MEDS Public Health and Preventive Medicine (2025)
Clausius Scientific Press, Canada
DOI: 10.23977/phpm.2025.050217
ISSN 2616-1915 Vol. 5 Num. 2

The Functional Localization of VTA in Ketamine-Induced Dysfunction of Auditory Decision-Making

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Keywords: Auditory Decision-Making, VTA, Ketamine, Acousma

Abstract: This study investigates the functional localization of the ventral tegmental area (VTA) in ketamine-induced disruptions of auditory decision-making in mice, with the broader goal of elucidating the neural mechanisms underlying perceptual disturbances, such as hallucinations. Although ketamine is increasingly used in clinical settings, particularly for treatment-resistant depression, the specific brain circuits mediating its adverse cognitive and perceptual effects remain inadequately characterized. To address this knowledge gap, we employed a multi-modal experimental approach integrating behavioral testing, stereotaxic microinjections, immunofluorescence staining, and in vivo neuroimaging techniques to assess the VTA's role in sensory-cognitive processing following ketamine administration. Mice were trained in a two-alternative forced choice auditory task designed to evaluate their ability to discriminate between different tone frequencies. Compared to controls, ketamine-treated mice exhibited significantly flattened psychometric function, indicative of impaired auditory discrimination and degraded stimulus-response mapping, resembling hallucinatory-like behavior. Immunofluorescence analysis revealed robust upregulation of c-Fos expression within the VTA, suggesting increased neuronal activity. In parallel, in vivo measurements of 8 auditory-evoked trunk signal intensity showed attenuated responses in the ketamine group, further implicating disrupted sensory signal transmission. These converging data indicate that ketamine induces both functional and molecular alterations in the VTA, a dopaminergic hub implicated in reward, motivation, and sensory modulation.

1. Introduction

The Ventral Tegmental Area (VTA) is a midbrain structure located ventral to the periaqueductal gray matter. It is one of the central components of the brain's dopaminergic system, primarily composed of dopaminergic neurons. The VTA projects through both the mesolimbic and mesocortical pathways to several brain regions, including the nucleus accumbens, prefrontal cortex, hippocampus, and amygdala. The VTA plays a critical role in regulating reward processing, motivational behavior, reinforcement learning, and emotional modulation. Dopaminergic activity within the VTA is essential for encoding reward-related cues, guiding goal-directed behavior, and supporting adaptive decision-making[1]. Moreover, the VTA interacts with stress-related circuits and

contributes to the long-term impact of chronic stress on brain function and structure[2].

Ketamine is a phencyclidine derivative primarily known for its role as a dissociative anesthetic. It exerts its pharmacological effects mainly by antagonizing the N-methyl-D-aspartate (NMDA) receptor, a subtype of glutamate receptor involved in excitatory neurotransmission and synaptic plasticity[3]. Clinically, ketamine has long been used for the induction and maintenance of anesthesia in both human and veterinary medicine, valued for its rapid onset, preservation of airway reflexes, and cardiovascular stability. However, beyond its anesthetic properties, ketamine has more recently emerged as a promising agent in the treatment of major depressive disorder and treatment-resistant depression. Sub-anesthetic doses of ketamine—administered intravenously or intranasally—have demonstrated rapid antidepressant effects, often within hours, in contrast to the delayed onset of traditional monoaminergic antidepressants[4].

Auditory decision-making—a cognitive process integrating sensory perception with executive control—relies on finely tuned neuromodulatory mechanisms. Disruption of this process, as observed in neuropsychiatric disorders and pharmacological interventions, provides critical insights into the neural substrates of sensory-cognitive integration. The non-competitive *N*-methyl-D-aspartate receptor (NMDAR) antagonist ketamine, while therapeutically valuable, induces dose-dependent impairments in auditory discrimination that recapitulate core features of schizophrenia-spectrum pathologies. Emerging evidence positions the ventral tegmental area (VTA) as a pivotal hub in this dysfunction[5].

Existing studies offer limited evidence to confirm the specific role of the ventral tegmental area (VTA) in ketamine-related impairments of auditory decision-making. In particular, the neural mechanisms linking VTA activity to these cognitive disruptions remain poorly defined. This project aims to strengthen the evidence for VTA involvement in ketamine-induced dysfunction and to investigate how VTA circuits contribute to these effects. A clearer understanding of this pathway may support the development of treatments for related psychiatric conditions.

2. Methods

2.1 Experiment design and animal groups

Blank control group: No injection treatment was administered. This group was used to assess the baseline condition and the potential effects of experimental procedures (such as grasping and injection).

