MEDS Public Health and Preventive Medicine (2025)
Clausius Scientific Press, Canada

DOI: 10.23977/phpm.2025.050219
ISSN 2616-1915 Vol. 5 Num. 2

Ketamine-Induced Activation of the TeA Region in Mice Correlates with Auditory and Visual Hallucinations

Xu Le

New York University, 70 Washington Square South, New York, NY10012, USA
lx2255@nyu.edu

Keywords: Ketamine, Temporal Association Cortex (TeA), Auditory Hallucinations, Calcium Imaging/ c-Fos Expression, Auditory Discrimination Deficits

Abstract: Ketamine, a non-competitive NMDA receptor antagonist, is widely used to model hallucination-like states in both humans and animals, yet the precise neural mechanisms underlying its auditory effects remain unclear. This study investigates how ketamine alters auditory perception by targeting the temporal association cortex (TeA), a higher-order auditory processing region. Using a multi-modal approach combining stereotaxic viral injection, fiber photometry-based calcium imaging, immunofluorescence for c-Fos expression, and behavioral analysis via a two-alternative forced choice (2AFC) task, we examined ketamine's impact on TeA function in C57WT mice. We found that ketamine significantly increased c-Fos expression in TeA, indicating elevated overall neuronal activity. Paradoxically, fiber photometry revealed a concurrent reduction in calcium signal intensity, suggesting impaired signal fidelity and auditory information processing. Behaviorally, ketamine-treated mice exhibited marked deficits in high-frequency sound discrimination, supporting a functional link between TeA dysfunction and auditory perceptual impairment. These results suggest that ketamine disrupts auditory cognition by destabilizing TeA circuit dynamics, offering new insights into the neural basis of hallucinations and laying the groundwork for future circuit-targeted interventions.

1. Introduction

Auditory hallucinations refer to the perception of sounds or speech that do not exist in the external environment, often described by patients as voices, music, or other auditory phenomena occurring without a corresponding stimulus. These experiences are prevalent in psychiatric disorders such as schizophrenia, substance use disorder, and certain neurodegenerative conditions (de Leede-Smith & Barkus, 2013)[1]. While hallucinations are primarily characterized by perceptual distortions, they also carry significant emotional salience and can severely impair an individual's social functioning and quality of life.

Understanding the neural mechanisms underlying auditory hallucinations is thus critical for advancing clinical treatments and developing targeted interventions. Previous research has identified the auditory cortex and its higher-order association areas, including the temporal, frontal, and parietal lobes, as key contributors to hallucination generation (Montagnese et al., 2021)[11]. Among these regions, the temporal association cortex (TeA) plays a crucial role in contextual auditory processing

and perception, integrating both sensory input and internal representations. Recent studies using in vivo calcium imaging and behavioral assays have shown that activity in the TeA is significantly disrupted in animal models of hallucination, especially after pharmacological manipulation (Komatsu & Ichinohe, 2020; Zeng et al., 2023)[5][14].

One of the most widely used pharmacological tools in hallucination research is ketamine, a non-competitive NMDA receptor antagonist that can induce hallucination-like states in both humans and animals. Ketamine has been shown to disrupt the excitatory–inhibitory balance, increase cortical noise, and alter auditory discrimination in a dose-dependent manner (Kulikova et al., 2022; Winterer et al., 2023)[7][12]. These effects are believed to stem from ketamine's suppression of fast-spiking GABAergic interneurons, leading to disinhibition of pyramidal neurons and aberrant information processing (Homayoun & Moghaddam, 2007). Notably, several studies have linked ketamine administration to elevated spontaneous activity and a reduced signal-to-noise ratio in association cortices, which may contribute to perceptual distortions characteristic of hallucinations (Zeng et al., 2023)[14].

However, despite these advances, several fundamental questions remain unresolved. First, the precise circuit mechanisms by which ketamine alters TeA function have not been fully delineated. It is unclear how NMDA receptor antagonism in the TeA affects top-down auditory modulation or whether specific projection pathways (e.g., TeA → primary auditory cortex or hippocampus) are selectively disrupted. Second, although prior studies suggest that ketamine exerts cell-type-specific effects, the identity of vulnerable neuronal populations within the TeA, and their role in generating hallucination-like behaviors, remain to be clarified. These gaps limit our understanding of how region-specific and cell-specific disruptions translate into complex perceptual symptoms such as hallucinations.

