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LETTER

Spatial transcriptomic profiling of neuronal adaptations to zero-magnetic field: Alterations in Amygdala–PVT–Hypothalamus

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Received: April 28, 2025; Accepted: August 12, 2025; Published Online: August 18, 2025; https://doi.org/10.59717/j.xinn-life.2025.100153

Citation: Liu Q., Wang C., Zhong Q., et al. (2025). Spatial transcriptomic profiling of neuronal adaptations to zero-magnetic field: Alterations in Amygdala–PVT–Hypothalamus. *The Innovation Life* **3**:100153.

Dear Editors,

The Earth's magnetic field is a fundamental environmental factor that has shaped biological evolution, yet its role in shaping brain function at the cellular and molecular levels remains underexplored. Recent studies suggest that geomagnetic fields may play a role in neuronal activity, neurotransmitter dynamics, and behavioral regulation.[1] However, how the absence of a magnetic field—a zero-magnetic environment (0 nT. Here, "zero magnetic field" refers to an extremely low magnetic field environment—approaching ±1.5 nT—that represents the lowest achievable level under current experimental conditions)—affects neuronal cells, contributing to brain function, remains poorly understood. This knowledge gap is particularly relevant in the context of space exploration, where astronauts are exposed to significantly altered magnetic conditions that may influence from neuronal cells to higher cognitive function, emotional regulation, and autonomic control.

One critical brain network involved in these processes is the amygdala–paraventricular nucleus of the thalamus (PVT)–hypothalamus axis, which integrates emotional, cognitive, and autonomic signals to regulate stress responses, mood, and metabolic homeostasis.[2] The amygdala is central to emotional processing,[3] the PVT serves as a relay for stress and arousal-related signals,[4] and the hypothalamus governs autonomic and endocrine functions.[5] Disruptions in the excitatory–inhibitory balance within this axis can profoundly affect emotional states, cognitive function, and physiological stability. However, how a zero-magnetic environment influences this balance in specific brain regions remains largely unexplored.

In this study, we investigated the effects of prolonged zero-magnetic exposure (60 days), simulating the space magnetic field environment, on neuronal and glial populations within the mouse brain through an integrated analysis of spatial transcriptomics and single-nucleus RNA sequencing (snRNA-seq) data. Our analysis revealed that neurons, rather than glial cells, exhibit greater sensitivity to magnetic field deprivation. Notably, the amygdala, PVT, and hypothalamus showed significant alterations in neuronal excitability and synaptic gene expression, indicating a potential cellular mechanism underlying the emotional and anxiety-related changes induced by zero-magnetic exposure. These findings provide the first cellular-resolution insights into the potential neurobiological risks associated with space travel, highlighting the increased sensitivity of specific brain regions to the absence of geomagnetic field. Furthermore, region-specific disturbances in the neuronal excitatory/inhibitory balance within these areas highlight the critical role of the Earth's magnetic field in maintaining neural homeostasis, particularly in circuits involved in emotional memory, stress responses, and physiological regulation.

ZERO-MAGNETIC MOUSE CHAMBER

This study was conducted using the advanced ground-based zero-magnetic environment simulation platform developed under the National Science Project at Harbin Institute of Technology, which provides a unique experimental setting to mimic the magnetic field conditions of space. To assess the magnetic field distribution, four measurement probes were positioned around the cage shelf in both the zero-magnetic and geomagnetic chambers (Figure 1A). The zero-magnetic chamber can achieve nanotesla (nT)-level magnetic field precision, with regular fluxgate magnetometer calibrations performed every three days to ensure continued accuracy. Magnetic field measurements consistently showed values below 1 nT within the chamber, confirming excellent zero-magnetic field conditions. In contrast, the control environment—representing the normal geomagnetic field—exhibited magnetic field strengths ranging from 51 to 54 μT, reflecting typical terrestrial geomagnetic properties (Figure 1B). Mice were housed in either the zero-magnetic or geomagnetic chamber for 60 days to simulate prolonged space travel exposure, followed by transcriptomic profiling analyses.

