ISSN 2959-409X

Exploring the Mechanisms and Functions of the Impact of Ketamine on Spatial Orientation Decision Making

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Abstract:

The aim of the study is to explore the impact of ketamine on sound perception and spatial orientation decision making, and the mechanism of how ketamine impacts perception and decision abilities. The methods used include behavioral and histology approaches. From the results, it can be concluded one of the brain regions affected by ketamine is TeA(temporal association area), which plays a vital role in sound perception and spatial orientation. An unexpected finding is that ketamine impacts left turning and right turning decisions at different degree, which requires further research on ketamine's different impact on two cerebral hemispheres. In all, the study's findings help people further understand how ketamine impacts spatial and sound perception.

Keywords: ketamine, sound perception, spatial orientation, TeA(temporal association area).

1. Introduction

1.1 Background of the Research

Ketamine($C_{13}H_{16}CINO$) has been mostly used for anesthesia, analgesia, sedation and treatment of chronic pain syndromes[1], all of which achieved by its role as an NMDA receptor antagonist. By blocking NMDA receptors, ketamine inhibits the action of the neurotransmitter glutamate which plays a crucial role in pain perception, cognition and body movement[2]. Moreover, the inhibition of NMDA receptors leads to an increase in the release of dopamine in the brain, for it inhibits inhibitory neurons which exert an inhibitory control over dopamine neurons, such as GABAergic interneurons and PY neurons, and therefore exert overall excitatory signals[3]. Another feature of ketamine is its dissociative properties. High levels of ketamine in human body can lead to distortion of sights, colors, sounds, and the sense of space, creating powerful visual hallucinations, vivid dreams and an "out-of-body" hallucinogenic experience[4]. With its ability to increase dopamine and cause hallucinations, ketamine is often abused and has thus been listed as a controlled substance in many countries[5].

Among all hallucinations caused by ketamine, the distortion of spatial orientation is especially fatal, for it can lead to drug-takers to fall off buildings. After years of research, researchers have identified the specialized cells important for identifying directions: grid cells and head direction cells[6]. Grid cells help the brain map out the environment, while head direction cells help maintain a sense of direction and orientation relative to the surrounding environment by firing when the head is oriented in a specific direction[7]. These cells locate mostly in entorhinal cortex within the hippocampal formation, Therefore making hippocampus essential for navigation and understanding spatial relationships[8]. Besides direct spatial information, auditory information also plays a crucial role in spatial orientation, for the way sound waves bounce off objects in an environment allows the brain to understand the size and shape of a space[9]. Therefore, another reason why ketamine causes spatial distortion might be closely related to the sound hallucination people endure after having ketamine. Adequate researches have found out the brain regions that play a vital role in sound perception, including the TeA brain region[10]. However, though the sound perception, the spatial perception and orientation and the general mechanism of how ketamine causes changes in neural system by blocking NMDA receptors are separately well explained, it has not been deeply explored how ketamine impacts spatial perception and orientation through cell specific mechanisms.

1.2 Target of the Research

Among different sensory hallucinations caused by ketamine, this study focuses specifically on its auditory hallucination and how it impacts spatial perception. By conducting behavioral experiment on mice injected with

ketamine and recording their neural activities, this study aims to explore the cell specific mechanisms and functions of the impact of ketamine on sound perceiving and spatial orientation decision making. Based on studies conducted before, it is deduced that such impact take place specifically by inhibiting PV neurons and increasing dopamine levels[11]. Whether this prognosis can be proven true and whether there exist other cell specific mechanisms and functions involved in the process of ketamine impacting spatial orientation decision making are the conclusions aimed to be drawn out through this study.