To address these gaps, the present study employs a fiber photometry-based calcium imaging approach combined with a two-alternative forced choice (2AFC) auditory discrimination task in mice. By examining both neuronal activity in the TeA and behavioral performance following ketamine administration, we aim to elucidate how ketamine interferes with auditory perception and identify the underlying neural mechanisms contributing to hallucination-like phenomena.

2. Experiment Methods

To investigate the effects of Ketamine on auditory perception in mice, we used a combination of stereotactic injections, fiber optic implantation, virus-based neuronal activity labeling, and behavioral assays. This comprehensive approach allowed us to assess how Ketamine influences neuronal activity in the temporal association cortex (TeA), a region crucial for auditory processing, and how these changes correlate with behavioral impairments in sound discrimination.

The first step involved preparing the mice for surgery. Isoflurane anesthesia was administered to ensure a pain-free state, with the appropriate dose tailored to maintain deep anesthesia throughout the procedure. The mice were then placed in a stereotaxic frame, which ensured precise positioning of the head for subsequent brain surgery. To prepare the brain, the head hair of the mice was shaved using an electric clipper, and the skin was cleaned and sterilized. To prevent any damage to the inner ear and stabilize the animal, the ear bars were symmetrically placed in the cochlear recess, ensuring the head was firmly secured without undue pressure.

After the mouse was fully prepared, we performed a craniotomy to expose the brain for targeted injections. The skull was carefully punctured at predefined coordinates, based on the brain atlas, using a fine drill. This allowed for precise access to the TeA region, where experimental manipulations were to occur. A fiber optic probe was then implanted at the injection site, positioned slightly shallower than the viral injection point to avoid compressing the viral diffusion area. The probe was

secured with dental cement to ensure stability throughout the experiment.

Next, we performed a virus injection to express the calcium indicator GCaMP6S in the TeA neurons. Using a microinjector, we carefully injected the virus solution into the TeA region at a controlled rate to avoid any mechanical damage. Once the virus was injected, we allowed a brief waiting period to ensure sufficient absorption. This was followed by the implantation of the fiber optic probe to monitor neuronal activity through real-time calcium imaging during subsequent experiments.

After surgery, the mice were transferred to a warm recovery box to monitor their recovery from anesthesia. During this period, they were observed for any signs of postoperative complications, and the incision site was carefully inspected to ensure there was no infection.

After recovery, we conducted cardiac perfusion to prepare the mouse brain for tissue processing. Under deep anesthesia, the mouse were perfused with PBS to flush out blood and tissue debris, followed by a 4% paraformaldehyde solution for fixation. The brain was then extracted and post-fixed for 4-24 hours before being cryoprotected in a 30% sucrose solution at 4 ℃ overnight. This step ensured the preservation of tissue integrity for subsequent sectioning. The fixed brain tissue was embedded in OCT compound and rapidly frozen using liquid nitrogen.

The brain was sectioned into 10-30 μm thick slices using a cryostat. These sections were mounted onto pre-cooled slides and stored for further analysis. To assess neuronal activation, we performed immunofluorescence staining for c-Fos, a marker of immediate early gene expression indicating short-term neuronal activation. Under baseline conditions, the TeA region showed minimal c-Fos expression, suggesting low neuronal activity. However, following Ketamine injection, a significant increase in c-Fos expression was observed, reflecting enhanced neuronal activity in the TeA.

In parallel, we performed calcium imaging to directly observe changes in neuronal activity in real time. Mice were injected with Ketamine or saline (as a control) and then exposed to auditory stimuli (5000 Hz and 20000 Hz tones). Fiber optic probes allowed us to monitor calcium dynamics in the TeA region while the mice were engaged in the task. Our results showed a marked reduction in calcium signal intensity following Ketamine injection, suggesting that Ketamine interferes with auditory processing at the level of the TeA.

