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An Immersive Virtual Reality System for Rodents in Behavioral and Neural Research

Li Liu[1] Zi-Yang Wang[1] Yu Liu[1] Chun Xu[2,3]

[1] Institute of Automation, Chinese Academy of Sciences, Beijing 100190, China
[2] Shanghai Center for Brain Science and Brain-inspired Intelligence Technology, Shanghai 200031, China
[3] Institute of Neuroscience, Chinese Academy of Sciences, Shanghai 200031, China

Abstract: Context cognition involves abstractly deriving meaning from situational information in the world and is an important psychological function of higher cognition. However, due to the complexity of contextual information processing, along with the lack of relevant technical tools, little remains known about the neural mechanisms and behavioral regulation of context cognition. At present, behavioral training with rodents using virtual reality techniques is considered a potential key for uncovering the neurobiological mechanisms of context cognition. Although virtual reality technology has been preliminarily applied in the study of context cognition in recent years, there remains a lack of virtual scenario integration of multi-sensory information, along with a need for convenient experimental design platforms for researchers who have little programming experience. Therefore, in order to solve problems related to the authenticity, immersion, interaction, and flexibility of rodent virtual reality systems, an immersive virtual reality system based on visual programming was constructed in this study. The system had the ability to flexibly modulate rodent interactive 3D dynamic experimental environments. The system included a central control unit, virtual perception unit, virtual motion unit, virtual vision unit, and video recording unit. The neural circuit mechanisms in various environments could be effectively studied by combining two-photon imaging and other neural activity recording methods. In addition, to verify the proposed system′s performance, licking experiments were conducted with experimental mice. The results demonstrated that the system could provide a new method and tool for analyzing the neural circuits of the higher cognitive functions in rodents.

Keywords: Virtual space, flexible control, multi-sensory interactions, visual programming, context cognition.

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1 Introduction

The core issue in neuroscience is the study of higher cognitive function, which involves not only the study of molecules, cells, and neural circuits, but also that of multi-sensory integration and comprehensive cognition. Context cognition is one of the higher cognitive functions which serves to imbue meaning to surroundings. Our memories of events are always tied to the context in which they occurred. The context here refers to the sum of all elements of the external environment that people or animals are in at the time of the event, including various visual, auditory, tactile, and olfactory information[1]. People′s actions and reactions in daily life are always closely related to the context. In other words, people or animals are often able to exhibit a very "appropriate" behavior or reaction in their favor according to the characteristics of the situation. For example, if a person sees a snake in the wild that is free to move, they will show fear and avoid it. However, if the person sees a snake in a box at the zoo, they may behave very differently. Context cognition serves essential cognitive functions in the field of neuroscience. Therefore, it is important for improving individual learning and memory, and it even plays a vital role in individual survival and disease treatment. However, due to the complexity of the problem itself and the current lack of relevant technical means, little is known about context cognition and its neural mechanisms of behavioral regulation. Fortunately, it is believed that the behavioral training of rodents using virtual reality techniques may be a potential key to uncovering the neurobiological mechanisms of context cognition.

It has been found that in order to analyze the neural circuitry of higher cognitive functions, accurate activity recordings and the manipulation of specific cells in neural circuitry are mandatory processes. Two-photon microscopy can be used to observe neurons in deep brain regions[2]. It can also be used to perform three-dimensional optical imaging of specific nerve cells in the living brain, and allows photogenetic stimulation of specific cells. Its

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resolution can exceed that of a single neuron. Light stimulation under a two-photon microscope is currently the only feasible way to achieve single-cell resolution of light stimulation in living animals[3]. It can stimulate even a range as small as the size of dendritic spines of neurons ($< 2\,\mu m$)[4, 5]. The experimental study of cognitive behavior usually needs to be carried out on animals with free movement, however, the fact that two-photon microscopy can only be applied to animals with fixed heads significantly restricts the application of two-photon microscopy. Fortunately, virtual reality (VR) systems can effectively overcome these obstacles. VR is a multi-sensory information fusion, interactive three-dimensional dynamic space, and behavior training system[6, 7]. It can quantitatively control the sensory information of rodent's visual and spatial perception. When the head is fixed, the rodent can still perceive the change of environmental information. This makes it possible to study the activity of nerve cells in context cognition. In previous related research investigations, VR technology combined with two-photon microscopy and light stimulation technology was used to establish comprehensive experimental platforms. These platforms were then utilized for training the cognitive behaviors of head-fixed rodents[2, 8] and scan their neural activities.

Recently, VR technology has been widely used in the field of neuroscience[9–11]. VR systems could potentially be adopted to simulate virtual spaces, in which each component element of the virtual space could automatically be controlled. The experimental research conditions could then be strictly controlled, and scenes quickly switched many times during the experiments without artificial interference. Moreover, VR systems have rich scenario batteries which can effectively save physical space. In addition, more comprehensively realized and timely experimental data for analyses and research study can be acquired using VR systems[12]. Therefore, it is considered that VR systems have greatly enhanced both neuroscience and behavioristic research. VR has not only been adopted in research related to context cognition in rodents through virtual scenarios but also used to establish a series of rodent behavioral paradigms. In addition, the frontier issues in neuroscience may be further examined, such as decision making, spatial navigation, learning, and memory[13, 14]. Hölscher et al.[15] trained rats to navigate to cylinders hanging from the ceiling of a VR scene. The cylinders were placed with regular spacing in a large 2D virtual space, and the rats were rewarded when they entered the area below a cylinder. Rats learn spatial tasks in this VR quite readily. This setup creates new opportunities for investigations of information processing in navigation[15, 16].