Spatial RNA-seq profiling on the coronal mouse brain sections detected 254,267 bins (bin size: 16 μm) and identified 38,047 transcriptomic profiles in total. For the coronal section from the earth magnetic environment (Control), 19,022 profiles were identified at depths of 1794 unique molecular identifiers (UMIs) per bin. While, for the coronal section from Zero-Magnetic Space Chamber (0 nT), 19,025 profiles were identified at depths of 2186 UMIs per bin. Integration of Spatial transcriptomics and snRNA-seq data were performed to determine the cell identities in each bin, and to annotate in 39 clusters (Figure 1C). We aligned each cluster with anatomical brain regions defined according to the Allen Mouse Brain Atlas.[6,7] These clusters displayed regional segregation of brain tissues and were consistent with histological annotations including the cortex (cx), field CA1, CA2, CA3 and dentate gyrus (DG) of hippocampus, fimbria of the hippocampus (fi), stria terminalis (st), cerebral peduncle (cp), external medullary lamina (eml), medial lemniscus (ml), thalamus and Hypothalamus regions.

The major non-neuronal cell types in the brain regions were identified and annotated by analyzing the expression patterns of known gene markers, including microglia (marked by *Tmem119*, etc.), astrocytes (*Gfap* etc.), oligodendrocytes (*Mbp*, etc.), oligodendrocyte progenitor cells (OPCs, marked by *Olig2*, etc.), endothelial cells (*Cldn5*, etc.), leptomeningeal cells (*Col1a1*, etc.), and arachnoid barrier cells (*Ocln*, etc.). For neuronal cells, 32 clusters were identified based on marker gene expression patterns, excitatory (marked by *Slc17a7*, *Slc17a6*, etc.) and inhibitory (marked by *Gad1*, *Gad2*, etc.) characteristics, and spatial distribution across the cortex, hippocampus, and thalamus (Figures 1C-E). These cell type annotations and neuronal spatial distribution patterns were used to quantify the ratios of cell types in different brain regions, assess neural cell responses to the 0 nT environment, and localize region-specific neuronal responses to the 0 nT environment.[8]

Among the non-neuronal clusters, no significant differences in cell type ratios or spatial distribution were observed between the experimental groups, indicating that the zero-magnetic environment had no detectable impact on microglia, astrocytes, oligodendrocytes, or OPCs. Additionally, no region-specific distribution patterns of these glial cells were identified in the brain. Similarly, for neuronal populations, the ratios of excitatory and inhibitory neurons remained unchanged in the hippocampus and most cortical regions, except for the amygdala. In contrast, significant alterations in neuronal ratios were observed in the amygdala, PVT and hypothalamus, suggesting a distinct, region-specific neuronal response to the zero-magnetic environment.

Changes in Amygdala

A subcluster of excitatory neurons in the amygdala exhibited a significant increase following exposure to a 60-day zero-magnetic environment compared to the Earth's magnetic field control group (Figure 1F(f1)). This increase was further validated through VGLUT (the protein encoded by *Slc17a7*) immunofluorescence and *Slc17a7* mRNA expression analyses in the amygdala, confirming significant differences between the control and 0 nT groups (Figures 1G(g1) & 1I). Integration of snRNA-seq data revealed that this excitatory neuronal subcluster was characterized by high expression levels of *Il1rapl2, Dpp10, C1ql3, Hs3st2,* and *Galnt14*, among other genes. Gene Ontology (GO) enrichment analysis revealed that these genes are

