 consecutive trials on the second day. To demonstrate the usability of the attachable walls and treadmills, three rats were then tested on a delayed version of the spatial alternation task by partially reconfiguring the maze: a runway in the center of the stem in the figure-8-shaped maze was replaced by a treadmill (Figure 4B). To force the rats to run on the treadmill for a 7-s delay period, movable walls were set at the gaps in front and behind the treadmill. The rats achieved a mean score of greater than 70% correct choices over 50 consecutive trials at the beginning, and their performance improved after 10 days of testing (Figure 4D).

After three mice were familiar with the linear track and rectangular maze in the mouse version of the reconfigurable maze, the maze shape was morphed to a T-maze (Figures 4E and 4F). The mice were trained to alternate between left and right at two branch points for 1 h per day over 5 days. All mice gradually learned the spatial alternation task (Figure 4G). They achieved a mean score of greater than 75% correct choices over 20 consecutive trials on the second day. These results and maze reconfigurations demonstrate that the reconfigurable maze system can consistently replicate the existing standard mazes and serve as tests of spatial learning and memory.

Place Field Remapping during the Maze Morphing Experiment
Several lines of evidence suggest the hippocampal place coding partially or completely changes in response to external or internal cues (Muller and Kubie, 1987; Leutgeb et al., 2005). This phenomenon is called the remapping of place fields. To demonstrate the repeatability and reproducibility of our reconfigurable maze system over existing mazes, we provide here a novel morphing experiment to examine the remapping of place fields between different shapes of a maze with an identical path length when the maze shape morphs from square to cruciform to square. As the cruciform maze is an internal structure of the square maze, the total path length does not change during morphing. Moreover, the four outermost runways of the cruciform maze physically overlap with the path of the square-shaped maze. As our maze realizes the morphing in one enclosure, it enables the identification of factors influencing hippocampal place coding: path integration and spatial reference frame (Figure 5A).

To examine the remapping of the place fields, we examined 129 place cells monitored from the dorsal hippocampal CA1 of four rats. The total path length of the runway and available external landmarks in the
Figure 3. Interlocking Gaps Do Not Alter Hippocampal Place Coding

(A) Left, normalized firing rate map of two representative place cells that have place fields around gap locations in the hippocampal CA1 on the square-shaped maze configuration. Right, normalized firing rate maps of 65 hippocampal place cells simultaneously monitored from a rat (CA) ordered by the latency of their peak firing rates. Each line is a single unit. Gap locations are indicated at the top. Red indicates maximum firing rates, and blue indicates silent.

(B) Distribution of the average width of place fields randomly shuffled from the original sample covering the gaps (3,000 shuffles). Red line indicates the average width of the place fields on the gaps. Dotted lines indicate 5th and 95th percentiles for the shuffled data.

(C and D) Left, the number of place fields (C) and the firing rate of multi-unit activity (MUA) in the hippocampal CA1 (D) as a function of linearized location on the square-shaped maze. Right, the average number of place fields (C) (z = 0.14, p = 0.89) and the average firing rate of MUA (z = -1.51, p = 0.13) on the gaps (#3, 4, 9, 10) (green shaded area) and between them (orange shaded area).

(E) Representative posterior probability of locations decoded by the Bayesian decoder from 89 simultaneously monitored cells. Values are indicated by the color bar (right).

(F) Left, the difference between actual and decoded locations as a function of linearized location on the square-shaped maze. Right, the average difference between actual and decoded locations on the gaps (#3, 4, 9, 10) (green shaded area) and between them (orange shaded area) (z = 1.34, p = 0.18). Bin width is set at 3 cm.

All error bars indicate SEM. All were from two-tailed Wilcoxon rank-sum test, n.s.: p > 0.05.
mazes were nearly identical across maze shape morphs, whereas the spatial correlation of the firing rate map of place cells within the overlapping runways in the square-shaped maze between the first and second exposures was significantly larger than that between the square-shaped maze and the cruciform maze (Figures 5B and 5C). This suggests that the maze shape can be a cue capable of inducing remapping of the location of place fields as a spatial reference frame. Moreover, the difference in the maximum firing rate of place fields within the overlapping runways in the square-shaped maze between the first and second exposures was similar to that between the square-shaped maze and the cruciform maze (Figures 5Ba and 5D). These results suggest that maze morphing from square to cruciform is represented by a difference in place field locations without a change in place field firing rate.