At present, most rodent VR systems construct only a single stimulation, while Hölscher et al.[15, 16] used a panoramic display to provide virtual visual stimulation. Such a display was the crucial component in designing a functional rat VR. A panoramic display covers a substantial part of a rat's field of view and rats perceive their surroundings as an interactive environment. Sofroniew et al.[17] developed a tactile VR system for head-fixed rodents, where running through corridors was simulated by movable walls on both sides of the rodent[16, 17]. Rodents were fixed on top of a ball, and their movement was measured and translated into the position of the walls, such that if a rodent run to the right, then the wall on the right would come closer. In their setting, two walls always kept a certain distance (30 mm) from each other, simulating a fixed-width corridor. In order to simulate curvatures in the virtual corridor, the walls were moved accordingly. Rodents successfully steered the ball to follow the turns in the corridor. Using this tactile VR, Sofroniew et al.[17] studied whisker-guided locomotion. Radvansky and Dombeck[18] developed olfactory virtual reality system[18], they introduced a system to control and maintain a virtual olfactory landscape. This system uses rapid flow controllers and an online predictive algorithm to deliver precise odorant distributions to head-fixed mice as they explore a virtual environment. They established an odor-guided virtual-navigation behavior that engaged hippocampal CA1 “place cells” that exhibit similar properties to those previously reported for real and visual virtual environments, thereby demonstrating that navigation based on different sensory modalities recruits a similar cognitive map. This method opens new possibilities for studying the neural mechanisms of olfactory-driven behaviors, multi-sensory integration, innate valence, and low-dimensional sensory-spatial processing.

Higher cognitive functions involve multi-sensory integration and comprehensive cognition[19]. The context includes various types of visual, auditory, tactile, and olfactory information within an entire external environment when events occur. Although, some laboratories throughout the world only construct virtual visual spaces, just as shown in Fig. 1. The full integration of virtual scenes with multi-sensory information remains challenging[8, 14, 20–23]. Therefore, in order to provide a common platform for neuroscience and behavioristic studies, an immersive VR system was developed, which had the ability to record multi-sensory information with high flexibility for rodents. This system provided a new method and tool for analyzing the mechanisms of the neural circuits of higher cognitive functions.

The immersive virtual reality system proposed for the rodent's study operated on a visual programming platform. The system consisted of the following eight modules: virtual auditory module, virtual olfactory module, virtual tactile module, virtual stimulus module, reward and punishment module, monitoring module, virtual motion device, and virtual vision device. These modules comprised an interactive, three-dimensional dynamic construction space for the experimental rodents, with the ability to record the multi-sensory information of the ro-

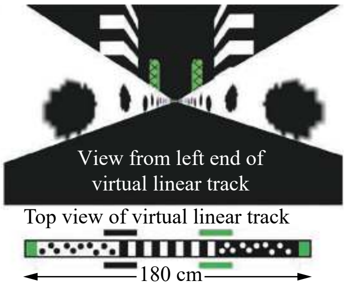

Fig. 1 Virtual visual space map[23]

dents. The system solved the problems related to the authenticity, immersion, and interactivities of the previously used VR systems. The virtual motion unit was designed based on a table tennis suspension device combined with a photoelectric positioning technique. For example, an environment was constructed using seven table tennis balls installed in a blower conditioning system, which was then used to suspend a foam ball. Then, head-fixed rodents were allowed to run freely in the environment. The running speeds and virtual positions of the rodents were recorded in real-time using an optical mouse device. The experimental rodents were able to interact with the environment efficiently and accurately.

The aforementioned system was oriented to the fields of neurobiology and behaviorism. It provided a high-flexibility experimental scheme that could be extended and optimized according to the different experimental requirements of the neurobiological researchers. The users who had limited program development foundations could also design, optimize, and develop the behavioral experiment by utilizing the control modules according to their requirements in the visual programming interface. Several custom blocks were used to convert all hardware and virtual environmental instructions into executed codes. This resulted in the experimenters with limited programming experience being able to easily set up and configure flexible experimental programs, as well as designing complex behavioral training schemes according to their requirements.

The remainder of this study is organized as follows. Section 2 illustrates the structure used in the proposed system and describes the design of every module and the associated software in detail. Section 3 presents the experimental results and the discussion of the results obtained. Finally, in Section 4, the conclusions are presented, and future work is outlined.

2 System design

2.1 Structure of the proposed system

According to the context cognition research requirements, it was necessary to achieve a multi-modalities interaction. Therefore, integrated control and methods were considered. In the present study, virtual auditory, virtual olfactory, virtual tactile, and virtual vision modules were designed to create virtual training surroundings. In addition, speed and direction detection, lick counting, reward control, electric stimulation, and blowing stimulation functions were designed to support the modules mentioned above. The system consisted of a head-fixed mechanical module that could be used with two-photon imaging, optical fiber imaging, and multi-channel electrophysiological recording processes.

It was found that, in order to achieve flexible and compatible control of the multi-modalities, an independent modular design should be considered. As shown in Fig. 2, six controlling modules were combined to construct a virtual perception unit, which utilized one hardware controller to coordinate the different operational sequences. Due to the fact that the experimental designing processes had only allowed for a serial method, there were no command conflicts observed in the proposed system. In other words, each module was implemented independently, and the various modules did not affect each other. A motion unit and a visual unit were independently connected to the virtual scene program using universal serial bus (USB) and high definition multimedia interfaces (HDMI), respectively. The main control program connected the Scratch and virtual scene programs using the hypertext transfer protocol (HTTP). It was also found that this structure is conducive to extending and optimizing the different experiments carried out in this study.

2.2 Central control system

In order to ensure that the proposed system was easy to operate for the researchers who lacked programming backgrounds, a user interface (experimental design interface) was built using the Scratch modularization programming platform. However, the Scratch platform had not provided any hardware driver interfaces. Therefore, in order to solve this problem, a central control system was designed which could be used to drive the hardware and data transmissions.

