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The Impact of Local Ketamine on Spatial Cognition Through Dopamine

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Abstract:

The dopamine system plays a crucial role in maintaining spatial cognitive functions. Ketamine, a non-competitive NMDA receptor antagonist, indirectly affects dopamine release and function by modulating glutamatergic neuronal activity, leading to spatial cognitive impairments. This study aims to explore the specific mechanisms through which local ketamine administration affects spatial cognition. By injecting ketamine into the hippocampus of mice and combining behavioral experiments with brain tissue analysis, we found that ketamine-induced dopamine damage significantly impairs the mice's spatial orientation abilities. This discovery offers new insights into the negative impact of ketamine addiction on cognitive function.

Keywords: Ketamine, Hippocampus, Dopamine, VTA, Spatial Cognition

1 Introduction

Ketamine is commonly used as a dissociative anesthetic and for pain treatment. In addition to this it can increase dopamine levels making it a useful antidepressant to fight against depression. Therefore, ketamine is addictive and abused because of its ability to stimulate the hypothalamus, where dopamine is released in the brain, and have pleasurable effects[1]. Ketamine abusers usually are represented by adolescents and young adults. According to the Drug Abuse Warning Network, 74 percent of ketamine emergency department mentions are made up of people ages 12 to 25 in the United States[2]. The abuse of ketamine and the psychotomimetic effects of ketamine are linked to the dopaminergic system leading to outputs of irregular levels of dopamine causing dopamine damage. Dopamine is a key neurotransmitter in the brain, although in previous research by researchers have found that dopamine is used for motor and limbic functions such as hunger, aggression, and arousal, dopamine also has a role in cognitive functions such as spatial localization[3].

In cognitive psychology, spatial cognition refers to the ability to use knowledge of the spatial environment to understand how to act and move in space. In 2016, a group of researchers used a6-hydroxy-dopamine rat modeling the Parkinson's disease to discover that dopamine is an essential neurotransmitter in spatial cognitive learning. Their results show that dopamine depletion results in spatial impairments and mistakes in the neural encoding of spatial memory and decision-making processes in the hippocampus[4-5].

Spatial cognition is a higher field of cognition that both animals and humans have. Using this connection allows researchers to use invasive animal studies to infer similar brain activity in humans[6]. This study will use brain stereotactic injection and optical fiber embeddings to inject virus into the hippocampus. This virus will be activated when given blue light via the embeddings on the mouse's head, using this we will track the reaction of the mouse and its effect on its spatial cognition. In addition, this study will use the brain of a ketamine effected mouse by staining the neuronal activity to locate where it effects the brain. Using this information, we will conclude it effects on the spatial cognition of mice.

2 Materials and Methods

2.1 Brain stereotactic injection and optical fiber embedding

2.1.1 Materials

1) Viruses, laboratory animals (mice), disinfectants, ketamine

2) Stereotactic apparatus, grinding drill, microinjection pumps, sodium chloride injection, ophthalmic erythromycin ointment.

2.2.2 Methods

1) Surgery preparation
Anesthetize mice with an appropriate anesthetic, usually by inhalation or intraperitoneal injection (IP injection). After making sure that the mouse is completely anesthetized, the hair on top of the head, the hair between the eye line and the ear line, is shaved with a razor.
2) Head fixation
Use ophthalmic erythromycin ointment to protect the cornea of the mouse from the surgical light. The head of the mouse is fixed to the stereotactic apparatus, so that the mouse incisors are stuck in the apparatus's incisor clip. The height of the apparatus and the position of the front and back are adjusted to facilitate the access of the ear rods to the external acoustic meatus. The tips of the two ear rods are aimed at the skull depression in front of the external acoustic meatus, and the head is fixed by adjusting the ear rods so that the head of the mouse was kept in the center of the U-shaped opening.
3) Position
The skin of the mouse head and the fontanelle is disinfected before and after exposure of the skin of the mouse's head is cut with scissors. Expose the front and back fontanelle, clean the periosteum with sterilized cotton head or blade. Use a micro syringe to withdraw the virus and ensure no air bubbles. Using micro injector tip point, locate the anterior fontanelle as zero point, and leveling the needle, before and after leveling and left and right leveling, through the brain atlas to determine the coordinates. A micro syringe is used to determine the location of the injection on the surface of the skull and marked with ink.
4) Drilling injection
Using a grinding drill, create a hole at the marked point and remove the skull and dura mater. Lower the syringe, zeroing when the tip touches the brain surface. Inject at 0.2 μL/min, then leave the needle for 15 minutes before slowly withdrawing. If optical fiber implantation is needed, clean and roughen the skull, then lower and secure the fiber 200-300 μm above the injection site with dental cement. After the cement hardens, monitor the mouse on a heating pad until it wakes, then return it to its cage.