To evaluate how these changes in TeA activity affected auditory perception, we used a two-alternative forced choice (2AFC) task. This task involved training mice to discriminate between high- and low-frequency sounds by selecting the appropriate water dispenser as a reward. Mice injected with Ketamine showed a significant decline in their ability to discriminate between high-frequency sounds, with accuracy dropping notably in the high-frequency range compared to saline-injected controls. This suggests that Ketamine disrupts the mice' auditory discrimination ability, further supporting the hypothesis that Ketamine interferes with auditory processing.

The data analysis included the assessment of accuracy, response times, and behavioral biases in the 2AFC task. Statistical analysis revealed significant differences in the accuracy of sound discrimination between the Ketamine and control groups. Mice injected with Ketamine displayed impaired auditory judgment, further corroborated by the observed changes in neuronal activity measured by calcium imaging and c-Fos expression.

3. Data Recording and Analysis

3.1 Behavioral Data Recording

During the experiment, the following behavioral parameters will be recorded in each trial to evaluate how Ketamine affects auditory perception and decision-making:

Correct Choices: Number of trials in which mice correctly selected the water dispenser matching the presented sound frequency.

Incorrect Choices: Number of trials in which mice selected the wrong dispenser.

Choice Bias: Whether the mice show lateral bias (preference for one side) regardless of sound cues.

Response Time (RT): Time interval between sound onset and choice initiation, used to assess changes in decision speed.

Abort Trials: Number of trials in which no choice was made within a predefined time window, possibly indicating attention deficits or behavioral suppression.

Total Trials: Number of trials each mouse completed per session, used to check engagement and data reliability.

Behavioral events will be video-recorded and time-stamped using a tracking system or manually coded, allowing frame-accurate extraction of behavioral metrics.

3.2 Statistical and Comparative Analysis

To evaluate the behavioral effects of Ketamine, statistical comparisons will be performed between pre- and post-injection conditions as well as between the Ketamine and control groups.

(1) Accuracy Metrics

The behavioral accuracy (ACC) of each mouse will be calculated as:

$$\text{Accuracy (\%)} = (\text{Correct Choices} / \text{Total Trials}) \times 100$$

Accuracy will be compared across conditions to assess whether Ketamine impairs auditory discrimination ability.

(2) Response Time (RT) Analysis

Mean RTs under different drug conditions will be computed. Paired or unpaired t-tests will be used depending on the within-subject or between-subject design. If the data are non-normally distributed, non-parametric tests such as the Wilcoxon signed-rank test will be applied.

(3) Choice Bias Analysis

Chi-square tests or binomial tests will be used to evaluate whether mice develop side bias in decision-making under different conditions.

(4) Abort Trial Rate Analysis

The proportion of abort trials will be statistically compared to assess the potential attention or motor suppression effects of Ketamine.

(5) Longitudinal Analysis

Mixed-effects linear models (MLMs) or Generalized Estimating Equations (GEE) will be employed to model changes in accuracy and RT across multiple testing sessions, evaluating the short-term and long-term impact of Ketamine.

(6) Group-Level Comparison

If multiple cohorts are tested (e.g., Ketamine vs. saline controls), s two-way ANOVA or mixed ANOVA will be used to test for the main effects and interactions between drug condition and session. Post-hoc pairwise comparisons will be adjusted using Bonferroni correction.

All data will be visualized through bar graphs (mean ± SEM), RT histograms, and individual learning curves to depict trends and variability.

4. Experimental Results and Analysis

4.1 Ketamine Affects Neuronal Activity in the TeA Region

C-Fos protein is a commonly used marker for immediate and early gene expression, indicating short-term neuronal activation. By detecting c-Fos expression in the TeA region, we can indirectly assess the effect of Ketamine on neuronal activity in this area. Under baseline conditions, the c-Fos fluorescence signal in the TeA is weak or nearly undetectable, indicating low neuronal activity. However, after Ketamine injection, the c-Fos fluorescence signal in the TeA is significantly enhanced,

reflecting strong neuronal activation. This comparison is visually presented in Figure 1, where the left panel shows the brain of a Ketamine-injected mouse with high fluorescence intensity, while the right panel shows the control mouse with minimal signal. This finding suggests that Ketamine may alter the neural activity pattern in the TeA through direct or indirect mechanisms. In combination with behavioral experimental results, mice injected with Ketamine showed impaired auditory perception and abnormal behavioral responses, further confirming that Ketamine may deeply impact auditory processing and related cognitive functions by modulating neuronal activity in the TeA region.