**DISCUSSION**

We demonstrate our reconfigurable maze system’s ability to provide flexible, scalable, repeatable, and reproducible tests by configuring standard existing mazes in a single real environment and by examining spatial navigational behavior and neuronal activity during learning and memory performances in the mazes. Our maze system has the potential to become an invaluable, scalable tool for the study of learning and memory, including working and reference memory, spatial navigation, and decision-making, as well as anxiety. Using the reconfigurable maze, experimenters can consistently replicate a variety of mazes in their...
Currently, a variety of maze tests are routinely used to gain a greater understanding of learning and memory in animals and for screening behavioral phenotypes in animal models of diseases. Although evidence is being accumulated, it is not necessarily available to contribute to our understanding of learning and memory mechanisms in the brain, because the underlying neuronal signatures are not necessarily similar, even when identical maze shapes are used across different conditions. For instance, if a behavioral phenotype for spatial memory deficits emerged in two maze tests, each conducted in a different room, the tests may only specifically show the remapping of hippocampal place coding across the different rooms, as demonstrated in the maze morphing experiment of the present study. Indeed, recent studies suggest that place cell activity sequences are linked to future planning (Pfeiffer and Foster, 2013) and episodic-like memory retrieval (Takahashi, 2015), as well as neurodegenerative diseases (Cheng and Ji, 2013). Therefore, such neuronal dissociations should be minimized in a comparative study across maze tests. Place cells show an irregular firing pattern in passive VR experiments (Aronov and Tank, 2014). Therefore, we designed our maze based on the concept that various shapes can be constructed in a single real environment, where several types of learning and memory tests can be performed. Recently developed active VR can markedly minimize such dissociations, specifically for proximal cues (Aronov and Tank, 2014; Stowers et al., 2017).
Although our maze system and the active VR are not exclusive, the combination will enable the production of unlimited experimental situations without the neuronal distortions arising from both proximal and distal cues in the near future.

Our reconfigurable maze system is compatible with techniques for monitoring neuronal activity such as extracellular multiple single-unit recording (Wilson and McNaughton, 1993) and single-photon calcium imaging (Ziv et al., 2013) for freely behaving rats or mice. As demonstrated by the remapping of place fields across morphed mazes, the combination must be accelerated to allow understanding of the neuronal underpinning of learning and memory. Furthermore, our maze enables rapid prototyping of tasks by reconfiguring the coordination of the parts, including runways, rewards, and obstacles, within a few minutes, accelerating studies on learning and memory.

The reconfigurable maze is thus a promising maze platform for testing the performance of learning and memory, including working, reference and episodic memory, decision-making, spatial navigation, and anxiety, as well as for screening behavioral phenotypes of mice, including transgenic models of disease.

Limitations of the Study
The initial setup cost and laboratory space for introducing the reconfigurable maze may be barriers to widespread distribution. However, the scalable features minimize these barriers. For instance, a minimal setup realized by removing optional modules from the full configuration (demonstrated by a square-shaped maze without movable walls or shut-off sensors) will reduce the cost. In addition, the modular architecture allows the configuration of small mazes to fit limited laboratory space. Although the minimal setup may not be a compelling reason for laboratories to invest in the reconfigurable maze, the complexity can be expanded incrementally by adding supplementary parts. Moreover, the setup and validation for novel configured mazes within a laboratory may be time consuming. Provided that the reconfigurable maze will be widely distributed and standardized, the overall cost will be lowered and information resources describing how to set up new configurations of the maze and validate their performance for specific aims will be shared between researchers. In this way, implementation of the reconfigurable maze will ensure reproducibility and repeatability.

Despite its flexibility, reproducibility, repeatability, and scalability, our maze has a few limitations. Although oblique runways at an angle of 45° could be embedded into our maze as demonstrated by the configured radial arm maze (Figure 1E), the angle cannot be flexibly modified because the coordinated grid assignment of the parts requires the runways to be placed at a fixed angle. Unlike the recently developed honeycomb maze (Wood et al., 2018), the tower supporting the runway cannot be raised and lowered automatically. Although adjustable mechanisms are capable of overcoming those limitations, they increase the damage rate of the physical system during behavioral and neuronal recordings, reducing the major advantage of reproducibility and repeatability.

METHODS
All methods can be found in the accompanying Transparent Methods supplemental file.