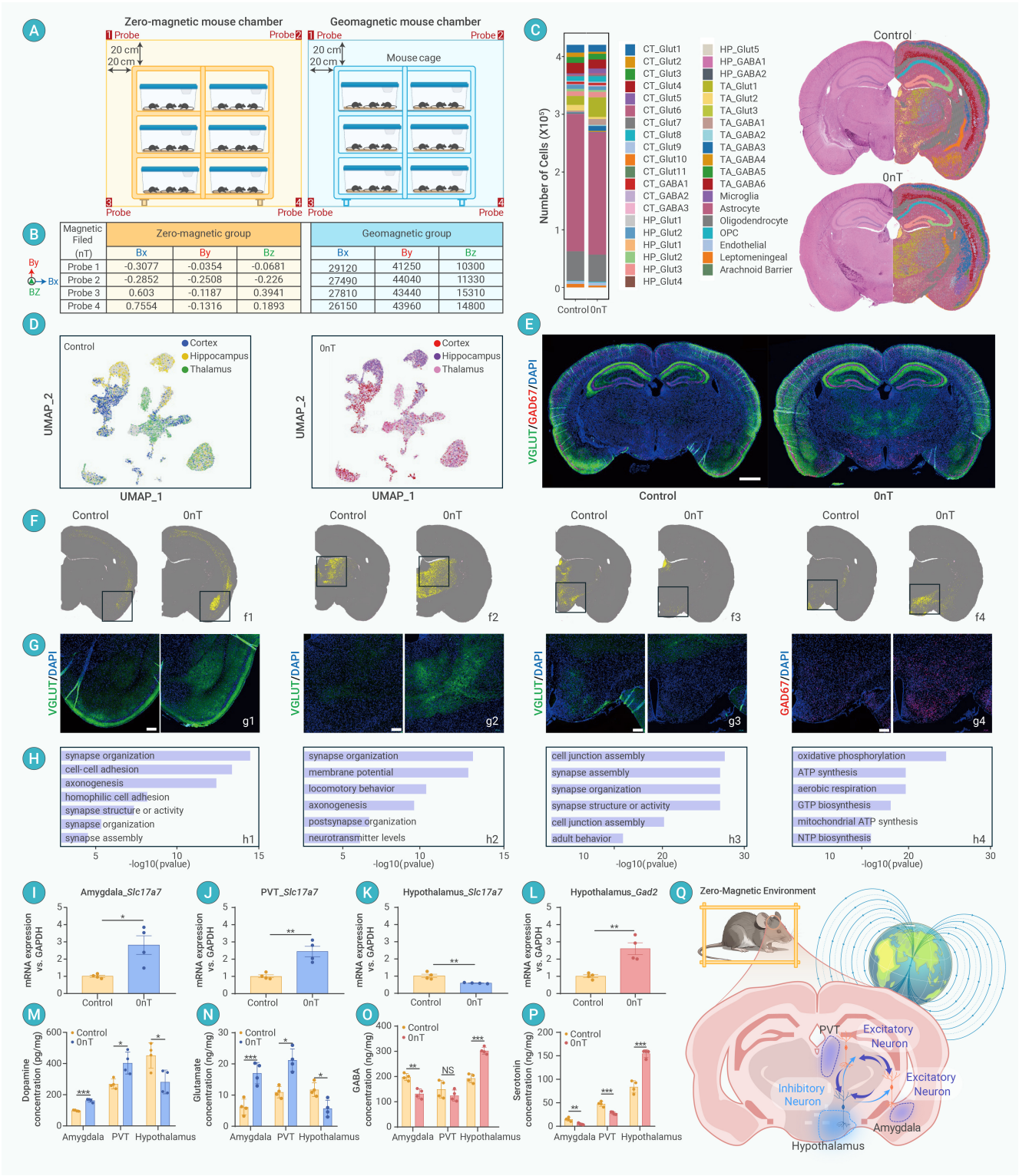

Figure 1. Spatial Transcriptomic Profiling of Neuronal Adaptations to a Zero-Magnetic Environment (A–B). Schematic diagram of the zero-magnetic space chamber for mice (A) and magnetic field measurements within the zero-magnetic and geomagnetic chambers (B). Mouse cages used are made of non-magnetic materials. The unit of magnetic field was nT. Mice from each group were housed in either the zero-magnetic or geomagnetic environment for 60 days to simulate space-like exposure, followed by transcriptomic profiling analyses. (C). Integration of snRNA-seq data with spatial transcriptomics. Each color represents a distinct cell type mapped onto the brain section, showing the spatial localization of different cell populations. (D). UMAP of neuronal clusters in the cortex, hippocampus and thalamus, separately in control and 0 nT group of mouse brain. (E). Immunofluorescence of VGLUT (green) and GAD67 (red) on whole piece of the mouse brain slices. Scale bar: 1 mm. (F). Distribution and cell numbers of identified cell types from snRNA sequencing. Spatial projection of the neuronal cluster onto the brain section. Only cells identified as CT_Glut5 (f1), TA_Glut1 (f2), TA_Glut2 (f3) and TA_GABA1 (f4) neurons from the scRNA-seq analysis are mapped, showing their spatial distribution within the tissue. (G). Immunofluorescence of VGLUT (green) in amygdala (g1), PVT (g2), and hypothalamus (g3), and GAD67 (red) in hypothalamus(g4). n=3 mice for each group. Scale bar: 200 μm. (H). The pathway enrichment analysis of down streaming upregulated genes and pathways in CT_Glut5 (h1), TA_Glut1 (h2), TA_Glut2 (h3) and TA_GABA1 (h4), using Gene Ontology (GO) biological process terms. (I-L). mRNA expression of *Slc17a7* (excitatory neuronal marker gene) (in amygdala (I), PVT (J) and hypothalamus (K), and *Gad2* (inhibitory neuronal marker gene) in hypothalamus. n=4 mice for each group. (L). M-P. Neurotransmitter concentration of dopamine (M, excitatory neurotransmitter), glutamate (N, excitatory neurotransmitter), GABA (O, inhibitory neurotransmitter) and serotonin (P, inhibitory neurotransmitter) in amygdala, PVT and hypothalamus, by ELISA assay. n=4 mice for each group. (Q). Paragraph illustration of the finding, region-specific excitatory and inhibitory alterations in the amygdala–PVT–hypothalamus axis to Zero-magnetic environment. Data were presented as mean ± SEM. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ were considered as significantly different for 0 nT vs. Control.

