

MICRO REPORT

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Transcutaneous auricular vagus nerve stimulation in anesthetized mice induces antidepressant effects by activating dopaminergic neurons in the ventral tegmental area

Tae-Yong Choi^{1*†} , Jeongseop Kim^{1†} and Ja Wook Koo^{1,2*}

Abstract

Depression, a prevalent neuropsychiatric disorder, involves the dysregulation of neurotransmitters such as dopamine (DA). The restoration of DA balance is a pivotal therapeutic target for this condition. Recent studies have indicated that both antidepressant medications and non-pharmacological treatments, such as transcutaneous auricular vagus nerve stimulation (taVNS), can promote recovery from depressive symptoms. Despite the promise of taVNS as a non-invasive depression therapy, its precise mechanism remains unclear. We hypothesized that taVNS exerts antidepressant effects by modulating the DAergic system. To investigate this, we conducted experiments demonstrating that taVNS in anesthetized mice reduced depressive-like behaviors. However, this effect was abolished when DA neurons in the ventral tegmental area (VTA^{DA}) were inhibited. Additionally, taVNS in anesthetized mice enhanced VTA^{DA} activity, providing further evidence to support its antidepressant effects. Overall, our findings suggest that taVNS alleviates depression by augmenting VTA^{DA} activity, thereby contributing to a more comprehensive understanding of its therapeutic mechanisms.

Keywords Depression, Transcutaneous auricular vagus nerve stimulation, Antidepressant, Ventral tegmental area, Dopamine, Chronic social defeat stress, Forced swim test, Fiber photometry

Introduction

Depression, or major depressive disorder (MDD), is a prevalent and severe neuropsychiatric disease characterized by persistent low mood and diminished interest or pleasure in activities. Although numerous factors can contribute to depression, its principal determinants remain elusive. Emerging evidence has indicated that the dysregulation of neurotransmitters, notably dopamine (DA), particularly within brain regions governing emotions, cognition, sleep, and appetite, plays a pivotal role in its etiology [1]. As such, restoring the balance in brain chemistry may alleviate depression.

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It has been widely recognized that the activity of DA neurons in the ventral tegmental area (VTA^{DA}) and DA release to various brain regions regulate mood-related behaviors. Indeed, research has shown that stress or aversive stimuli suppress VTA^{DA} activity [2], while the optogenetic inhibition of VTA^{DA} leads to a depressive-like phenotype. Conversely, phasic photoactivation of VTA^{DA} has been shown to mitigate chronic stress-induced depressive symptoms [3]. Overall, these findings underscore the importance of regulating VTA^{DA} as a primary therapeutic approach for depression.

Numerous antidepressant medications, including dopamine agonists, dopamine reuptake inhibitors, and norepinephrine and dopamine reuptake inhibitors, as well as non-pharmacological therapies, such as electroconvulsive therapy, repetitive transcranial magnetic stimulation, and vagus nerve stimulation (VNS), have all been employed in the treatment of depression, to regulate the DAergic system [4]. In particular, non-invasive VNS modalities, such as transcutaneous auricular VNS (taVNS), are increasingly being used to treat depression owing to their multifaceted advantages [5]. However, the precise mechanism by which taVNS mitigates depression remains unclear.

Results

We hypothesized that taVNS exerts its antidepressant effects by activating VTA^{DA} activity. Initially, we investigated whether taVNS induced this effect through conduction of the forced swim test (FST), a standard rodent behavioral test commonly used to assess the efficacy of antidepressant drugs or treatments in eliciting or preventing depressive-like states, 20 min after sham (i.e., off-site stimulation) or taVNS intervention in anesthetized mice (Fig. 1A, B) [6]. As a result, taVNS, but not sham treatment, in anesthetized mice led to a reduction

in immobility time during the FST, indicating its antidepressant effect (Fig. 1C).

Additionally, we explored whether taVNS could alleviate depressive symptoms induced by chronic social defeat stress (CSDS), an ethologically validated animal model of depression [7, 8]. CSDS markedly diminished social interaction with an aggressor mouse only in susceptible mice, while resilient mice were unaffected (Fig. S1). Notably, our findings revealed that taVNS treatment in an anesthetized state effectively reversed CSDS-induced social avoidance (Fig. 1D, E). These results suggest that taVNS may exert anti-stress or antidepressant effects under both normal and depressive conditions.