DATA AND CODE AVAILABILITY
The detail of the maze parts is freely available (https://github.com/TakahashiLab/ReconfigurableMazeParts). The controlling software is also freely available (https://github.com/TakahashiLab/ReconfigurableMazeGUI).

SUPPLEMENTAL INFORMATION
Supplemental Information can be found online at https://doi.org/10.1016/j.isci.2019.100787.

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AUTHOR CONTRIBUTIONS
S.T. conceived the project. S.H. developed control software. S.H., K.M., R.T., and Y.T. performed behavioral experiments. H.A. and K.I. performed the histological verification. S.H., H.A., and K.I. made apparatus. S.H.
performed electrophysiological experiments. S.H. and S.T. performed surgery, data analyses, and wrote the manuscript.

DECLARATION OF INTERESTS
S.T. is an inventor on Japanese unexamined patent application publication (No. P2019-115315A, applicant: Doshisha University) pertaining to the reconfigurable maze. All remaining authors declare no conflict of interest.

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Supplemental Information

The Reconfigurable Maze Provides Flexible, Scalable, Reproducible, and Repeatable Tests

Satoshi Hoshino, Riku Takahashi, Kana Mieno, Yuta Tamatsu, Hirotsugu Azechi, Kaoru Ide, and Susumu Takahashi
Figure S1. Top view of configured mazes, Related to Figure 1. (a–e) Top view of maze shape configured to T (a), W (b), figure-8 (c), plus (d), and radial arm (e) mazes in an enclosure. This view demonstrates that several shapes of the maze can be configured in the same space. (f) The plus maze configured with tall sidewalls for testing anxiety.
Figure S2. The reconfigurable maze for mice, Related to Figure 1. (a–d) The shape was configured to match T (a), W (b), figure-8 (c), and plus (d) mazes in an enclosure. (e–h) Top view of the mazes. Note that the central stem remains in the same position. (i–l) Example running trajectory of a mouse in each maze. Scale bar, 40 cm. (m) The runway is placed atop a tower on a breadboard with a grid of holes. (n) The baseplate of the tower is fixed by a bolt because the weight of the baseplate alone is too light to support the runway. The grid of the breadboard allows the flexible coordination of the runways and accessory parts in repeatable ways.
Figure S3. A short gap between runways is a prerequisite margin for the interlocking parts, Related to Figure 1. (a) Close-up photo showing the gap. (b) The runway (gray) is placed atop a tower (blue) on a breadboard (brown) with a grid of holes. The baseplate of the tower has protrusions (red) that coordinate the placement of the section on the breadboard. (c) Top view of a runway on the breadboard. (d) Two runways with a 1 cm gap. (e) This view demonstrates that the runways overlap only if the total dimensional error exceeds a precision of 1 cm.
Figure S4. The running speed and head direction on the runway with or without gaps, Related to Figure 2. (a) Schematic showing two gapless runways placed at the top and bottom of the square-shaped maze. R1 and R2 indicate food dispensers. (b–c) The running speed (b) and head direction (c) of five rats on the runways with gaps (solid line) and without gaps (dotted line) as a function of the examined gap locations. The data from individual animals are overlapped as color-coded dots. Neither running speed nor head direction were influenced by the presence of a gap (simple main effect: gap presence vs. running speed between all pairs of the examined gaps: $P > 0.05$, gap presence vs. head direction between all pairs of the examined gaps: $P > 0.05$). However, there were interactions between gap presence and location (running speed: $F_{1,4} = 2653.9$, $P < 10^{-6}$; head direction: $F_{1,4} = 302.3$, $P < 10^{-4}$) shown using two-way repeated-measures ANOVA. Error bars indicate SEM.
Table S1. Dimensions of the runway for the rat version of the reconfigurable maze, Related to Figure 1.

| Runway type         | Combination          |
|---------------------|----------------------|
| Straight            | A, B, C, D, E        |
| Right               | C, F, G, H, I, J, K, L, T |
| Left                | C, F, G, H, I, J, K, L, T |
| Central             | C, F, H, I, J, K, L, T |
| S-shaped            | H, M, N, O, P        |
| Right and Left      | C, Q, R, S, T        |
| Octagonal field     | W, X, T              |
| Long straight       | C, U, V, T           |