2.2 Calcium Signal Recording Process

Open the calcium signal recorder system to adjust the parameters, adjust the video and calcium signal record to record the data. Put the jumper in the dark box to record 5 minutes as the baseline value. Then the jump line connects the mouse's head fiber to continue the recording, after recording for a certain period of time, the mouse is removed and injected with medication, 30 mg kg ketamine or saline, after which the jump line continues the recording and observes the mouse behavior. The mouse's calcium signal is then recorded every certain time.

2.3 Cardiac perfusion

2.3.1 Materials

Infusion pump (if no infusion pump, injector may be used), scalp needle with soft catheter, scissors, forceps, hemostatic forceps, PBS, 4% paraformaldehyde.

2.3.2 Methods

1) Preparation before perfusion:
The mice are anesthetized by intraperitoneal injection, the limbs are fixed on the dissecting table with the chest and abdomen fully exposed. The thoracotomy—a surgical procedure, cutting between ribs to reach the lungs and heart—is performed. Cut open the subcutaneous tissue of the chest, then lift the xiphoid—bone in-between the ribs holding them together at center of chest—with tweezers and cut open the chest with scissors. Tear the pericardium (film that protects heart) with tweezers and expose the heart.
2) Perfusion:
The needle is inserted into the left ventricle and PBS is slowly pumped at a rate of 0.15 to 0.2ml per second until the drain from the right atrial appendage is almost colorless. At this time change to paraformaldehyde perfusion. When the mouse appears tail hanging and limbs twitching state, indicates that formaldehyde has entered the mouse's body, the stiffness of the mouse body indicates that perfusion success, on the contrary, continue to perfusion until the mouse body stiffens, after pulling out the needle.
3) Brain harvesting:
When the body of the mice is stiff and the internal organs turn white, the head of the mouse is removed, cut off the head, the neck and temporal muscles to expose the skull, cut off the cartilage coated on the medulla oblongata, insert the ophthalmic scissors from the cross between the eyes, break the skull apart, and then abstract the complete brain tissue. Keep the brain intact. Preserve the brain in PBS in fridge.

2.4 Immunofluorescent staining

Retrieve the brain, remove the brain from the PBS. Freeze the brain using OCT compound to put into the microtome machine. Observe the brain while cutting to locate the brain areas necessary for research. Preserve the cut brain in PBS for staining. The slide is washed in 0.1% PBST three times for 6 minutes, then in 0.3% PBST for 30 minutes, followed by three 6-minute washes. After drying, a water-blocking ring is drawn, and 5% BSA is added for 1 hour. The first antibody, diluted in 0.1% PBST, is incubated for 24 hours at 4°C. After warming to room temperature and washing, the second antibody is added and

incubated in the dark for 2 hours. Finally, the slides are washed, sealed with anti-fading solution containing DAPI, and observed via microscopy.

3 Results

3.1 Impact of Ketamine on Sensory Processing in Mice

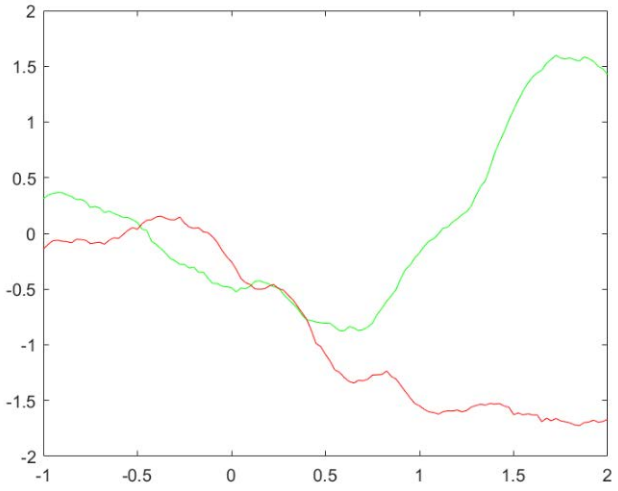

Figure 1 Brain activity during decision-making (left or right turn)

This figure 1 shows the calcium signal recording of the mouse when deciding when to turn left or right. The green line shows the want to turn left while red shows turning right. The higher the brain waves show the mouse turns in that direction. The amplitude of the brain waves indicates the likelihood of turning in that direction, with higher wave amplitudes correlating with the chosen direction. As observed, the green line (left turn) rises significantly around 1.5, suggesting the mouse's decision to turn left, while the red line (right turn) shows less amplitude, indicating a lower probability of turning right. This pattern demonstrates the neural basis of spatial decision-making in the mouse.