As shown in Fig. 3, this study′s central control system comprises both upper and lower software. The upper computer software included the user interface, main program, and virtual scene program. It was mainly used for human-computer interactions, device control, data analyses, and communication. The lower software was used to control the virtual perception unit. The corresponding data were transmitted between the upper and lower programs through a serial protocol interface.

In addition, in order to facilitate the design of the control instructions and experimental procedures, a user interface was built using the Scratch visual editing interface. The main program was written in Python, and it controlled the virtual scenes and the lower software. During this study′s experiments, the specific instructions were sent to the main program when some extension blocks on

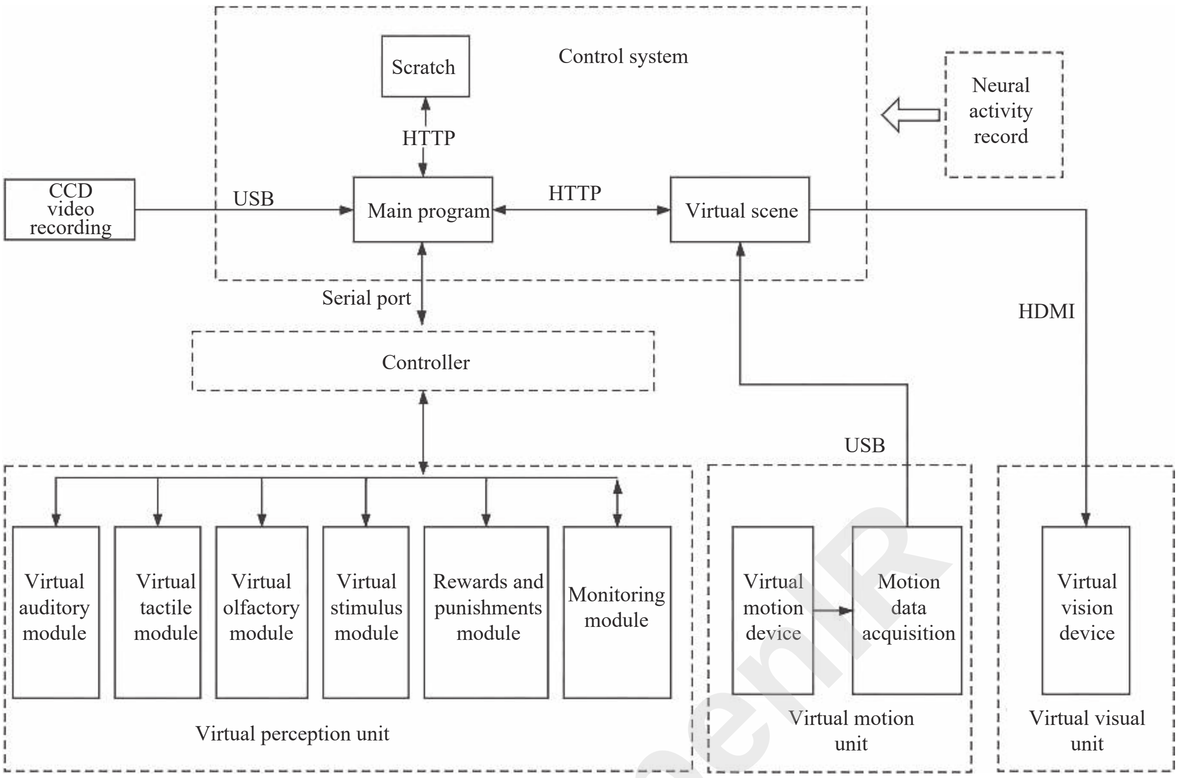

Fig. 2 System structure

the Scratch interface were clicked. Then, those instructions were decoded to several commands and sent to the lower software via serial. Subsequently, those commands triggered the virtual perception device to execute the block functions.

The upper software included the user interface, main program, and virtual scene program. These three independent modules were connected using HTTP, and the Scratch visual editing interface was used for the user interactions. Furthermore, it had the ability to create custom building blocks for different functions. In the present study, according to the Scratch extension communication protocol, a JSON-format description file was created to define the extension blocks and imported to Scratch through the "Import experimental HTTP extensions" menu. In this study's proposed system, in order to contain the functions used in the rodent behavioral training as much as possible, 27 custom building blocks were created, shown in Fig. 4. These custom-building blocks included 6 parameter setting blocks; 17 control instruction blocks; and 4 display blocks. Also, parameter setting blocks were used to select and configure the environmental parameters. Control command blocks were utilized to adjust the switch of each module, and display blocks were adopted to draw the collected data. For example, in order to observe the motion changes in a timely and clear manner, a real-time graph was drawn based on the motion data in the platform.

The custom blocks used in this study are detailed in

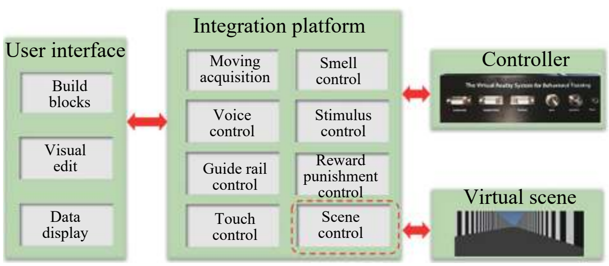

Fig. 3 Structural design of the central control system

Fig. 4 Custom blocks

Fig. 4. An example of a manipulation of the auditory experiment is provided as follows: When the virtual auditory module began, the start block of the module was first triggered, and the waiting block controlled the duration. Then, the type and source of the sound could be adjusted in the parameter setting block of the module. At the end of the experiment, the ending block was triggered. The division of the functional modules into different blocks allowed for the flexible manipulation of the control instructions and parameter settings, even in the cases of complex behavioral training. In addition, the processes were clear and intuitive for users designing various training programs since the different blocks could be easily dragged to the program editing bar.