| Basic component | Polygon type | Depth (mm) | Width (mm) | Height (mm) |
|-----------------|--------------|------------|------------|-------------|
| A               | Rectangle    | 5          | 490        | 100         |
| B               | Rectangle    | 5          | 490        | 45          |
| C               | Rectangle    | 2          | 100        | 5           |
| D               | Rectangle    | 5          | 100        | 5           |
| E               | Rectangle    | 5          | 440        | 10          |
| F               | Non-regular  | 5          | 485        | 100         |
| G               | Rectangle    | 5          | 485        | 45          |
| H               | Rectangle    | 5          | 100        | 45          |
| I               | Rectangle    | 5          | 260        | 45          |
| J               | Rectangle    | 10         | 480        | 15          |
| K               | Non-regular  | 10         | 480        | 15          |
| L               | Non-regular  | 2          | 230        | 10          |
| M               | Non-regular  | 5          | 480        | 100         |
| N               | Rectangle    | 5          | 305        | 45          |
| O               | Non-regular  | 10         | 480        | 15          |
| P               | Non-regular  | 2          | 180        | 10          |
| Q               | Non-regular  | 5          | 490        | 100         |
| R               | Non-regular  | 2          | 330        | 10          |
| S               | Non-regular  | 10         | 450        | 15          |
| T               | Rectangle    | 5          | 100        | 10          |
| U               | Rectangle    | 5          | 685        | 100         |
| V               | Rectangle    | 5          | 100        | 10          |
Runways can be assembled using a combination of basic components (A-X). All 3D models are freely available (https://github.com/TakahashiLab/ReconfigurableMazeParts).

| Runway type          | Combination          |
|----------------------|----------------------|
| Straight             | A, B, C, D           |
| Right                | A, B, C, D, G, H, I  |
| Left                 | A, B, C, D, G, H, I  |
| Right and Left       | A, C, D, G, H, I     |
| Central              | A, C, D, E, F        |

Table S2. Dimensions of the runway for the mouse version of the reconfigurable maze, Related to Figure 1.

| Basic component | Polygon type | Depth (mm) | Width (mm) | Height (mm) |
|-----------------|--------------|------------|------------|-------------|
| A               | Rectangle    | 3          | 391        | 40          |
| B               | Rectangle    | 3          | 391        | 28          |
| C               | Rectangle    | 10         | 40         | 10          |
| D               | Rectangle    | 3          | 40         | 5           |
| E               | Rectangle    | 3          | 140        | 28          |
| F               | Rectangle    | 3          | 115        | 5           |
| G               | Rectangle    | 3          | 40         | 15          |
| H               | Rectangle    | 3          | 80         | 5           |
| I               | Rectangle    | 3          | 311        | 28          |

Runways can be assembled using a combination of basic components (A-I). All 3D models are freely available (https://github.com/TakahashiLab/ReconfigurableMazeParts).
Table S3. Variability between rats: electrophysiological measurements, Related to Figure 5.

| RAT #       | ζA | δB | δA | εA | Total |
|------------|----|----|----|----|-------|
| No. of pyramidal cells | 89 | 40 | 46 | 35 | 210   |
| No. of interneurons    | 8  | 4  | 9  | 5  | 26    |

Analyses for the square-shaped maze for gap effects

| No. of place cells (spatial information > 0.1 bit/spike & peak firing rate > 1 Hz) | 28 | 18 | 14 | 2  | 62   |
| Spatial information (bits/spike, mean ± sem) | 0.41±0.05 | 0.30±0.03 | 0.66±0.13 | 0.22±0.06 |
| Unit isolation quality (isolation distance, Median ± QD) | 8.3 ±5.5 | 5.9 ±12.2 | 14.1 ±8.7 | 6.4 ±2.7 |

Analyses for the maze morphing from square to cruciform to square

| No. of place cells (spatial information > 0.1 bit/spike & peak firing rate > 1 Hz in any of three situations) | 60 | 24 | 28 | 17 | 129  |
| Spatial information (bits/spike, mean ± sem) | 0.67±0.11 | 0.24±0.03 | 1.06±0.17 | 0.96±0.34 |
| Unit isolation quality (isolation distance, Median ± QD) | 8.3 ±5.6 | 6.7 ±6.2 | 6.9 ±8.0 | 6.8 ±3.6 |

(*) The number of cells meeting the criteria had a large difference between the two conditions because some cells met the criteria either in the square-shaped maze or in the cruciform maze during morphing.
Supplemental Video legends

Supplemental Video 1. Morphing of a maze from square to cruciform by S.H. (expert), Related to Figure 1.

Supplemental Video 2. Morphing of a maze from square to cruciform by K.M. (expert), Related to Figure 1.