involved in essential neuronal synaptic functions, including synapse organization, synapse assembly, and synaptic organization, suggesting a potential impact on synaptic plasticity and neuronal signal transmission (Figure 1H(h1)).

Neurotransmitter analysis revealed that exposure to the zero-magnetic environment led to a significant increase in the concentrations of the excitatory neurotransmitters dopamine and glutamate, alongside a decrease in the inhibitory neurotransmitters GABA and serotonin in the amygdala (Figures 1M-P). These findings provide further evidence of heightened excitatory activity in the amygdala induced by the zero-magnetic environment.

Changes in PVT

Another subcluster of excitatory neurons in region of the PVT at thalamus also exhibited a significant increase following exposure to the zero-magnetic environment (Figure 1F(f2)). This increase was further validated through VGLUT immunofluorescence and *Slc17a7* mRNA expression analyses (Figures 1G(g2) & 1J). It revealed that this excitatory neuronal subcluster was characterized by high expression levels of *Ntng1, Plekng1, Vav3, Synpo3, Ptpn3* and *Tcf7l2*, among others. GO enrichment analysis indicated that these genes are involved in critical neuronal synaptic functions, including synapse organization, locomotory behavior, and postsynapse organization, which are directly linked to the regulation of locomotory behavior, anxiety, and cognition (Figure 1H(h2)).

Neurotransmitter analysis revealed a pattern similar to that observed in the amygdala, with increased concentrations of dopamine and glutamate, accompanied by a decrease in GABA and serotonin in the PVT region (Figures 1M-P). This suggests an excitatory induction in PVT regulation under the zero-magnetic environment.

Changes in Hypothalamus

Another subcluster of excitatory neurons located in the thalamus and hypothalamus exhibited a significant decrease following 60-day zero-magnetic environment exposure (Figure 1F(f3)). This decrease was further validated through VGLUT immunofluorescence and *Slc17a7* mRNA expression analyses (Figures 1G(g3) & 1K). This excitatory neuronal subcluster was characterized by high expression levels of *Ebf1, Rmst, Gpc5, Car10* and *Mgat4c*. GO enrichment analysis associated these genes with synapse organization and cell junction assembly, suggesting potential disruptions in synaptic connectivity and neuronal communication between the thalamus and hypothalamus (Figure 1H(h3)).