1.3 Purpose of the Research

Ketamine abuse has significant negative effects on both the brain and the body. Short-term effects include hallucinations, confusion, memory loss, high blood pressure, and slowed breathing that can potentially lead to death. In the long term, users may experience persistent psychotic episodes, respiratory depression, heart rate abnormalities, and severe bladder and kidney issues[12], as well as increased risk of accidents due to impaired coordination and judgment, and potential for severe psychological effects such as anxiety and depression during withdrawal. Some people abuse ketamine not only for the instant pleasure brought by it, but also because of the longing for vivid dreams and hallucinations caused along. The understanding of cell specific mechanisms and functions of ketamine-caused hallucination may help scientists further explore ways to inhibit such hallucination in order to help such people addicted to ketamine to come off the drug. Also, from becoming a safe anesthetic and sedation drug half a century ago, to being used as an antidepressant these days[13], ketamine is consistently providing new possibilities for medical treatment. Thus, it is necessary to reduce its side effects, such as hallucination, to the lowest possible degree, which has to be achieved by first understanding the formation of hallucination caused by ketamine. In all, the study aims to deepen and refine humans' understanding on the mechanism of how ketamine impacts neural system in specific ways such as altering auditory and spatial perception and to contribute to the further understanding of hallucination inhibition and ketamine side effect reduction.

2. Materials, methods and results

2.1 Animals

Adult wt mice (17g-20g) were divided into two groups. Mice from group one were labeled as KX mice while mice from group two were labeled as Saline mice.

2.2 Surgery

Some mice(n=16, 2n=32) from each group were conducted surgery for behavioral use while the rest of the mice(n=1, 2n=2) were conducted surgery for histology use.

2.2.1 Surgery for behavioral use

Both KX mice and Saline mice were anesthetized with an intraperitoneal injection of chloral hydrate (50/0.5, mg/kg) and mounted on a stereotaxic frame. After ensuring complete anesthesia of the mice, the hair on the top of their head (between the line connecting the eyes and the line connecting the ears) was shaved with scissors or a razor. The mice's head were then fixed on the stereotaxic device and erythromycin eye ointment was used to protect their corneas. Scissors were used to cut open the skin on the top of the mouse head to expose the anterior and posterior fontanels. The anterior and posterior fontanelle were exposed, and a disinfectant blade was used to scrape off the periosteum. The tip of the micro syringe was used to locate the front fontanelle as the zero point, and front and rear and left and right leveling was conducted to determine the coordinates through the brain atlas. The use a micro syringe to locate the injection site of TeA((in mm from bregma: ML ±3.4, DV 1.2; in mm from the outline of the skull: AP 0.65) on the surface of the skull and mark it with ink. After the skull was exposed and cleaned, craniotomies were made to allow for virus injection into and fiber-optic implant over TeA(by using a grinding drill to drill a circular hole around the marked point, removing bone debris, and using a needle to pick out the skull at the positioning point. Using a calibrated glass pipette attached to a stereotaxic injector, virus rAAV-CaMKIIa-GCaMP6s-WPRE-hGH PolyA was bilaterally injected into the VeA. The surface of the mouse skull was then scrubbed with a saline cotton ball and scratches were left on the surface with a razor blade to increase its roughness. The fiber pin was fixed to the brain stereotaxic instrument with a gripper, slowly lowered and gently punctured the dura mater. The tip of the syringe was reset to zero when it was on the surface of the brain so that the fiber could be lowered to an appropriate depth (in mm from bregma: DV 1.15) and fixed with dental cement. After the cement was completely solidified, the mice was placed in the heating pad while researchers wait for the mice to wake up and pay attention to the respiration of the mice. After the mice woke up, they were transferred to the breeding cage and raised separately in two groups for future behavioral use, their health condition monitored regularly.

2.2.2 Surgery for histology use

Mice(n=2) were evenly divided into two groups. Treat the KX mouse with an intraperitoneal injection of ketamine (25/1, mg/ml, 500nl) and Saline mice with an intraperitoneal injection of physiological saline(500nl). One and a

half hours after the injection, cardiac perfusion were conducted on the mice by pumping PBS and PFA throughout their systematic circulation. When the body of the mouse was stiff and the internal organs became white, the head of the mouse was removed with the head fur cut, the neck and temporal muscles cleaned to expose the skull, the cartilage coated on the medulla oblongata cut, and the cranium cut broken with ophthalmic scissors, so that the complete brain tissue could be removed with its integrity carefully maintained.

The mice brains were stored in 4% paraformaldehyde solution for 24 hrs at 4℃.