Supplemental Video 3. Morphing of a maze from square to cruciform by R.T. (expert), Related to Figure 1.

Supplemental Video 4. Morphing of a maze from square to cruciform by K.I. (beginner), Related to Figure 1.

Supplemental Video 5. Morphing of a maze from square to cruciform by H.A. (beginner), Related to Figure 1.

Supplemental Video 6. Morphing of a maze from square to cruciform by S.T. (beginner), Related to Figure 1.
Transparent Methods

Maze system implementation. The reconfigurable maze consists of interlocking runways (49 cm × 10 cm for rats, see Table S1; 39 cm × 5 cm for mice, see Table S2) and an array of accompanying parts including feeders, movable walls, shut-off sensors, and treadmills. Each runway is placed atop a tower mounted on a breadboard with a grid of holes (hole-to-hole spacing: 25 mm for rats, 25 mm for mice), that enables each section to be mounted independently of other runway sections. For rats, each runway made of 5 mm thick black polyvinyl chloride (PVC) (matte finish) is 55 cm above the breadboard (Table S1). For mice, 3 mm thick gray PVC is 34 cm above the breadboard (Table S2). The tower and its baseplate are made of aluminum. For rats, the baseplate has four protrusions that can be inserted into the holes of the breadboard to attach it. For mice, a bolt was inserted into the holes because the weight of the baseplate was too light to support the runway. Sidewalls (45 mm height for rats; 30 mm height for mice) around the top of the runway prevent the rats and mice from slipping off the runway. The elevated runways prevent the rats and mice from jumping out of the maze. The maze sits within a shielded enclosure (4 m × 5 m for rats; 1.8 m × 3.0 m for mice) covered by a copper mesh. All metal parts are grounded to reduce electrical artifacts in the electrophysiological recording.

An Arduino Mega controller was used to receive signals from shut-off sensors and to send activation signals to the actuators in the treadmills and feeders according to the user-defined sensor and actuator schedule. Custom-made scheduling software written in Matlab was used to monitor the location of rat or mouse via shut-off sensors and to control the actuators in the treadmills and feeders, which enables the feeders to be turned on and off according to the location of rats or mice.

Animals. Ten Long–Evans rats and four C57BL/6J mice purchased from Shimizu Laboratory Supplies, Co. Ltd. (Kyoto, Japan) were housed individually in cages (20 × 25 × 23 cm for rats, 14 × 21 × 12 cm for mice) where the light was maintained on a 12-hour light/12-hour dark schedule with the light phase starting at 8:00 am. The tests were performed in the light phase. The weight of all rats or mice was kept at 80% of free-feeding body weight. To examine hippocampal place coding on the maze, a custom-made microdrive was implanted into the dorsal hippocampal CA1 of both hemispheres (eight tetrodes each) of four rats to record multiple single-unit activities. All procedures were approved by the Doshisha University Institutional Animal Care and Use Committees.

Surgery, electrode preparation, and recording. Under isoflurane anesthesia, a custom-made microdrive with 16 independently movable tetrodes was fixed to the skull above the
hippocampus of both hemispheres (eight tetrodes each; AP 3.8 mm, ML 3.0 mm, DV 0.5–1.0 mm) of four rats (Table S3). After surgery, the electrodes were individually lowered into the pyramidal cell layer of the dorsal hippocampal CA1. The extracellular signals were amplified, buffered, digitized, and continuously sampled at 25 kHz using two 32-channel RHD2000 chips (Intan Technologies, Inc., CA) via a motorized commutator (AlphaComm-I; AlphaOmega Inc., Israel). The spikes and local field potential (LFP) were digitally filtered at 800–7.5 kHz and 0.1–200 Hz, respectively. The occurrence of sharp-wave ripple events in the LFP during immobility periods was used to estimate the pyramidal cell layer. After spike sorting using KlustaKwik, putative principal cells were distinguished from fast-spiking cells based on average firing rate (5 Hz). We defined place cells if the following criteria were met: the overall firing rate was >0.1 Hz, spatial information was > 0.1 bit/spike, and maximum firing rate was >1.0 Hz.