Meanwhile, while no significant changes were observed in inhibitory neurons within the amygdala and thalamus, a subcluster of inhibitory neurons in the hypothalamus exhibited a significant increase following 60-day zero-magnetic environment exposure (Figure 1F(f4)). This increased inhibitory neuronal subcluster was identified through spatial and snRNA-seq analysis and further validated by GAD67 (the protein encoded by *Gad2*) immunofluorescence and *Gad2* mRNA expression analysis (Figures 1G(g4) & 1L). This inhibitory neuronal subcluster was characterized by high expression levels of *Resp18, Gad2, Gnas, Fth1* and *Cadps2*. GO enrichment analysis linked these genes to neuronal functions related to mitochondrial ATP synthesis, cellular respiration, and oxidative phosphorylation, indicating a potential metabolic shift in inhibitory neuronal activity under the zero-magnetic environment (Figure 1H(h4)).

Neurotransmitter analysis provided further evidence that 60-day zero-magnetic environment exposure induced a significant upregulation of inhibitory neurotransmitters GABA and serotonin, along with a significant downregulation of excitatory neurotransmitters dopamine and glutamate, further supporting an overall shift toward increased inhibitory signaling in the hypothalamus (Figures 1M-P).

CONCLUSION

Our study presents the first integrated spatial transcriptomics and single-nucleus RNA sequencing analysis of prolonged zero-magnetic (0 nT) exposure effects on neuronal and glial populations in the mouse brain. We identified significant region-specific alterations in neuronal excitatory and inhibitory cell types within the amygdala, PVT, and hypothalamus, highlighting the sensitivity of these areas to magnetic field deprivation. Notably, an increased proportion of excitatory neurons was observed in the amygdala and PVT, whereas the hypothalamus exhibited elevated inhibitory neuron populations alongside a reduction in excitatory neurons. These shifts in neuronal subtypes were accompanied by changes in neurotransmitter-related gene expression consistent with altered excitatory/inhibitory balance. While glial populations showed comparatively less pronounced changes, their potential modulatory roles warrant further investigation. Collectively, these results provide novel cellular-level insights into how the absence of the Earth's magnetic field may perturb neuronal homeostasis in specific brain regions critical for emotional, cognitive, and autonomic functions (Figure 1Q).[9,10] These findings lay important groundwork for understanding the neurobiological risks posed by zero-magnetic environments, such as those encountered during space travel, and underscores the need for further mechanistic studies to elucidate the functional consequences of these cellular adaptations.

The effects of zero-magnetic exposure on excitatory and inhibitory neuronal circuits within the amygdala–PVT–hypothalamus axis warrant further detailed analysis and experimental validation in future studies. Such investigations will be essential to clarify the specific functional impacts and underlying mechanisms of these region-specific neuronal changes.

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FUNDING AND ACKNOWLEDGMENTS

This work was supported by the China National Key R&D Program (2022YFA1604502 and 2024YFF1206400); National Natural Science Foundation of China (U23A20393, 82174033, T2350710233); Guangdong Provincial Key Laboratory of Multimodality Non-Invasive Brain-Computer Interfaces (2024B1212010010); Shenzhen Science and Technology Key Program (JCYJ20220818101404009). We thank Climb Technology Co., Ltd. for their technical support in the 10x VisiumHD assay for the Spatial Transcriptome and snRNA-seq analysis. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

AUTHOR CONTRIBUTIONS

Q.L. and Y.W. designed and supervised the study. W.T. oversaw the Zero-Magnetic Space Chamber construction and implementation. B.S. and W.T. provided funding support. C.W., Q.Z. F.Z., B.G., K.Z., M.G., W.L., Y.Z., S.Z., J.W., X.X. and T.S. were responsible for animal experiments. All authors contributed to the manuscript and approved the final version

DECLARATION OF INTERESTS

The authors declare no competing interests.

DATA AND CODE AVAILABILITY

Data generated or analyzed in this study are available from the corresponding authors upon request.