2.3 Histology

The stored mice brains were taken out and 50-μm sections were sliced. Before C-fos staining, brain slices were fixed with cold acetone at 4 ℃ for 15 minutes and washed with PBS for 3 times, five minutes each time. Slices were then transferred and soaked in 3% H_2O_2 for 20 minutes in a light-free environment so that endogenous peroxidase were inactivated. After that, slices were again washed with PBS for 3 times, five minutes each time and then sealed with BSA at 4 ℃ for 1 hour without washing, absorbing or shaking off the blocking solution. Primary antibody were immediately added and stayed overnight at 4 ℃. Brain slices were washed with PBS for 3 times before secondary antibody was added and stayed at room temperature for one hour, away from light. Afterwards, brain slices were washed again with PBS for 3 times (5 minutes each), followed by adding DAPI staining solution which stayed for 1 hour at room temperature in the dark. Brain slices were then washed with PBS for 3 times, mounted and sealed with anti fluorescence quencher.

2.4 Behavior

2.4.1 Behavioral research on mice's auditory and spatial perception and orientation decision

In the behavioral experiment designed to study mice's spatial perception and decision, mice were put in a behavioral box under 2AFC(Two-Alternative Forced Choice) behavioral system. The inner structure of the box consists mainly of two parts: a water feeding system controlled by buttons and a hidden audio. The water feeding system includes three burettes with one button and a plate under each of them, put in a line. The box's walls have a layer of soundproof sponge so that the mice would pay sole attention to the sound given by the inner audio without being affected by outside noise. (Figure 1)

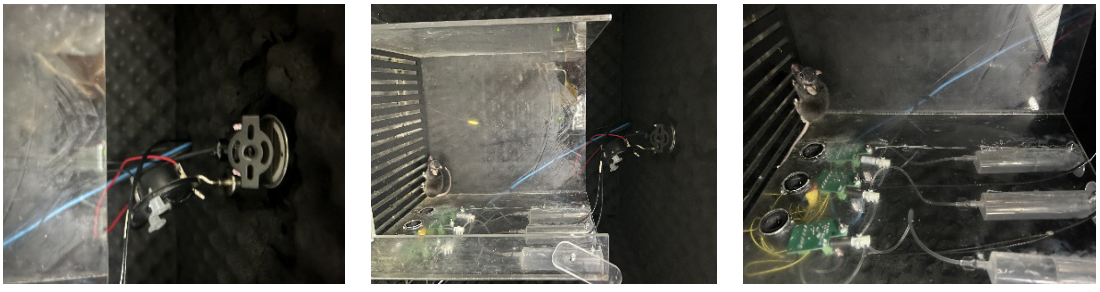


Fig.1 (a)Whole image of the box (b) the audio hidden in the dark (c) the three pokes with water

The experiment is conducted under the following steps:

1. Deprive the mice of water for 3~5 days.

2. Training Phase I: Train the mouse to push the buttons using water reinforcement. Mouse needs to push the button in the middle to so that the audio will give a sound. Upon hearing the sound, the mouse needs to push one button, either on the left or on the right, so that water will come out into the plate below the button pushed. After several times of 'trial and error', the mouse will eventually learn the procedure: press the button in the middle, hear a sound and press another button to get water. The mice needs to be trained like this until it is able to get the water for more than 700 times in one hour.

3. Training Phase II: The basic training method used is similar to Training Phase I, except that the mouse can not get water simply by pushing either button on the left or right. Instead, the mouse has to identify whether the sound pitch is low (below 10000 Hz) or high (above 10000 Hz), turn left to press the left button when hearing a low-pitch sound and turn right to press the right button when hearing a high-pitch sound. (Figure 2)