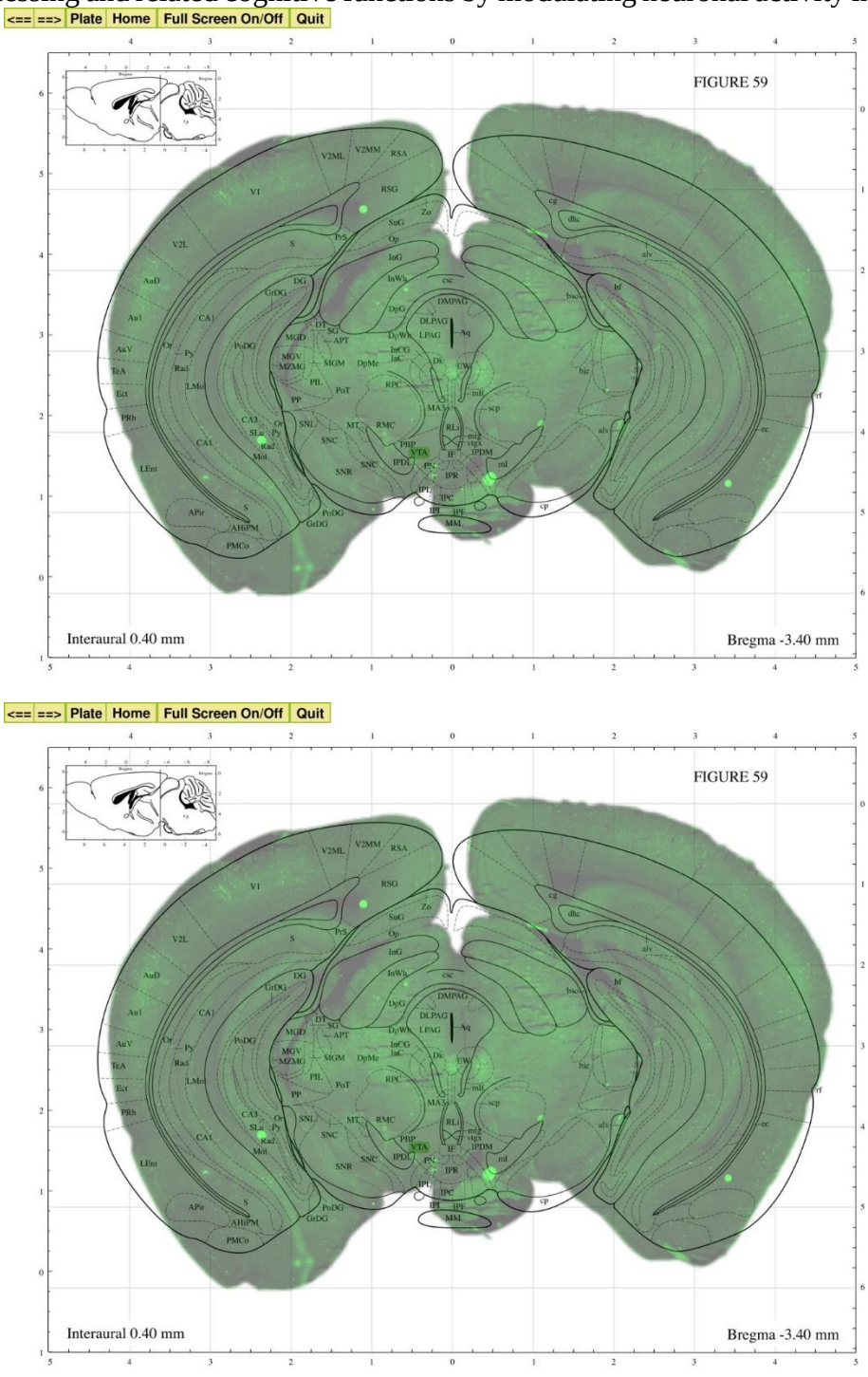

Figure 1 Immunofluorescence staining results of the mouse brain

We chose to implant the fiber optic in the TeA region because it is associated with auditory processing in the mouse cortex. Additionally, after immunofluorescence staining, the TeA region exhibited a significant increase in signal, supporting the choice to target this area for subsequent calcium imaging. A high-magnification view of the TeA region is shown in Figure 2, highlighting the fluorescent puncta used for quantitative analysis. Therefore, we injected the virus into the TeA region for calcium imaging to record the neural activity signals. Quantitative results of the puncta—including total number, mean size, and coverage—are summarized in Figure 3 and Table 1, supporting the observed increase in neuronal activity.

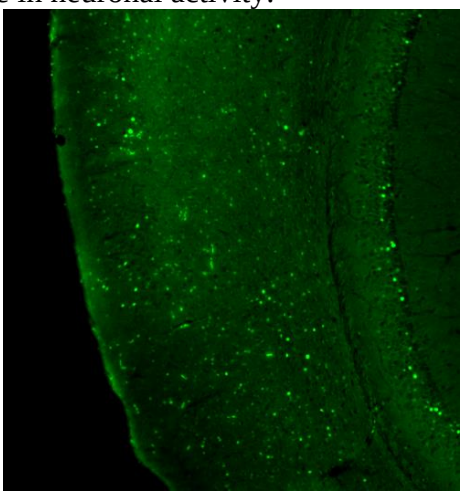

Figure 2 Mouse Brain TeA Region

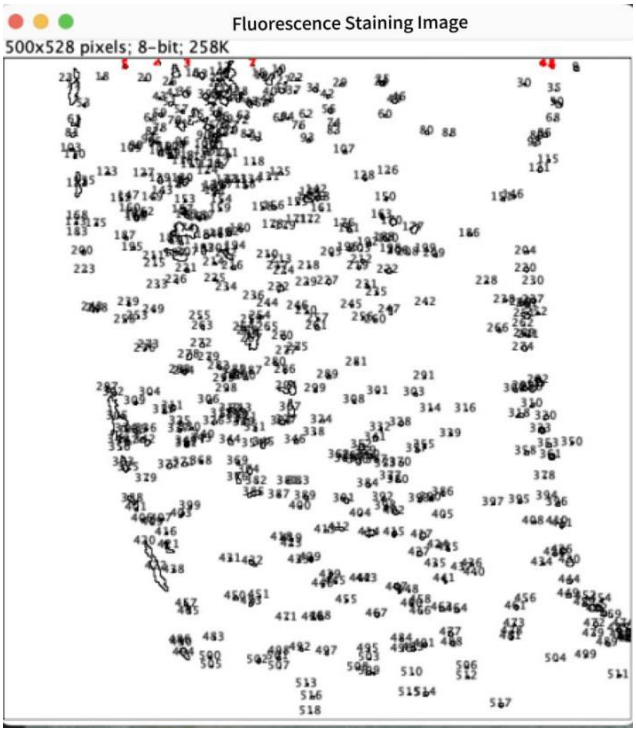

Figure 3 Quantification of Fluorescent Puncta in TeA Region

Table 1 Quantitative Analysis of Fluorescent Puncta in TeA Using ImageJ

Slice	Count	Total Area	Average Size	%Area	Mean
Fluorescence Staining Image	518	4600	8.880	1.742	255

4.2 Ketamine Affects Mice's Auditory Judgment and Choice

The red line represents the changes in brain fiber optic calcium signals in mice after Ketamine injection, while the black line represents the signal changes in mice injected with an equal amount of saline as the control group. As shown in Figure 4, the calcium signal drops sharply in the Ketamine group, confirming a disruption in auditory signal transduction at the neuronal level. As shown in Figure 4, after Ketamine injection, the brain fiber optic calcium signals in mice exhibit a significant downward trend. This change suggests that Ketamine may have an inhibitory effect on brain function, particularly in brain regions related to auditory processing. The sharp decrease in the signal likely reflects a reduction in the sensitivity of mice to external auditory stimuli, thereby disrupting the normal transmission and processing of sensory input. This result further supports the hypothesis that Ketamine interferes with auditory cognition by affecting calcium signals in neurons, providing experimental evidence for studying its mechanisms in neural regulation.