In this study, the main program was written in Python and was an essential part of the central control system. The main program connected the controller, user interface, and virtual scene program. Since the Scratch platform had not provided a direct interface for connecting the controller and editing platforms for the virtual scene device, it was necessary to build a program to transfer the commands and edit the virtual scenes before proceeding. The main functions of the designed program were to receive instructions, process data, and send commands. In the present study, the HTTP server was used to transfer the data and commands of the user interface and virtual scene. The function modules included virtual scene control, sound control, olfactory control, tactile control, stimulus control, reward and punishment control, guide control, data processing, and analysis. Also, the program contained charge-coupled device (CCD) data recordings which were used to monitor the rodents' movements. The main program flow chart is shown in Fig. 5. First of all, it detected whether or not custom blocks had been triggered. If there were triggered blocks, the program accepted instructions, collected data, and sent instructions to the lower software. Otherwise, the detection process continued. Furthermore, it detected whether the end block was triggered. If there was no end block triggered, it returned to the detect process. However, if an end block had been triggered, the program would end.

In order to accurately record the times of each behavior training of the rodents, as well as facilitate the analysis and study of the experimental results, the triggered times of each block were accurately recorded in the program log, with the times accurate to within milliseconds.

The virtual scene program was written in C++, and a "Graphic State Notation" method was used to break the behavioral training into sequential sessions. During each sequential stage, the program switched states according to different conditions, such as counting, timing, and specific parameters.

The lower software was designed for controlling the virtual perception equipment. The functions of the lower software were guide rail control, voice control, communication, etc.

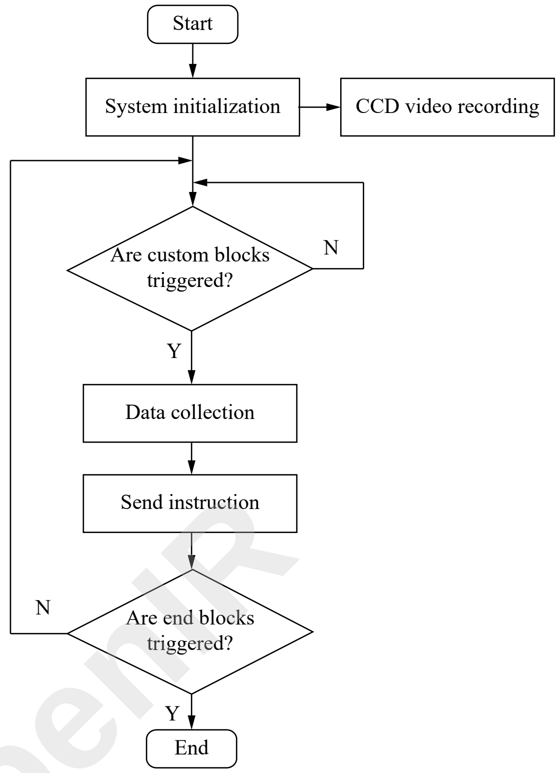

Fig. 5 Program flow chart

2.3 Controller

Since the proposed system was designed for rodent behavior research, in order to ensure that the rodents received immersive experiences, as well as realize accurate control signals and time synchronization between a variety of sensory information and improve the accuracy of the virtual reality system, an integrated controller was utilized for the flexible manipulation of multi-modal perceptions in the virtual scenes. The controller centrally controlled the following six functional modules: virtual auditory module, virtual olfactory module, virtual tactile module, virtual stimulus module, reward and punishment module, and the monitoring module. The main components of the controller included sound control, steering gear control, guide rail drive, relay, data acquisition, interface circuit, microcontroller, etc. For convenience purposes and to avoid electromagnetic interference, all of the circuit boards were fixed in a shielding case. The external devices were connected through the respective panel interfaces of the case. The connection diagram of the controller is shown in Fig. 6.

2.4 Virtual space

The virtual space was composed of the following three units: virtual perception unit, virtual motion unit, and virtual vision unit. The virtual perception unit was used to build a virtual training environment for the rodents, which contained the following six modules: virtual audit-