Next, we examined whether the antidepressant effects of taVNS in anesthetized mice are mediated by VTA^{DA} activity. To investigate this, we performed the FST with or without taVNS in mice that received AAV-rTH-Cre [9] and AAV-DIO-Kir2.1-EGFP [10] into the VTA to inhibit VTA^{DA} (Fig. 1F, G). As anticipated, inhibition of VTA^{DA} resulted in increased immobility time during the FST (Fig. 1H), consistent with findings from a previous study [3]. Importantly, taVNS in an anesthetized state did not reduce immobility time in these mice (Fig. 1H). These results indicate that the antidepressant effects of taVNS are mediated through VTA^{DA} activity.

Finally, we investigated whether taVNS influenced VTA^{DA} activity. To accomplish this, we measured VTA^{DA} activity via fiber photometry in mice that received AAV-rTH-Cre and AAV-Flex-jGCaMP8f [11] and implanted optic fibers into the VTA (Fig. 1I, J). Overall, we found that taVNS in anesthetized mice increased the frequency of Ca²⁺ transients of VTA^{DA}, suggesting an increase in the average activity of VTA^{DA} (Fig. 1K–M). Interestingly, the increased activity of VTA^{DA} induced by taVNS was further increased for 30 min following taVNS (Fig. 1K–M). These results indicate that the potentiation of VTA^{DA} activity by taVNS resulted in an antidepressant effect.

(See figure on next page.)

Fig. 1 taVNS induces antidepressant effects by increasing VTA^{DA} activity. **A** The FST procedure without (i.e., off-site stimulation or sham control) or with taVNS intervention. **B** A representative image of a mouse treated with taVNS, followed by the cymba and cavum concha of the vagus innervation area in the ear. **C** Immobility time during the FST. Animals were treated with or without taVNS. Values are shown as the mean \pm standard error of the mean (SEM) ($n=6$ mice per group; Student's t-test [unpaired, two-tailed]; * $p < 0.05$). **D** Schematic diagram depicting the experimental procedure for CSDS and social interaction test (SIT) with or without taVNS. **E** taVNS significantly reversed CSDS-induced social avoidance. ($n=5$ mice per group; Student's t-test [paired, two-tailed]; ns, not significant; * $p < 0.05$). **F** Schematic illustration of viral injection to inhibit VTA^{DA}. **G** Representative images of brain sections injected with AAV-rTH-Cre and AAV-DIO-EYFP (top) or AAV-DIO-Kir2.1-EGFP (bottom) into the VTA. DAPI, blue; EYFP (top) or Kir2.1-EGFP (bottom), green. Scale bars, 1000 μm . **H** Immobility time during the FST. Control and VTA^{DA}-inhibited mice were treated with or without taVNS. Values are shown as mean \pm SEM ($n=6$ mice per group; Two-way ANOVA with Fisher's LSD multiple comparisons test; ns, not significant; *** $p < 0.001$; **** $p < 0.0001$). **I** Schematic illustration of viral injection and optic fiber implantation to measure VTA^{DA} activity using fiber photometry. **J** A representative image of a brain section infected with AAV-rTH-Cre and AAV-Flex-jGCaMP8f in the VTA. A fiber optic cannula was implanted above the viral injection site. DAPI, blue; jGCaMP8f, green. Scale bar, 500 μm . **K** Procedure for fiber photometry recordings with taVNS. **L** Representative Ca²⁺ traces from a single animal. Data are presented as the percentage change in fluorescence over the mean fluorescence ($\Delta F/F$). Top, taVNS_OFF (before, 10 min); middle, taVNS_ON (10 min); bottom, taVNS_OFF (after, 30 min). **M** Peak analysis of Ca²⁺ imaging traces. ($n=6$ mice; repeated-measures one-way ANOVA with Tukey's multiple comparisons test; * $p < 0.05$; ** $p < 0.01$)