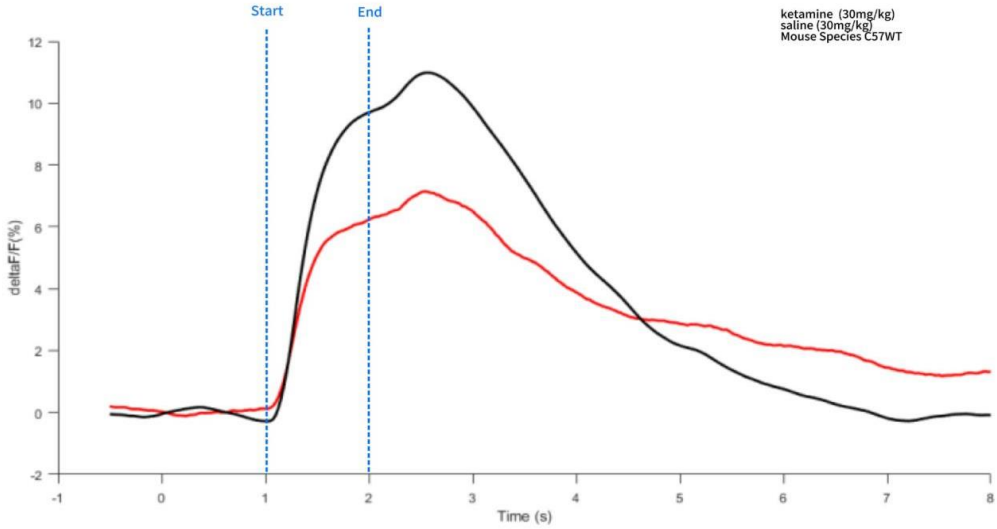

Figure 4 Brain fiber optic calcium signals in the mouse TeA region after virus injection.

4.3 AFC Experiment Results

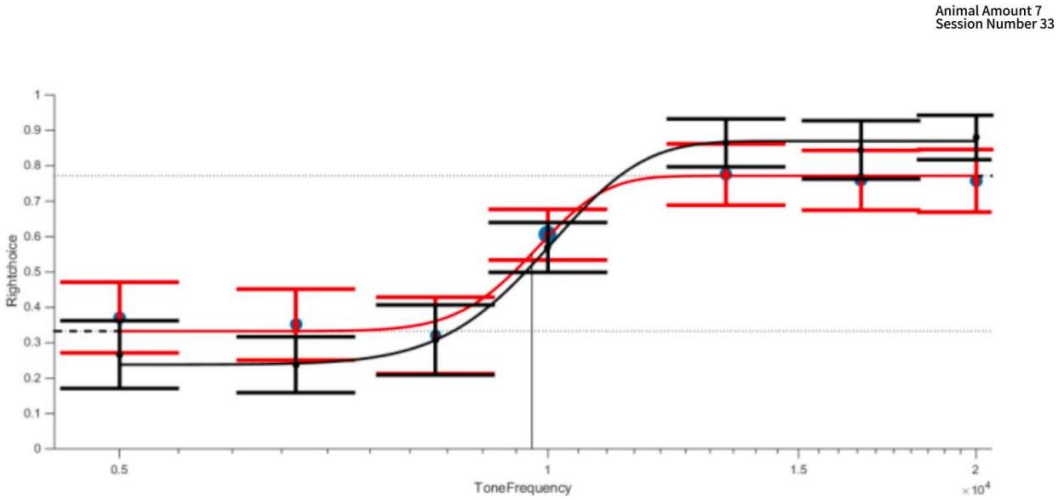

Figure 5 2AFC Experiment Results

The experiment trained dehydrated mice in sound frequency discrimination, and the data is shown

in Figure 5. The red line represents the data from mice injected with Ketamine, and the black line represents the data from mice injected with the same dose of saline as the control group. The X-axis corresponds to the frequency range from low to high, while the Y-axis shows the accuracy of the mice. The length of the bar reflects the range of values. In the low-frequency range (from 500 Hz to 10,000 Hz), both Ketamine-injected mice and saline-injected mice had low accuracy, approximately 0.3 to 0.4, indicating poor sound discrimination ability in this frequency range. In the high-frequency range (above 10,000 Hz), the accuracy of saline-injected mice significantly increased to around 0.8 to 0.9, showing strong sound discrimination ability. In contrast, Ketamine-injected mice showed lower accuracy in the same high-frequency range. These results indicate that Ketamine interferes with the auditory recognition and spatial judgment ability of mice.