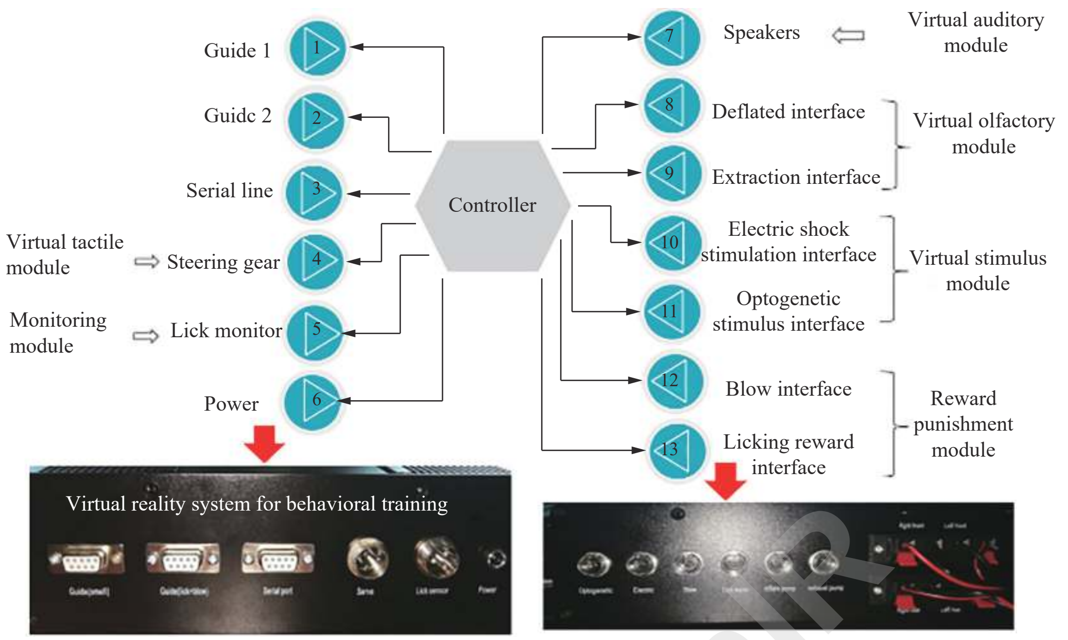

Fig. 6 Controller connection diagram

ory module, virtual olfactory module, virtual tactile module, virtual stimulus module, reward and punishment module, and monitoring module. The virtual motion unit was used to record the movement data of the training rodents, and the virtual vision unit was used to create a virtual visual scene for the training rodents. Their combinations formed interactive three-dimensional dynamic spaces with multi-sensory information fusion, as shown in Fig. 7.

2.4.1 Virtual perception unit

The virtual perception unit was composed of the following six independent functional modules.

Virtual auditory module. The ability to perceive surrounding information using their auditory senses is essential for rodents. They can build a cognitive map using the different sound sources and volumes. Cushman et al.[24] developed a virtual auditory system. Auditory cues were presented by a 7-speaker surround sound system. The intensity and orientation of the auditory stimulation could be modified. Therefore, it was important for the virtual auditory module to design a surround system that included different sound sources and could be adjusted to different voice volumes. As shown in Fig. 7, four speakers were placed at the four corners surrounding the virtual system. The sound intensity of each channel was controlled by the controller. In the system, six sound sources were designed in the virtual training space located in the edges of the front, rear, left front, right front, left rear, and right rear with respect to the virtual map. One or more sound sources could be turned on within the user interface according to the requirements of the training experiments. The volume of the sound around the training rodent was determined by the number of the selected sound sources and the distances between the rodent and each selected sound source in the virtual map. When a rodent changed direction in the virtual map, the sound sources changed accordingly.

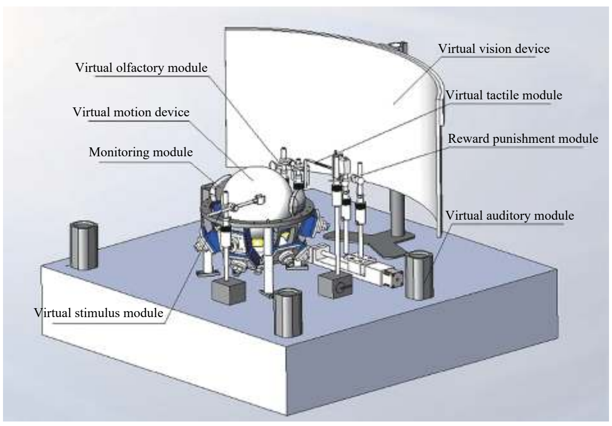

Fig. 7 Schematic diagram of the virtual space structure

For example, when the training rodent hears a loud, terrifying sound in front, it may potentially turn around in order to escape. Since the rodent's head is fixed, when it turns around in the virtual map, the frontal sound source will be turned off, and the sound source in the rear will be turned on automatically. Then, as the rodent goes further and further along the path, the terrifying sound will become progressively lower. In order to design sufficiently different experimental environments, the system built a sound library that included three types of sounds: pure sound, white noise, and natural sounds, with a total number of 50 choices. It was also able to play additional sounds through its extended storage.

Virtual olfactory module. Radvansky and Dombeck[18] developed an olfactory virtual reality system to control and maintain a virtual olfactory landscape[18]. This method successfully studied the neural mechanisms of olfactory-driven behaviors. The system also provided a virtual olfactory space which increased the authenticity of the immersive experience for training rodents. In this study, a set of multi-channel smell generation devices were used. A vacuum pump was utilized to accurately control, timely generate, and update the gaseous odors. As shown in Fig. 7, when the olfactory module was triggered, an electric guide automatically drove the exhaust pipe and deflated pipe to the area in front of the experimental rodent. A smell generation device accurately controlled the stimulation of the odor molecules through the airflow. Meanwhile, the device was coordinated with the air extraction vacuum pump in order to quickly clean any residual odor. The electrical guide is then automatically returned at the end of the process.

Virtual tactile module. In the virtual map, when the rodents were located against the virtual wall, the system needed to transfer the corresponding tactile information. In a natural setting, the rodents would also use the tactile system of their whiskers to explore the entire space along a corner. Sofroniew et al.[17] studied whisker-guided locomotion. Rodents successfully steered the ball so as to follow the turns in the corridor in the tactile VR. Therefore, to increase the sense of reality in the virtual space, tactile feedback to the rodents' whiskers was generated when the rodents approached the boundaries of the virtual space. In this study's proposed system, the aforementioned tactile feedback was realized using a swinging metal rod arm driven by a digital steering gear (model: 55G KY62) which touched the rodents' whiskers. As shown in Fig. 7, the metal rod swinging arm was fixed on a steering gear, which was fastened to a bracket through the frame. When the rodents came up against the virtual barrier, the steering gear automatically controlled the metal rod swinging arm to touch the rodents' whiskers.