Ketamine treatment group(KT group): Intraperitoneal injection of a specific dose of ketamine solution (dissolved in sterile normal saline).

2.2 Behavioral Experiment

We conducted an auditory decision-making task in mice using a custom transparent chamber placed inside a sound-attenuating box. The chamber had three side ports, each fitted with sensors and water delivery tubes for rewards. Auditory stimuli were played through a speaker (ES1, Tucker-Davis Technologies), and mouse behavior was recorded with an overhead infrared camera. The task was controlled by the Bpod system, an open-source MATLAB-based platform. Training included three stages: Stage 1: Mice hear 5 kHz and 20 kHz tones. Incorrect choices were not punished. Stage 2: White noise was introduced as a punishment for incorrect responses. Stage 3: Mice were trained to respond left for low tones, right for high tones, and showed ~50% rightward choice at 10 kHz—an ambiguous cue between categories.

2.3 Stereotactic Injection

Glass micropipettes (inner diameter: 0.3–0.5 mm) were pulled using a laser micropipette puller to produce tips ~10–13 μm in diameter. After backfilling the pipette with a viral solution, it was mounted onto the stereotaxic apparatus. Mice were anesthetized with 5% isoflurane for induction and maintained at 0.5% during surgery. After securing the mouse in a stereotaxic frame, erythromycin eye ointment was applied, and the scalp was disinfected with iodine. The skull was exposed and a small craniotomy was performed at the target coordinates (TeA: AP -3.4 mm, ML ±4.7 mm, DV -1.15 mm). Virus was injected at 1 nL/s using a microinjection pump. The pipette was left in place for 20 minutes post-injection to allow diffusion, then slowly withdrawn. Mice were placed on a heated pad post-surgery until recovery and housed individually. A minimum of two weeks was allowed for viral expression.

2.4 Mouse Brain Frozen Sectioning

Following fiber photometry, retrograde tracing, or optogenetics, brain tissue was collected to verify injection and implantation sites. Mice were deeply anesthetized with 5% chloral hydrate and perfused transcardially with 0.1 M PBS followed by 4% paraformaldehyde (PFA). Brains were post-fixed in 4% PFA at 4 ℃ for 24 hours, then cryoprotected in 30% sucrose until sinking. Tissues were embedded in OCT and sectioned at 45 μm using a cryostat (CM1950, Leica). Sections were stored for immunofluorescent staining or anatomical validation.

2.5 Immunofluorescence

To verify injection and fiber placement as well as assess molecular expression, brain sections were processed for immunofluorescence following fiber photometry, retrograde tracing, or optogenetics. Mice were anesthetized with 5% chloral hydrate and perfused with 0.1 M PBS followed by 4% paraformaldehyde (PFA). Brains were post-fixed in PFA at 4 ℃ for 24 h, then cryoprotected in 30% sucrose. Tissue was embedded in OCT and sectioned coronally at 45 μm (CM1950, Leica). Staining Procedure: Sections were washed in PBS (3 × 5 min). We permeabilized the cells in 0.5% Triton X-100 (PBST) for 30 minutes and blocked them in 5% fetal bovine serum in 0.1% PBST for 40 minutes. We incubated the cells at 4 ℃ for 48 hours with primary antibodies: Rabbit anti-c-Fos (1:5000), Rabbit anti-CaMKII (1:400), Rabbit anti-PV (1:500), and Rabbit anti-SOM (1:500). After washing with PBS (3 × 5 min), we incubated them in Alexa Fluor 488-conjugated secondary antibody (1:1000) at room temperature for 2 hours in the dark. We washed the cells again in PBS (3 × 5 min) and mounted the slides using anti-fading mounting medium. Slides were air-dried and cleaned for imaging.

2.6 Imaging the Brain Slices

Brain sections were digitized using the VS120 imaging system and EVOS7000 microscope, saved in 16-bit TIFF format at 2048×2048 resolution. Confocal imaging was performed with an Olympus Fluoview 3000 using 20× (NA 0.75) and 40× (NA 0.95) objectives, with a 1 μm z-step. ImageJ was used for manual neuron counting and area measurement. Images were converted to 8-bit, the background subtracted, and thresholded to binary. Target regions were delineated using the "Freehand selections" tool, and areas were measured with the "Measure" function. Data were exported to Excel for further analysis.

2.7 Statistical analysis

Data are presented as mean ± SEM. Statistical comparisons between groups were performed using GraphPad Prism 9.0 and custom scripts. Calcium imaging data were analyzed in MATLAB. One-way ANOVA followed by multiple comparison tests was used for analyses involving three or more groups. Behavioral data from auditory decision-making tasks were analyzed using rank-sum tests.