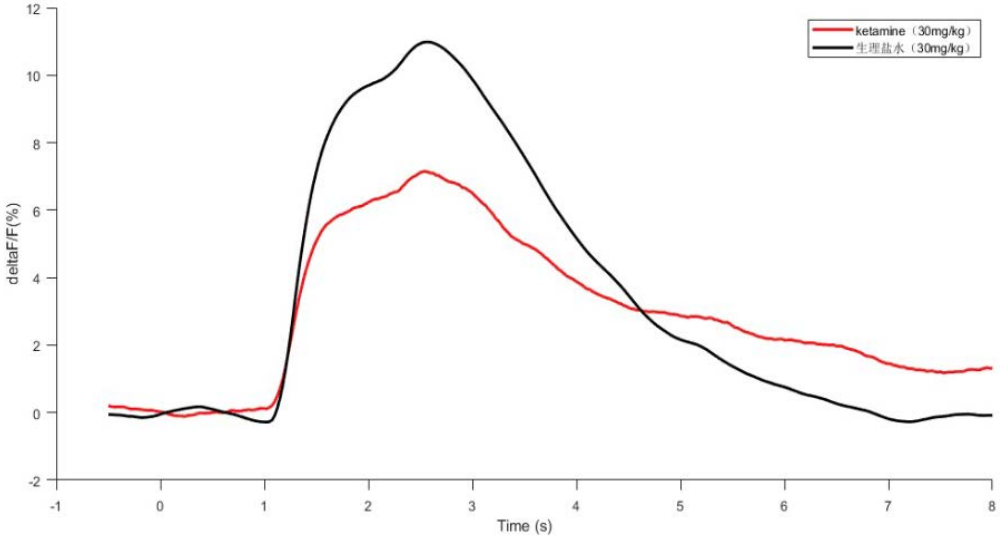

Figure 2 DA signal intensity during sound processing

To assess the effect of ketamine injection on the auditory processing function in mice, we used the DA biosensor dLight to detect DA signal intensity. The black line represents normal mice, while the red line represents mice injected with ketamine through stereotactic brain injection. The sound was given at 1 second and stopped at 2 seconds. The graph shows the response to the sound onset and offset, with two peaks: the first peak corresponds to the mouse's reaction when the sound was played, and the second, higher peak corresponds to the mouse's response

when the sound was turned off. As shown in the figure, compared to normal mice, the dopamine levels in the ketamine-injected mice were significantly lower when reacting to the sound. This indicates that ketamine has impaired dopaminergic signaling in the brain, reducing the mice's ability to process and respond to auditory stimulation. (Fig.2).