Virtual stimulation module. The system also provided a light stimulation interface and a current stimulation interface. A transistor-transistor logic (TTL) trigger was used to control the external Arduino controller, and then the Arduino controller determined the precise locations of the optical genetics according to the preset modes. The electrical stimulus worked as a signal of danger and was used to simulate a dangerous situation for the experimental rodents. As shown in Fig. 7, a resistance wire wrapped around the tail of a rodent was used to create a small electric current stimulus for the rodent during the experimental processes.

Reward and punishment module. Reward and punishment functions are the most important features of a behavioral training system. This study designed a licking water reward and a blowing punishment, respectively. For example, the rodents would be rewarded with water after a correct learning behavior training in the virtual scene. As shown in Fig. 7, the system provided both reward and punishment stimuli (such as a sugar-water reward and face-blowing punishment). The licking and blowing devices were controlled by the opening and closing of pipes with solenoid valves. When the reward and punishment module was triggered, an electric guide rail automatically drove the licking pipe and the blowing pipe to the area in front of the experimental rodents. Then, the solenoid valves of the licking pipe or blowing pipe were turned on in order to complete the reward or punishment stimulations.

Monitoring module. In the behavioral training experiments, it was important to monitor the number of rodent licking rewards. Therefore, a licking detection module was built using a combination of a capacitive touch sensor and a solenoid valve. The capacitive touch sensor was combined with an Arduino microcontroller. The number of licking actions could be extracted sensitively and effectively through the touch sensor signals. The switch of the water supply reward was built using a solenoid valve combined with a suspended gravity natural water supply method. The accuracy of the water supply could reach between 1 and 3 microliters per drop. The sensor transmitted digital signals to the central control system through a serial port, and the number counts of the licking actions were displayed on the user interface.

2.4.2 Virtual motion unit

The structure of the virtual motion unit is detailed in Fig. 8. A virtual motion device was built using an expandable polystyrene ball, hemispheric and support frame, and seven inflatable tube interfaces. In the present study, to ensure that the motion device was stable, seven table tennis balls were embedded into inflatable tubes to support a polystyrene ball. The experimental rodents were able to run on the top of the ball. The air pressure of each inflatable tube could be artificially regulated according to the weight of the rodent. This virtual motion device for the rodents was similar to a treadmill. The motion data acquisition module monitored the changes in the ball in real-time using two optical mouse devices (model: Ra poo

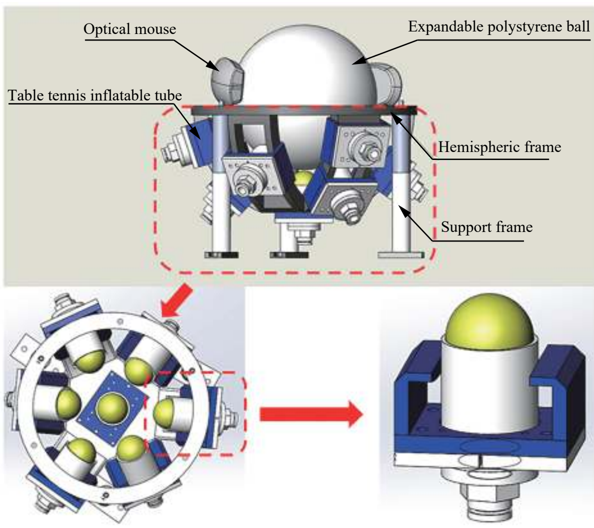

Fig. 8 Structure diagram of the virtual motion device

V310). The rodents interacted with the virtual scene in real-time and their running speeds, and orientation on the virtual motion device were monitored.

The seven table tennis balls in the inflatable tubes were supported by an air conditioning system which was composed of a fan, rotary joint, regulating valve, and an air pipe. The seven table tennis balls were evenly distributed at the bottom of the polystyrene ball in order to maintain stability and reduce the force of friction. As a result, the experimental rodents could run freely. This structural design effectively prevented the off-center and inertial motion of the polystyrene ball. Then, by adjusting the effective lengths and positions of the inflatable tubes, a polystyrene ball of various diameters could be obtained. The structure of this motion device was inspired by bearing rollers, and the table tennis balls were used as the intermediary. The table tennis balls floated in the air, and the polystyrene ball was able to roll freely while bearing the weights of the rodents. The head-fixed rodents could run freely on the polystyrene ball and also drive the polystyrene ball to roll at different speeds. Two optical mouse devices were used to monitor the changes in the rolling speeds of the ball. Then, according to the changes in the rolling actions of the ball, the rodents′ positions in the virtual scene changed accordingly. In that way, the rodents′ interactions with the virtual scene were achieved in real-time.

A specific interface driver was built in order to distinguish the two optical devices from the computer mouse. The integration program was able to dynamically connect library files in order to obtain the rodent information in real-time. The location information of the rodents in the virtual space was updated in real-time using the two optical mouse devices. The location information included the X/Y coordinates, angles and times. The speeds of the rodents′ movements were calculated according to the X/Y coordinates and time. Then, after the calculations and analyses were completed, the real-time position information and movement speeds of the rodents in the virtual space were displayed on the user interface.

2.4.3 Virtual motion unit

Hölscher et al.[15] used a panoramic display to provide virtual visual stimulation[12, 16]. A panoramic display covers a substantial area of a rat′s field of view. Rats learn spatial navigation in this VR quite readily. The system designed in this study adopts this method. The virtual visual unit included a virtual visual device and a virtual scene file. The virtual scene file was displayed on the virtual visual device. The images displayed on the virtual visual device were updated in real-time according to the rodents′ movement tracking data, and the presets of the virtual scene model. In addition, in order to cover the entire visual range of rodents, the virtual visual device utilized a curved screen format to present the visual stimuli and adopted curved orthotics to perform real-time hardware corrections of the images on the screen.