Behavioral training. All rats and mice were food-restricted and reduced to 80% of their ad libitum body weight over a two-week period before training. During this time, they were handled daily. To train rats or mice to obtain pellets from the food dispenser, they were initially placed on a box (48 cm × 24 cm, 32 cm height for rats; 34 cm × 24 cm, 19.5 cm height for mice) with the food dispenser at a corner. Rats. The rats were trained on L, C, or G-shaped mazes configured by the reconfigurable maze system in the testing enclosure where they were habituated to the sounds made by the movable walls being raised and lowered. The rats performed a small, square-shaped maze task (overall: 120 cm × 49 cm) in which they ran in a clockwise direction to obtain a pellet. The training lasted until the rat learned to obtain at least one pellet per minute within a 25-minute experimental period. Next, the rats trained to run in a clockwise direction in a large square-shaped maze (overall: 170 cm × 148 cm) to obtain pellets from two food dispensers located at the left and right sides of the maze. The training lasted for at least 25 minutes per day and continued until the criteria of at least one trial per minute was achieved over one week. After the initial training, rats were trained to perform in the morphing experiment and the spatial alternation tasks described below.

Mice. Unlike the rats, the mice were trained on a linear track with a movable wall where they obtained food pellets at both sides and were habituated to the sounds of the movable wall. The mice performed a rectangle-shaped maze task (overall: 49 cm × 80 cm). The training lasted until they learned to obtain at least one pellet per minute for 25-minute intervals. After the initial training, they were trained to run on the figure-8 shaped maze, double T-maze, plus maze, and W-maze to obtain food pellets.

Morphing experiment. Four rats were trained to run in a clockwise direction on the maze morphing from square to cruciform to square. They ran within ~1 hour in the following sequence:
square maze, cruciform maze, and square maze. The 15-minute long sequences were spaced at
~5-minute intervals, and the rats were rewarded each time they arrived at the food dispensers
located at both the left and right sides of the maze. Each maze morphing was done within
approximately 5 minutes. The unit recording was made after the rats had experienced the maze
morphing over a few days.

**Spatial alternation task.**

Rats. Five rats were trained to alternate between left and right at a decision point on the figure-
8 shaped maze until they achieved an 85% correct rating for 25 minutes. A delay period was then
incorporated. During the delay period, rats were locked between two movable walls in front and
behind a treadmill. The treadmill rotated at a constant speed (~20 m/min) during the delay period.
The delay period was incremented from 1 sec to 7 sec every testing day.

Mice. Three mice were trained to alternate left and right at two decision points on the double T
maze until they achieved a 75% correct rating for an hour (Figure 4g).

**Animal trajectory and head direction.** The tip and root of the head were tracked from images
captured at 50 or 100 frames per second by a USB3.0 digital video camera mounted on the ceiling
of the enclosure using *DeepLabCut* (Mathis *et al.*, 2018). Initially, 200 annotated images were
used to train the pre-trained ResNet-50 network using transfer learning. A few additional iterations
of training were then performed with the goal that all Euclidian distances between tracked
locations in adjacent frames would be under 50 pixels. Running trajectory was reconstructed by
concatenating the tracked root of the head. Head direction was computed from the tip and root of
the head by the inverse of the tangent function.

**Analyses.** The rat’s trajectory was linearized for each trial by projecting the actual trajectory onto
a predefined idealized trajectory using nearest-neighbor Delaunay triangulation. Spatial bins had
a resolution of approximately 3 cm.

Rate map. A firing rate map of well-isolated neurons was constructed in a standard manner by
dividing the total number of spikes in a bin (3 cm \( \times \) 3 cm) at a given location by the total amount
of time that the rat has been in that bin. Each value was smoothed with a Gaussian filter with a
variance of three.

MUA. MUA was calculated by summing the firing of all monitored cells including low-firing cells
and fast-spiking cells.

Spatial information. The spatial information (bits per spike) was used to measure how much
information a spike conveys about the rat’s location on the maze (Skaggs *et al.*, 1993). This is
calculated by the following formula:
where \( i \) indexes over the position bins, \( P_i \) is the probability that the rat was in bin \( i \), \( R_i \) is the mean firing rate in bin \( i \), and \( R \) is the overall mean firing rate. On the basis of the spatial information, we identified the place cells.

Spatial and rate similarity measures. The spatial similarity is expressed by calculating the spatial correlation of place fields between the spatially overlapping paths of the square and cruciform mazes. The difference in firing rates was expressed by calculating a difference/sum score (Leutgeb et al., 2005). The unsigned difference between the two maximum firing frequencies was calculated, and the difference was divided by the sum of the two rates to obtain the score for a set of conditions during maze morphing. Both similarities were also expressed using the cumulative distribution function, \( f(X < x) \), which gives the probability that the variable \( X \) will be \( X < x \) for each real number \( x \).