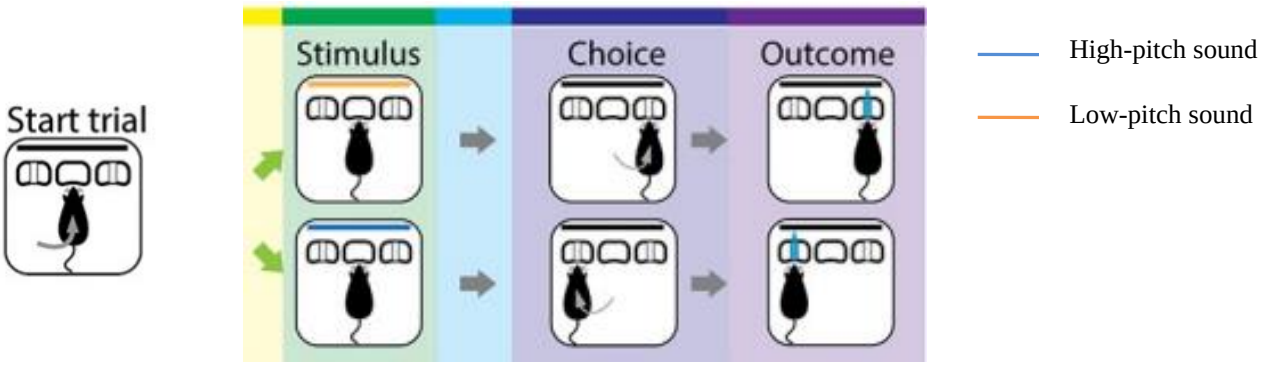


Fig.2 the experiment procedure

The mice needs to be trained like this until it is able to get the water for more than 700 times in one hour. The mice are put in the box and trained for one hour every day, and their only source of water is the reinforcement they get from the box. However, if a mouse continues failing and can not get water, a small amount of water is fed to it to make sure it stays alive.

4. Behavioral Experiment: KX mice received intraperitoneal injection of ketamine (30/1, mg/kg) while Saline mice received intreperitoneal injection of physiological saline. They were then put into the behavioral box to perform the procedures for one hour. Their choice accuracy were recorded and analysed.

2.4.2 Research on left-turn and right-turn brain signals in mice brain

Normal wt mice were used for this experiment. After injecting virus and implanting fiber-optic into the mice brain, mice were transported into cages and raised for 2 weeks before conducting their experiment.

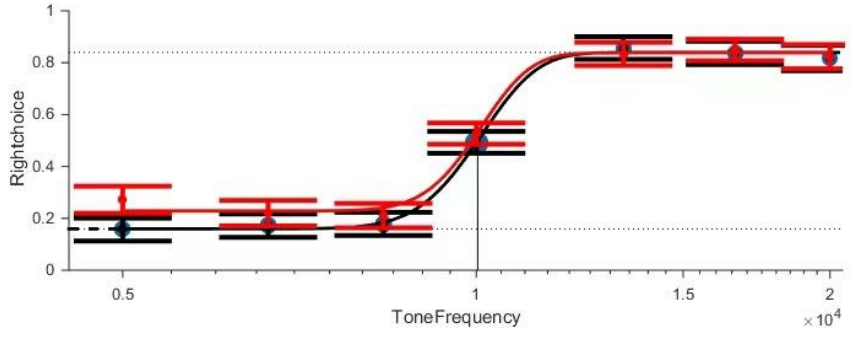
During the experiment, a mouse was put into a letter-"T"-shaped box for one hour so that it had to take turns to move around. The video of the mouse moving around in the box was later analysed by researchers, the time when it took left turns and right turns labeled separately. Using these labels, researchers graphed the average signal of left turn and right turn separately using Matlab.

2.5 Confirmation

After the behavioral experiment were conducted, cardiac perfusion were conducted on both KX and Saline mice and their brains were taken out to make sure that the position of the fiber optic was in the TeA region and the virus had been activated.

3. Results

32 sessions of the 2AFC behavioral experiment were completed by 32 mice, 16 of them Saline mice and another 16 of them KX mice, and their average likelihood of choosing the right side is shown in the graph. When the tone frequency was above 10000Hz, the higher likelihood of choosing the right side was, the more accurate the mice were at distinguishing sounds and making spatial orientation decisions. On the other hand, when the tone frequency was below 10000Hz, the lower the y-axis value is, the more accurate the mice were. The black curve represents the average performance of the Saline mice while the red curve represents the average performance of the KX mice. It can be deduced from the graph that ketamine has a higher impact on mice's left turn decision, and this pattern is consistent in repeated experiments as well. (Figure 3)



Red: ketamine group

Black: saline group

Fig.3 the average accuracy curve

To understand such a difference, researchers first identified which sound-perception-related and space-percep-

tion-related brain regions are activated by ketamine by examining the scanning copies of brain slices previously stained with C-fos stain.