The virtual scene file first built a 3D scene model[25]. A realistic model was the basis for realizing the virtual system. In this study, 3D Max software was used to construct a 3D scene model. The program, written in C++ language, had the ability to read the virtual scene model and change the position and perspective in the virtual scene according to the input of two optical mouse devices, which controlled the point of view. At the same time, the video was output in real-time. The key parameters of the virtual scenes could be stored and modified through configuration files. The virtual scene program formed a strong universality of the independent files which communicated with the main program (Python) by HTTP. Consequently, experimenters could choose different virtual scene files according to their training requirements.

3 Experiment method

The system uses a sufficiently large field of vision, multi-modal sensory stimulation, and real-time interaction to form a controllable virtual environment similar to the real world. It can conduct long-time and stable behavior training for rodents. Through long-term behavior training, rodents can learn various related tasks such as context cognition. In the experimental process, the rodent is first fixed on the experimental platform. The device is opened. Then, experimental programs are set up and configured in the visual programming interface by dragging blocks. After clicking Start, all steps will be completed automatically. Data recording and analysis are also completed automatically by the program. Behavioral information can be displayed in real-time. At the same time, control and imaging experiments are carried out by combining photo genetics and a two-photon microscope.

Throughout the experiment, we carried out relevant studies in strict accordance with the ethical rules for experimental animals. The experimenters have high scientific research quality, have worked many years in research on neurobiological experiment and behavior, and possess rich experimental experience in the operation of living rodents.

4 Results and discussions

The system proposed in this study could potentially be used to observe the neuron activities of mice combined with two-photon calcium imaging. First, the mice were trained to run through a simple tunnel in order to obtain water as a reward, as shown in Fig. 9(a). The mice adapted to the virtual visual space environment on the polystyrene ball. They obtained a water reward by running. At that point, the hippocampal CA1 cells were recorded by calcium imaging. From the example, the mice all showed a good firing pattern of the cells in the position shown in Fig. 9(b). This indicated a pattern of discharge consistent with that of the hippocampal CA1 cells in free-moving mice. The results shown in Figs. 9(c) and 9(d) were found to be consistent with the successful use of virtual space to record hippocampal location cells previously reported in related literature[26]. These preliminary results also showed that the mice had generated the expected location cell coding in the virtual reality system and have good virtual space perception.

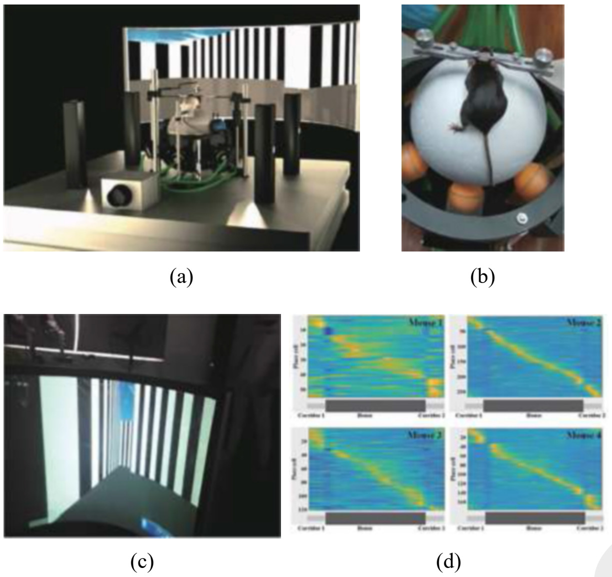

Fig. 9 Behavioral training: (a) Behavioral training in a virtual scene; (b) Head-fixed training mouse; (c) Virtual visual behavioral training of the mice; (d) Cell activation patterns.

In addition, a classic Go/no-Go behavior experiment was conducted in order to test the performance of the proposed system, as detailed in Fig. 10. Different scenes components were displayed by the VR system. The experimental mice learned that in Context 1, licking was a correct performance in order to receive rewards. However, in Context 2, licking was the wrong performance, and the mice learned that punishment would result.

In Figs. 10(d) and 10(e), the behavioral results of two mice are shown. The performance accuracy indicated that the mice had always maintained high water licking motivation from their previous training. The licking accuracy of Context 1 was close to 1. These results were consistent with those of previous reports[7, 26]. Meanwhile, regarding Context 2, the mice still maintained a high frequency of licking during the early stages of training, making its accuracy close to 0 at the beginning. However, as the training time increased, the mice gradually learned the rule of no reward in Context 2. Subsequently, the number of licks significantly decreased, and the accuracy rate steadily increased. These findings indicated that the mice had become more relaxed and have successfully learned the no-Go rules under the conditions of the virtual reality interactions. Therefore, the test and condition optimization of the mice behavior training had been successfully completed in the virtual space system. Furthermore, the immersive rodent virtual reality system with visual programming was able to expand the behavioral training. At the same time, the program was able to meet the needs of