Bayesian decoding. A memoryless Bayesian decoder (Zhang et al., 1998) was used to decode the rat’s locations on the basis of place cell activity. First, the probability of an rat’s location given place cell firings within a time window was estimated as follows:

\[
\text{Prob}(\text{Pos} | \text{spikes}) = \left( \prod_{i=1}^{N} f_i(\text{Pos})^{n_i} \right) \exp \left( -\sum_{m} \sum_{k=1}^{M} \frac{\text{Prob}(\text{Pos}_k | \text{spikes})}{\sum_{k=1}^{M} \text{Prob}(\text{Pos}_k | \text{spikes})} \right)
\]

where \( f_i \) and \( n_i \) represent the place map and the number of spikes of the \( i \)-th place cell within the time window, respectively, \( N \) indicates the total number of place cells, and \( \tau \) represents the duration of the time window.

The probability within each time window was normalized for every location as follows (Pfeiffer and Foster, 2013):

\[
\text{nProb}(\text{Pos} | \text{spikes}) = \frac{\text{Prob}(\text{Pos}_k | \text{spikes})}{\sum_{k=1}^{M} \text{Prob}(\text{Pos}_k | \text{spikes})}
\]

where \( \text{Prob}(\text{Pos}_k | \text{spikes}) \) represents the probability at the \( k \)-th location bin within the time window, and \( M \) represents the total number of location bins.

The time window was set at 300 ms. A point estimation of the location was made using the maximum likelihood estimation.

Statistical analyses.
Analyses of rat’s behavior around the gaps. Any value of running speed or head direction that exceeded more than three times the local scaled median absolute deviations (MAD) away from the local median within a sliding window (~72 cm) was defined as an outlier. The two-tailed
Wilcoxon rank-sum test was used to assess the gap effect on rat's behaviors in terms of running speed and head direction (Fig. 2b–c). Differences in running speed and head direction between gap locations between runways and the corresponding locations on the long gapless runway were assessed by two-way repeated-measures ANOVA (Fig. S4b–c). Differences in occupancy time at gap locations were assessed by one-way repeated-measures ANOVA (Fig. 2d). The occupancy time was calculated as the duration when the rat occupied the gap location. The duration while the rat paused (running speed < 5 cm/s) was excluded from the analysis.

Analyses of neural activity around the gaps. To examine whether the width of the place field was specifically changed at the gap locations, the Monte Carlo method was used. For each cell, the original place field location was shifted by a pseudo-random interval between 20 bins and 20 bins less than the length of the entire linearized track, with the end of the track wrapped to the beginning. This procedure was repeated 3,000 times for each cell. For each shuffling, the width of place field that is defined as the length of the firing field whose firing rate is over one-third of its maximum firing rate was calculated. Whether the place field on the gaps had a width greater than the 5th percentile or lower than the 95th percentile of the shuffled data was examined (Fig. 3b). Two-tailed Wilcoxon rank-sum test was used to assess the number of place fields, firing rates of MUA, and Bayesian decoding errors on the gap locations as compared with those on the non-gap locations (Fig. 3c-d, f).

Analyses of learning performance. The differences in learning curves were assessed using one-way repeated-measures ANOVA (Fig. 4). Two-way mixed ANOVA was used to ascertain the effect of expertise and experience on assembly time, and their potential interaction (Fig. 1j-k). The difference in spatial and rate similarities was assessed using the two-tailed Wilcoxon signed-rank test (Fig. 5c–d).

**Analysis software.** All analyses were performed using custom-made programs based on Matlab functions (v9.6; MathWorks, Natick, MA).

**Histology.** To identify the final recording locations, four rats were deeply anesthetized with isoflurane (Pfizer Japan Inc., Tokyo, Japan) and then given an overdose of pentobarbital sodium salt (50mg/kg, intraperitoneal (i.p.); Nacalai Tesque Inc., Kyoto, Japan) and transcardially perfused with phosphate-buffered saline (PBS), followed by 10% phosphate buffered formalin fixative (3.5-3.8% formaldehyde). Their brains were cut coronally at 40 μm and stained with cresyl violet. The final location of the tip of each electrode was around or below the pyramidal cell layer of the dorsal hippocampal CA1.
Supplemental References

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