3. Results

3.1 Auditory Behavioral Task Reveals Ketamine-Induced Perceptual Disruption

To evaluate the potential hallucinatory effects of ketamine on perceptual decision-making, we employed an auditory two-alternative forced choice task in trained mice. This behavioral paradigm assesses the animals' ability to discriminate between different tone frequencies and make directional decisions accordingly.

In the task, mice were trained to associate low-frequency tones with a leftward response (left water port) and high-frequency tones with a rightward response (right water port), with intermediate frequencies (e.g., 10 kHz) serving as ambiguous stimuli to probe perceptual boundaries. Figure 1 displays the psychometric performance of the two experimental groups. The black line represents the control group (mice injected with PBS), and the red line corresponds to the ketamine-treated group. The vertical axis indicates the probability of selecting the right water port, while the horizontal axis reflects the frequency of the auditory stimulus presented during each trial. Control mice exhibited a characteristic sigmoid-shaped psychometric function, demonstrating reliable and frequency-dependent decision-making. At lower frequencies (<8000 Hz), control mice showed a lower probability of choosing the right port, favoring leftward responses. As tone frequency increased, its choice probability shifted progressively toward the right port, indicating successful discrimination of tonal cues and a consistent mapping between stimulus and behavioral output. In contrast, mice treated with ketamine showed a flattened psychometric curve, with markedly less differentiation in choice behavior across the frequency spectrum. This attenuated slope reflects impaired sensitivity to frequency differences and a reduction in decisional certainty. Notably, in the low-frequency range—where a correct behavioral response would be a leftward choice—ketamine-treated mice exhibited a near-random distribution of left/right responses, suggesting a breakdown in stimulus-response mapping. This disruption in auditory decision-making likely reflects ketamine-induced alterations in perceptual processing.

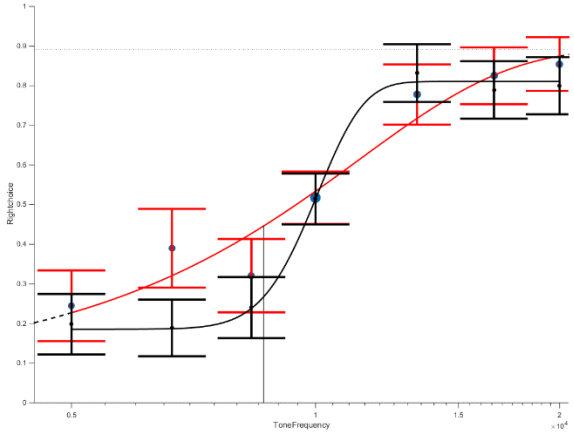

Figure 1 Behavior analysis of ketamine and saline injected groups

Given ketamine's known antagonistic effects on NMDA receptors, the results support the interpretation that cortical and subcortical circuits involved in auditory discrimination—potentially including the auditory cortex, thalamus, and midbrain dopaminergic regions such as the VTA—are functionally compromised. The inability of ketamine-treated mice to appropriately interpret low-frequency tones may be analogized to hallucinatory-like symptoms, in which external sensory input is misperceived or misinterpreted, leading to erroneous behavioral outputs. These findings align with clinical and preclinical literature suggesting that ketamine can induce perceptual distortions and cognitive dysregulation resembling psychosis.

3.2 Immunofluorescence Evidence of VTA Activation under Ketamine Exposure

Immunofluorescence staining (Figure 2) was performed to evaluate protein expression and neuronal activation in the ventral tegmental area (VTA) following ketamine administration. In brain sections obtained from control mice (non-injected group), the VTA region did not exhibit notable fluorescent labeling, suggesting a low baseline level of activity or target protein expression under physiological conditions. In contrast, brain sections from ketamine-treated mice displayed a pronounced accumulation of fluorescent signals specifically localized within the VTA. The concentrated fluorescent labeling observed in the VTA of ketamine-injected mice indicates a marked upregulation of activity-dependent markers, such as c-Fos or other related proteins, in response to ketamine exposure. This elevation in fluorescence intensity suggests that ketamine induces significant neuronal activation or molecular signaling events within this dopaminergic midbrain region.