3.2 2AFC behavioral experimental results

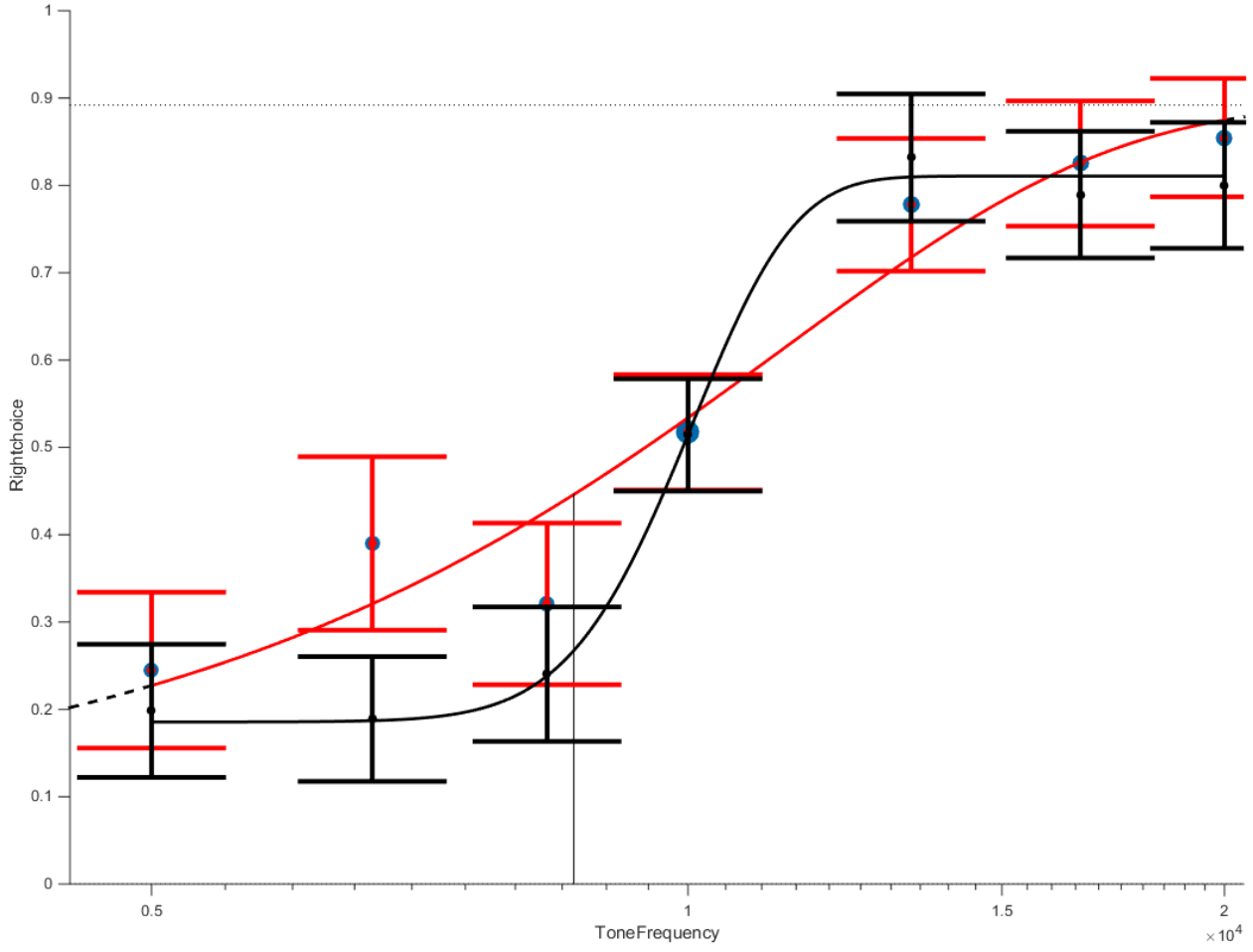

Figure 3 Accuracy in Tone Frequency Differentiation in Normal and Ketamine Infected Mice

This graph shows the effect of ketamine of spatial cognition (Fig.3). The red line represents a mouse who was injected with ketamine and the black line shows a regular mouse. Both mice are performing a task where a tone is played, depending on the frequency of it the mice will go to the right or left. This graph shows the accuracy of the mice when they move to the right corresponding with higher frequency sounds. As the black line suggests, the regular mouse has no trouble distinguishing between the sounds although there will be some mistakes. There is a sharp curve upwards as the frequencies increase and the accuracy increases as well. Compared to the normal mouse, the mouse injected with ketamine has more difficulty differentiating between the lowest frequency sounds and the highest frequency sounds but have trouble with the ones in between. This may be due to ketamine impairing the auditory processing system or impairing spatial cognition.