5. Conclusion

This study demonstrates that ketamine disrupts auditory perception by modulating neuronal activity in the temporal association cortex (TeA). We observed increased c-Fos expression and decreased calcium signaling in the TeA, alongside impaired auditory discrimination in a two-alternative forced choice (2AFC) task—particularly for high-frequency sounds. These converging results suggest that the TeA plays a central role in ketamine-induced sensory disturbances, including hallucination-like phenomena.

5.1 Methodological strengths and limitations

Our multi-modal approach combining immunohistochemistry, calcium photometry, and behavioral testing provides a comprehensive view across molecular, neural, and behavioral levels. The use of fiber photometry enables real-time monitoring of population-level activity in freely moving mice, while c-Fos mapping captures longer-term cumulative activation. However, these methods have limitations. Fiber photometry lacks single-cell or layer-specific resolution, and c-Fos reflects post-hoc activation rather than real-time dynamics. Additionally, the simplicity of the 2AFC task may not capture the full spectrum of perceptual abnormalities linked to hallucinations. Sample size and species specificity (e.g., C57BL/6J strain) may also limit generalizability.

5.2 Mechanistic contribution and novelty

Our findings help fill a mechanistic gap in the existing literature on ketamine-induced hallucinations. Previous studies have shown elevated TeA activity under ketamine (Hu et al., 2024)[3], but few have directly linked this to calcium dysregulation and perceptual behavior. We demonstrate that ketamine's blockade of NMDA receptors likely destabilizes the PSD95–NMDAR–PMCA complex, impairing calcium clearance and contributing to glutamate spillover (Lisek et al., 2017)[10]. This dual disruption—hyperactivity with hypocalcemia—suggests a breakdown in signal fidelity within TeA circuits, a novel insight into how disordered sensory encoding might emerge from molecular dysfunction.

Beyond its role as a pharmacological tool in basic neuroscience, ketamine occupies a unique position in translational psychiatry. As an NMDA receptor antagonist, it is one of the few compounds capable of reliably inducing hallucination-like states in both animals and humans (Krystal et al., 1994; Homayoun & Moghaddam, 2007)[6][2]. This makes ketamine an invaluable model for probing the neural mechanisms of perceptual disturbances such as those seen in schizophrenia. Furthermore, its emerging use as a rapid-acting antidepressant highlights the dual nature of its pharmacological profile—therapeutically beneficial at low doses, yet capable of inducing severe sensory distortions at higher doses (Zarate et al., 2006)[13]. Understanding the circuit- and cell-type-specific effects of

ketamine on regions such as the TeA is therefore critical, not only for advancing fundamental neurobiology but also for informing safer clinical applications and developing AI-based tools for detecting and mitigating hallucinatory episodes.

5.3 Comparisons and conflicts with prior work

While our results align with literature identifying the TeA as a top-down hub in hallucination models, they contrast with reports of uniform suppression or general sensory dulling under ketamine (Komatsu & Ichinohe, 2020)[4]. For instance, we found frequency-specific behavioral impairments (high-frequency discrimination decline), whereas some nonhuman primate studies observed broad-spectrum auditory degradation. These discrepancies may stem from differences in species, ketamine dosage, delivery route, behavioral paradigms, or strain-specific cortical tuning properties. Additionally, our observed calcium signal reduction despite increased c-Fos expression may seem paradoxical, but aligns with known NMDA receptor-mediated calcium pump disruption and network-level overactivation (Kulikova et al., 2022; Lisek et al., 2017)[8][9].

In summary, our data reconcile conflicting observations in the field by showing that ketamine can simultaneously increase gross neural activation while impairing precise sensory processing—a pattern potentially relevant to hallucinatory states.