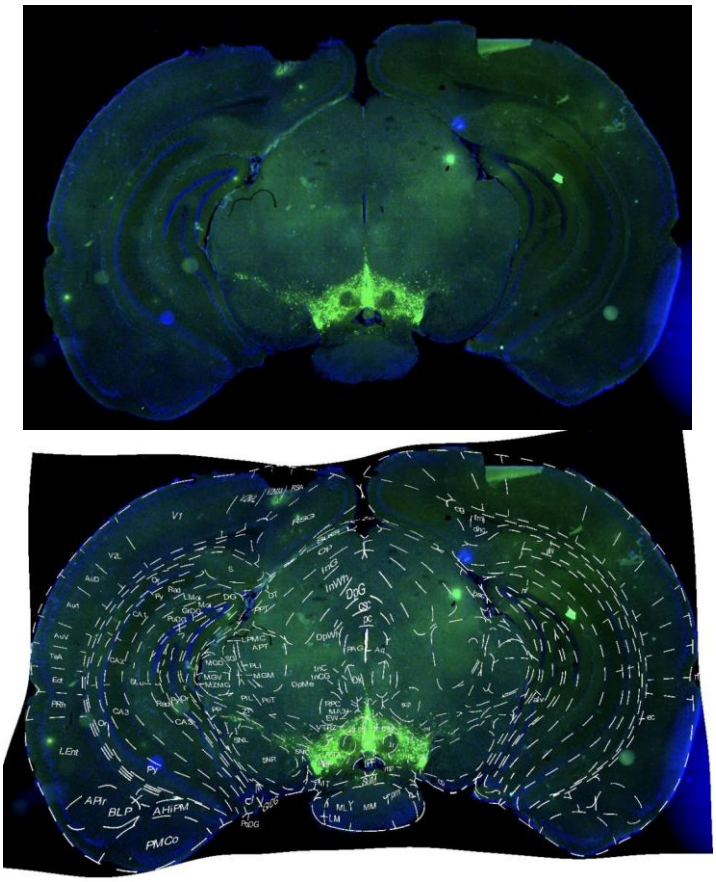

Figure 2 Immunofluorescence of VTA after ketamine injection

Given the VTA's critical role in modulating reward, motivation, and decision-making, the

enhanced protein expression following ketamine administration implies that this region may be centrally involved in the drug's disruption of auditory and cognitive processes.

The data support the hypothesis that ketamine alters the functional state of the VTA, possibly contributing to the auditory decision-making deficits and perceptual abnormalities observed behaviorally. These findings further underscore the VTA as a key neural substrate mediating the neuropsychiatric effects of ketamine.

3.3 Weakened Neural Responses after Ketamine Treatment

To investigate the impact of ketamine on auditory-related neural activity, we conducted a series of in vivo recordings to measure neural trunk signal intensity in the mouse brain under a range of sound intensities. The results are illustrated in Figure 3, where the red curve represents data from the control group (mice injected with phosphate-buffered saline, PBS), and the blue curve corresponds to data from mice administered with ketamine. The vertical axis denotes the magnitude of neural trunk signal intensity, while the horizontal axis represents increasing levels of auditory stimulus intensity.

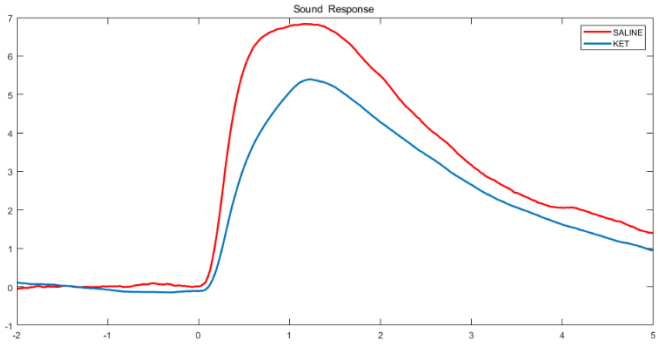

Figure 3 Neural activity of VTA in ketamine and saline injected groups

Analysis of the data revealed a robust neural response to sound in the control group. As auditory stimulus intensity increased, a corresponding rise in neural trunk signal strength was observed, reflecting a typical stimulus-dependent activation pattern within the auditory pathways. This pattern indicates intact auditory signal transduction and neural integration in control animals. In contrast, ketamine-treated mice demonstrated a markedly blunted neural response across a range of sound intensities. The overall neural trunk signal was consistently lower compared to controls, with significantly attenuated responses even at higher sound levels. This suggests that ketamine disrupts the fidelity or amplification of auditory signaling at the neural circuit level.