After neurons and brain cells undergo C-fos staining, they glimmer green under blue light if they are activated, which distinguish them from other inactivated cells. By comparing the number and scale of green glimmering cells in certain brain regions between saline group and ketamine group, researchers identified the brain regions and areas apparently activated by ketamine, which include TeA and hippocampus region, corresponding to former speculation. (Figure 4)

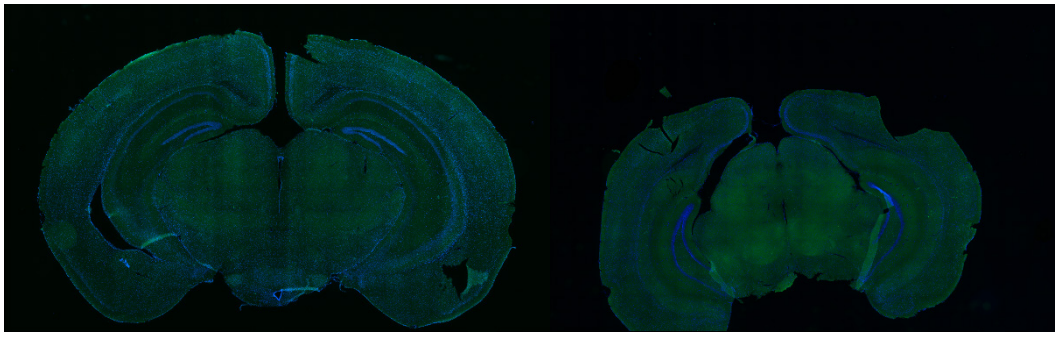


Fig.4 brain scanning copies; left:saline mice; right: ketamine mice

Researchers decided to focus primarily on TeA brain region, recording brain signals to determine whether difference exist between left-turning and right-turning.

The graph of the average signal of left turn and right turn is shown below. It can be seen from the graph that left-turn and right-turn signals have obvious difference in the mice's right brain hemisphere, thus providing one possible explanation for the difference of impact ketamine has on left-turning decisions and right-turning decisions, which is that ketamine impacts left and right hemispheres in different ways. (Figure 5)

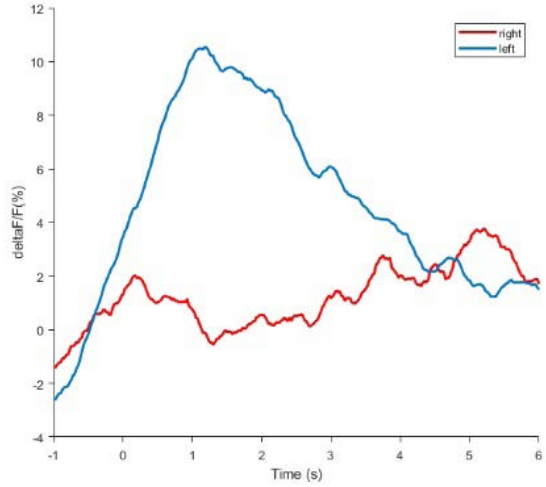


Fig.5 the average signal of left turn and right turn

4. Conclusion

From the difference in left-turning and right-turning signals in mice's TeA brain region and a peak-shaped activation curve for left-turning signals, it can be concluded that TeA region also plays a role in spatial orientation decision. The higher level of cell activation in TeA region in C-fos stained brain slices from KX mice compared to Saline mice proves the impact of ketamine on TeA. The lower accuracy of KX mice in the behavioral experiment shows the impact of ketamine on mice's sound perception and spatial orientation from the behavioral perspective. Therefore, the conclusion of the research is that ketamine influences mice's sound perception and spatial orientation by affecting the signals in TeA brain region.

However, it is still not studied the cell specific mechanism of ketamine in TeA region, which is a field for future studies. It can also be seen from the brain slices that other brain regions related to spatial perception are activated by ketamine, such as entorhinal cortex and the hippocampus, which requires further research to find out their role in ketamine impact. Last but not least, the different impact of ketamine on left and right hemispheres needs to be explored and explained further in future studies.

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