In conclusion, by identifying how ketamine disrupts auditory processing via TeA dysfunction and calcium dysregulation, our study provides foundational insight for developing AI-based systems that can better model, detect, and ultimately help mitigate hallucination-like symptoms in psychiatric disorders such as schizophrenia.

References

[1] De Leede-Smith, S., & Barkus, E. (2013). A comprehensive review of auditory verbal hallucinations: lifetime prevalence, correlates and mechanisms in healthy and clinical individuals. Frontiers in Human Neuroscience, 7, 367. https://doi.org/10.3389/fnhum.2013.00367

[2] Homayoun, H., & Moghaddam, B. (2007). NMDA receptor hypofunction produces opposite effects on prefrontal cortex interneurons and pyramidal neurons. Journal of Neuroscience, 27(43), 11496–11500. https: //doi. org/10. 1523/JNEUROSCI.2213-07.2007

[3] Hu, X., & al., et. (2024). TeA activity increases under NMDA receptor blockade in ketamine models of hallucination. Nature Communications, 15, 1297. https://doi.org/10.1038/s41467-021-21735-x

[4] Komatsu, M., & Ichinohe, N. (2020). Effects of ketamine administration on auditory information processing in the neocortex of nonhuman primates. Frontiers in Psychiatry, 11, 826. https://doi.org/10.3389/fpsyt.2020.00826

[5] Komatsu, Y., & Ichinohe, N. (2020). The role of association cortices in hallucination-like states: A multisensory perspective. Frontiers in Systems Neuroscience, 14, 24. https://doi.org/10.3389/fnsys.2020.00024

[6] Krystal, J. H., Karper, L. P., Seibyl, J. P., Freeman, G. K., Delaney, R., Bremner, J. D., ... & Charney, D. S. (1994). Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans: psychotomimetic, perceptual, cognitive, and neuroendocrine responses. Archives of General Psychiatry, 51(3), 199–214. https: //doi. org/10. 1001/archpsyc.1994.03950030035004

[7] Kulikova, S. P., & al., et. (2022). NMDA receptor dysfunction alters cortical calcium pump mechanisms and glutamatergic signal fidelity. Neuropharmacology, 208, 108933. https://doi.org/10.1016/j.neuropharm.2022.108933

[8] Kulikova, S., Qin, Y., & Izhikevich, E. (2022). Ketamine found to increase brain noise. European Journal of Neuroscience. https://doi.org/10.1111/ejn.15845

[9] Lisek, M., & al., et. (2017). Ketamine dysregulates PSD95–NMDAR–PMCA complex, disrupting Ca2+ homeostasis in cortical neurons. Molecular Neurobiology, 54(3), 2334–2346. https://doi.org/10.1007/s12035-016-9815-0

[10] Lisek, M., Ferenc, B., Studzian, M., Pulaski, L., Guo, F., Zylinska, L., & Boczek, T. (2017). Glutamate deregulation in ketamine-induced psychosis—A potential role of PSD95, NMDA receptor and PMCA interaction. Frontiers in Cellular Neuroscience, 11, 181. https://doi.org/10.3389/fncel.2017.00181

[11] Montagnese, M., et al. (2021). A review of multimodal hallucinations: Categorization, assessment, theoretical perspectives, and clinical recommendations. Schizophrenia Bulletin, 47(1), 237–245. https: //doi. org/10. 1093/schbul/sbaa101

[12] Winterer, G., & al., et. (2023). Ketamine and auditory processing: Cortical network modeling and

neurophysiological evidence. Biological Psychiatry, 93(6), 479–487. https://doi.org/10.1016/j.biopsych.2022.11.003
[13] Zarate, C. A., Singh, J. B., Carlson, P. J., Brutsche, N. E., Ameli, R., Luckenbaugh, D. A., ... & Manji, H. K. (2006). A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. Archives of General Psychiatry, 63(8), 856–864. https://doi.org/10.1001/archpsyc.63.8.856
[14] Zeng, H., Yu, D., & Li, K. (2023). Disruption of sensory precision by ketamine in mouse auditory cortex. NeuroImage, 275, 120161. https://doi.org/10.1016/j.neuroimage.2023.120161