These findings provide compelling evidence that ketamine administration impairs the brain's ability to process auditory information by suppressing neural trunk signal intensity. Given the essential role of these neural signals in encoding and transmitting sensory input from peripheral auditory structures to higher-order brain regions, the observed reduction in signal intensity may underlie the behavioral impairments in auditory decision-making previously reported. The data further support the hypothesis that ketamine exerts its neurophysiological effects through modulation of sensory and integrative pathways, particularly within circuits implicated in auditory perception and cognitive evaluation.

4. Conclusion

In the investigation of the functional localization of the ventral tegmental area (VTA) in ketamine-induced dysfunction of auditory decision-making, a comprehensive experimental

approach was employed. Specifically, I conducted a series of behavioral assays, immunofluorescence analyses on brain slices, and stereotaxic injection experiments to achieve precise anatomical localization of the VTA within the murine brain. Utilizing these methods, I was able to successfully identify and target the VTA region with high spatial accuracy. Following the administration of ketamine, a significant increase in the number and intensity of fluorescent signals was observed within the VTA region of the experimental group compared to the control group. These fluorescent markers, which were indicative of neuronal activation and protein expression changes, suggest an enhanced level of neuronal engagement or structural remodeling within the VTA following ketamine exposure. The behavioral test results further supported this neuroanatomical finding. Mice treated with ketamine exhibited altered auditory decision-making behaviors, consistent with patterns observed in hallucinatory-like states.

Taken together, these results indicate that ketamine exerts a stimulatory effect on the VTA, a key dopaminergic nucleus involved in reward processing and sensory integration. Moreover, the observed neurobiological alterations in this region likely contribute to the disruption of auditory perception and the emergence of hallucinatory-like behavior in mice. In conclusion, the data strongly support the hypothesis that the VTA plays a pivotal role in mediating ketamine-induced auditory processing deficits. These findings provide valuable insights into the neurobiological underpinnings of hallucination-like phenomena and highlight the VTA as a potential target for further research into the mechanisms of psychotomimetic drug action.

The present study aims to investigate the functional effects of ketamine, a well-known dissociative anesthetic, within a specific region of the murine brain: the ventral tegmental area (VTA). Through a combination of behavioral paradigms, neuroanatomical localization, and molecular visualization techniques, this research endeavors to elucidate the role of the VTA in ketamine-induced alterations of auditory decision-making processes in mice. The findings, while rooted in animal models, offer valuable implications that may be extended to enhance our understanding of ketamine's neurophysiological effects on the human brain. This line of research is of considerable clinical and pharmacological relevance. Ketamine, despite its therapeutic potential in anesthesia and treatment-resistant depression, is also associated with a range of adverse neuropsychiatric effects, including hallucinations and cognitive impairments. By clarifying how ketamine modulates activity within the VTA—a core dopaminergic hub implicated in motivation, reward, and sensory integration—this study contributes to a more nuanced understanding of the mechanisms through which ketamine may induce hallucination-like phenomena. Ultimately, this may inform the development of strategies aimed at mitigating the drug's undesirable side effects in clinical contexts.

However, it is important to acknowledge several limitations inherent in this study. Firstly, the experimental data are derived exclusively from murine models, which, although informative, do not fully replicate the complexity of the human brain. The anatomical and functional differences between rodent and human neural systems necessitate caution when extrapolating findings across species. Secondly, the scope of the current research is relatively narrow, focusing solely on the VTA without accounting for potential interactions with other brain regions known to be affected by ketamine, such as the prefrontal cortex, hippocampus, and thalamus. In addition, potential confounding variables—such as dosage variance, behavioral state, and inter-individual variability among test subjects—were not comprehensively examined in this initial investigation.

Looking toward future directions, this research provides a strong foundation for broader neuropharmacological inquiries. Follow-up studies could expand the scope of investigation to encompass whole-brain analyses of ketamine's effects, employing techniques such as whole-brain c-Fos mapping, optogenetics, or functional MRI in rodents and, eventually, in non-human primates or human-derived organoid models. Furthermore, a comparative analysis of ketamine's impact

across various brain regions would provide a more holistic view of its neuropsychiatric effects. In the translational domain, the insights gained from this research could support the development of novel anesthetic agents with improved safety profiles, offering alternatives to ketamine that preserve therapeutic efficacy while minimizing adverse effects. Additionally, understanding the neurobiological basis of ketamine-induced hallucinations may contribute to the design of targeted pharmacotherapies for auditory hallucination-related disorders, such as schizophrenia, thereby offering potential clinical benefits to individuals suffering from severe psychiatric illnesses.

In summary, while preliminary and limited in scope, this study represents a meaningful step toward unraveling the specific neural circuits affected by ketamine. Its implications span both basic neuroscience and translational medicine, offering pathways for future research and therapeutic innovation.

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