3.3 Immunofluorescence result of c-Fos

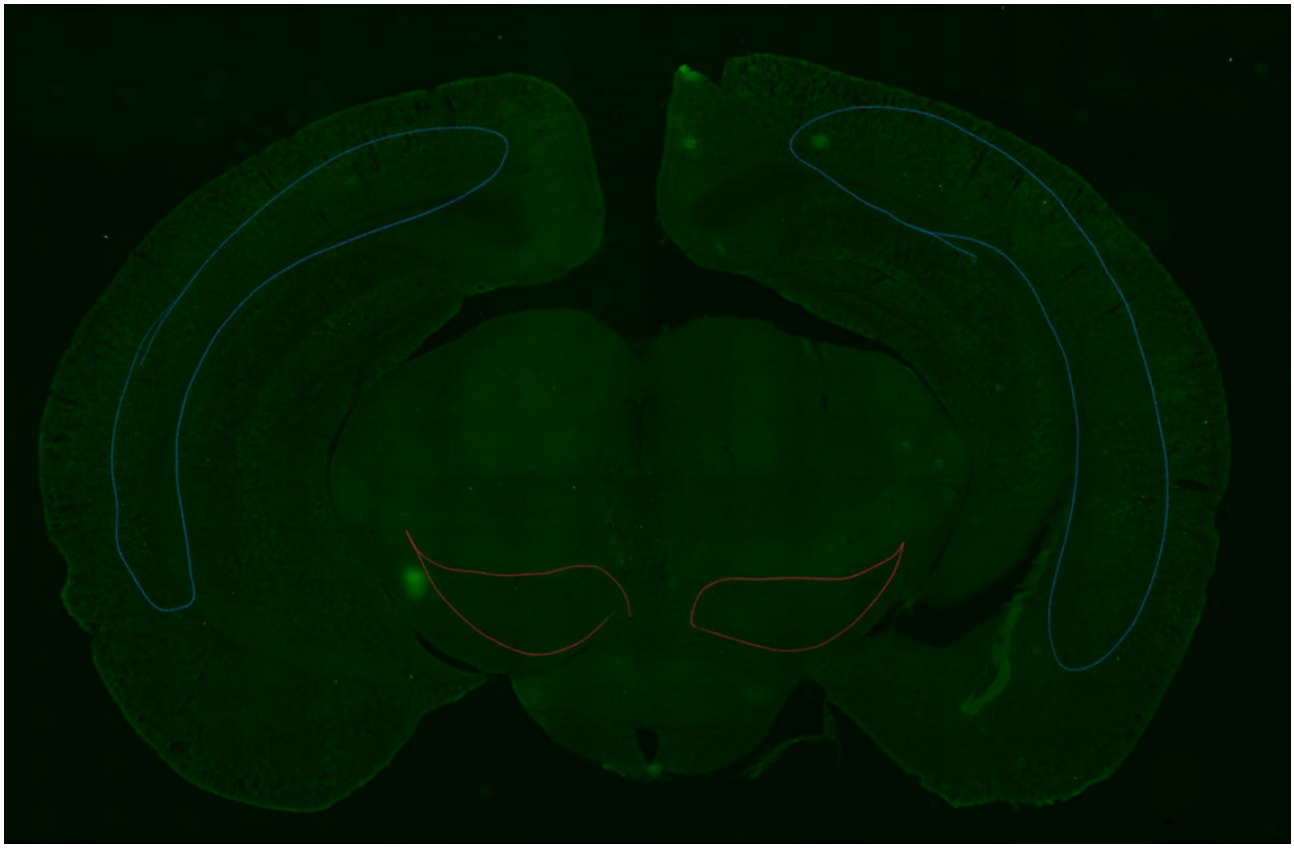

Figure 4 Ketamine Infected in Hippocampus and VTA

This is a picture (Figure 4) of the brain of a mouse who was injected with ketamine using brain stereotactic injection. The parts marked—the blue and red—are the hippocampus and the VTA respectively. In those marked areas, there are faint green glowing spots. These represent the nerves that have been affected by the ketamine. The picture shows that the hippocampus was more effected by the ketamine than the VTA by comparing the number of green spots in both areas. Although the VTA was not affected a lot but there are still spots of green in the red areas. Both of these brain areas are responsible for dopamine regulation so impairment in either one could lead to impairment in spatial cognition. This evidence supports the graph in figure 3 where the ketamine induced mouse had trouble correctly differentiating between high and low frequencies. Impairment in spatial cognition makes it so that the mouse has trouble distinguishing between left and right therefore leading to the wrong decision made.

4 Conclusion

Ketamine administration leads to unstable dopamine levels by impacting the hippocampus and VTA causing spatial cognition impairment. Additionally, it causes decreased sensory input as shown through the calcium signal recordings. A decease in spatial cognitive abilities cause us to lose awareness of the environment and space, therefore losing the ability to navigate the world. This is shown through the graphs of the experiments. This study will hope to inform people, especially abusers of ketamine of the effects of the drug. Abuse of ketamine impairs many important functions needed for everyday survival and abuse of ketamine decreases functionality in everyday life.

Further research should explore the long-term impact of ketamine on spatial cognition and its interaction with other neurotransmitter systems. Understanding the precise neural circuits affected by ketamine could inform the development of targeted therapies to mitigate these cognitive deficits. Additionally, public health initiatives should focus on educating at-risk populations about the cognitive risks associated with ketamine use, potentially reducing its abuse and the associated cognitive decline.

References

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