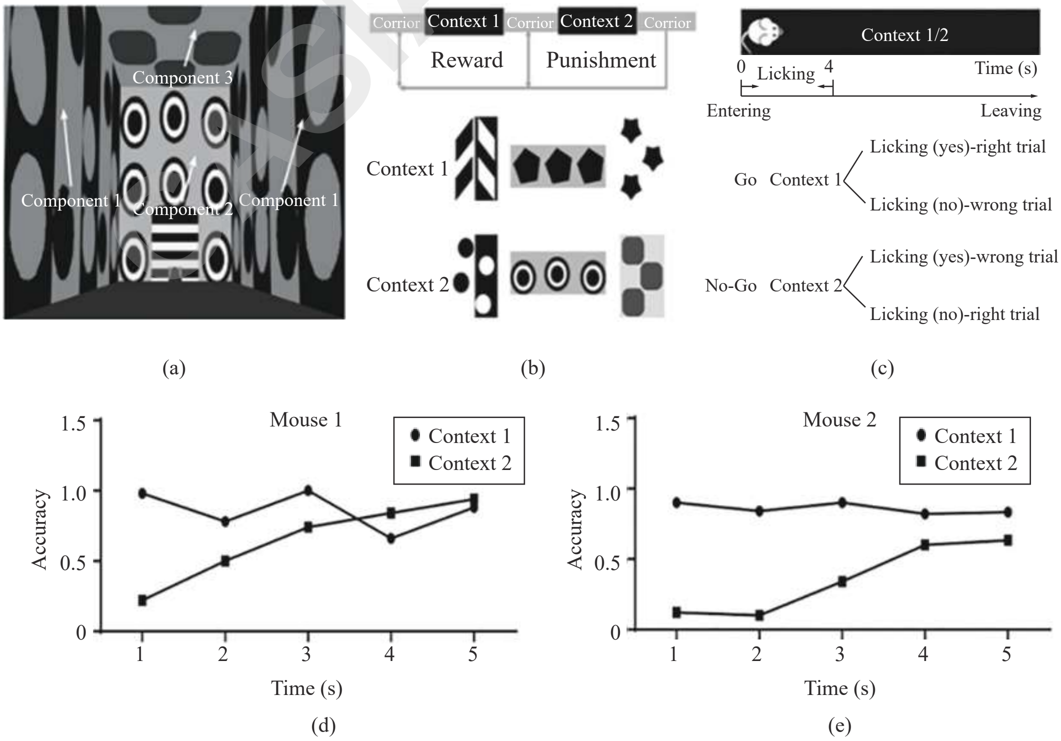

Fig. 10 Behavioral paradigm: (a)−(c): Go/no-Go behavior patterns of the virtual scenes; (d) and (e): Accuracy of the mice behaviors in context.

a variety of experimental requirements for head-fixed rodents, suggesting a wide range of potential applications.

5 Conclusions

In this research study, eight modules were adopted, which constituted a flexible control, multi-sensory information fusion in the rodents, and interactive three-dimensional dynamic space. The experimental test results confirmed that improvements had been made in the authenticity, immersion, and interactivity processes of a virtual reality system for rodents. By means of the centralized and flexible control of its controller, the system realized the time synchronization between the control signals and various perception information data. As a result, the accuracy of the virtual reality system was improved. The virtual motion unit adopted a table tennis ball suspension device and photoelectric positioning technology. It was able to realize the real-time interactions between the rodents and the virtual scene through the speed and orientation of the running movements of the rodents. As a result, the immersion, interaction, and stability of the virtual reality system were enhanced. The central control system adopted a Scratch visual editing interface which developed custom building blocks and converted all commands into custom building blocks. The central control system could thereby allow various users to set up and configure flexible experimental programs and design complex behavioral training schemes according to their experimental requirements. The flexibility of the virtual reality system was further improved by the aforementioned design changes. Therefore, the proposed immersive virtual reality system for rodents based on visual programming provided a new method and tool for analyzing the mechanisms of the neural circuits of advanced cognitive functions. Its wide application and promotional potential will help drive future research in relevant fields in China to reach the international forefront.

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Li Liu received the B. Sc. degree in mechatronic engineering from Hebei University of Science and Technology, China in 2001. She was awarded senior engineer by Beijing Municipal Human Resources and Social Security Bureau, China in 2015. Currently, she is a developer at Institute of Automation, Chinese Academy of Sciences, China.

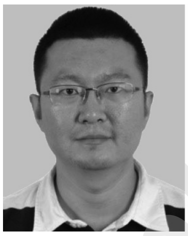
Her research interests include virtual reality and brain cognition.

E-mail: li_liu@ia.ac.cn
ORCID iD: 0000-0002-9436-7035

Zi-Yang Wang received the B. Sc. degree in electronic information science and technology from China Agricultural University, China in 2013, and the Ph. D. degree in agricultural electrification and automation from China Agricultural University, China in 2019. Currently, he is a post-doctor at Institute of Automation, Chinese Academy of Sciences, China.

His research interests include electrical signals processing, electrophysiology, brain-computer interface, and cognitive assessment.

E-mail: ziyang.wang@ia.ac.cn
ORCID iD: 0000-0003-2161-7504



Yu Liu received the B. Sc. degree in automation, the M. Sc. degree in pattern recognition and intelligent systems, from Beijing Institute of Technology, China in 2001 and 2004, respectively, and the Ph. D. degree in pattern recognition and intelligent systems from Institute of Automation, Chinese Academy of Sciences, China in 2010. He is currently a professor of Institute of Automation, Chinese Academy of Sciences, China.

His research interests include artificial intelligence and cognitive assessment.

E-mail: yu.liu@ia.ac.cn (Corresponding author)
ORCID iD: 0000-0001-5951-8478

Chun Xu received the B. Sc. degree in biology from Xiamen University, China in 2003, and the Ph. D. degree in neurobiology from Institute of Neuroscience, Chinese Academy of Sciences, China in 2009. He conducted postdoctoral research with Dr. Andreas Lüthi in the Friedrich Miescher Institute, Switzerland in 2009–2016. He joined Institute of Neuroscience, Chinese Academy of Sciences, China as a principal investigator in January 2017. He is the head of the Laboratory of Neurobiology of Context and Behavior, Institute of Neuroscience, Chinese Academy of Sciences, China.

His research interests include context perception, spatial navigation and emotional memory.

E-mail: chun.xu@ion.ac.cn (Corresponding author)