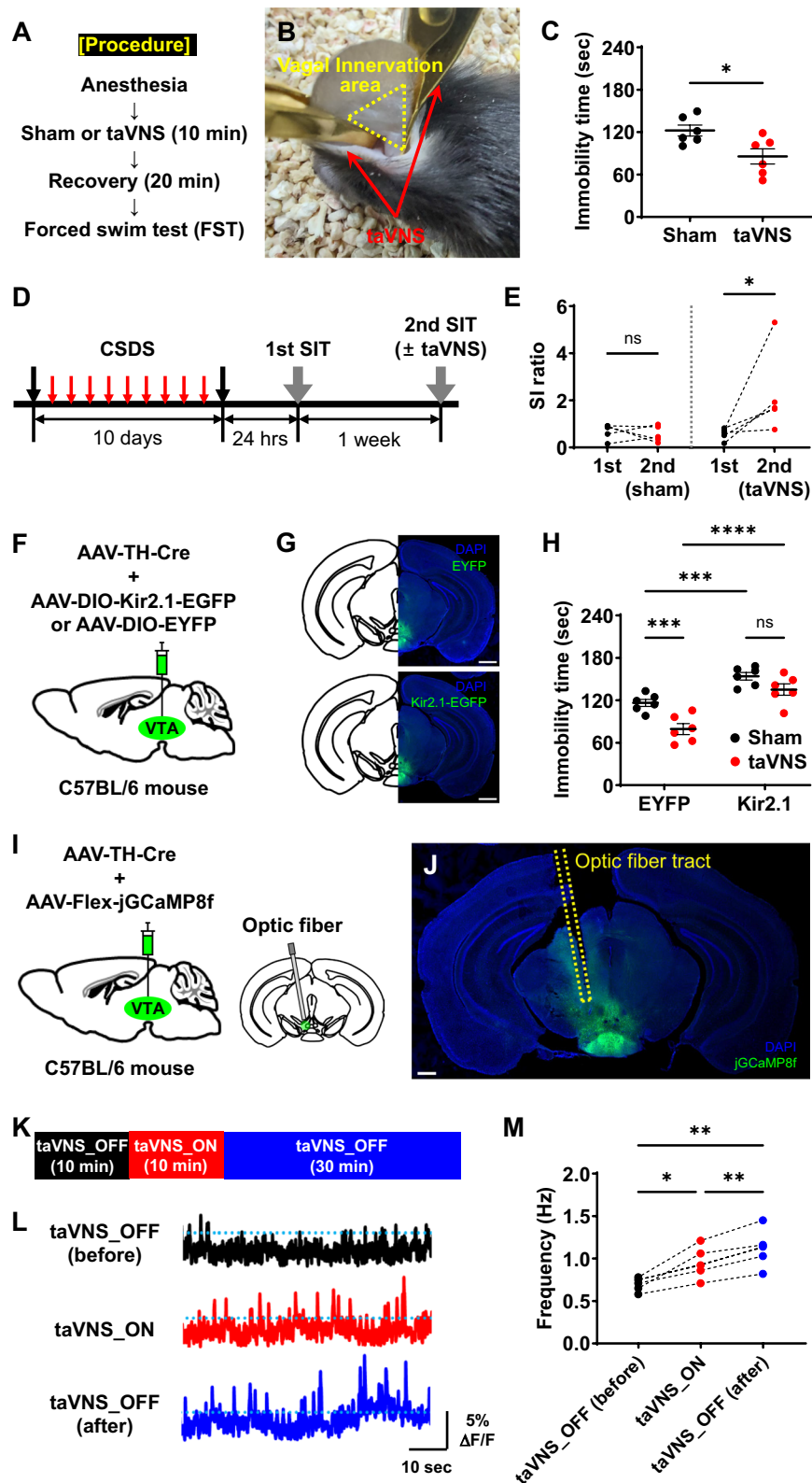


Fig. 1 (See legend on previous page.)

Discussion

The vagus nerve, also known as the tenth cranial nerve, is one of the 12 cranial nerves that emerge directly from the brain. It plays a crucial role in the parasympathetic nervous system, which regulates numerous unconscious bodily functions such as heart rate, digestion, and respiratory rate. Further, the vagus nerve has been implicated in the body's response to stress. The activation of the vagus nerve can induce relaxation and calmness [12]. Consequently, despite the use of VNS for depression treatment, its precise mechanisms remain incompletely understood.

Information from several peripheral organs is relayed to the brain, particularly to the nucleus of the solitary tract (NTS) in the brainstem. The NTS is interconnected with various brain regions responsible for synthesizing specific neurotransmitters, including the locus coeruleus, which produces norepinephrine, the dorsal raphe nucleus, which synthesizes serotonin, and the VTA, which produces DA [13]. This suggests that VNS can be used to treat various neuropsychiatric disorders, including depression, by modulating these neurotransmitter systems throughout the brain.

Although previous studies have reported that invasively implanted VNS induces an antidepressant effect [14, 15] and activates VTA^{DA} [16], whether and how non-invasive taVNS elicits an antidepressant effect currently remains unclear. Invasive VNS is effective for treating various neuropsychiatric disorders and has sustained therapeutic effects. However, it carries the risks of surgery, including infection, pain, and high cost [17]. Conversely, taVNS offers a non-invasive approach without the need for surgical procedures, along with high levels of safety, ease of use, and relatively low cost [18].

Overall, our findings suggest that taVNS in anesthetized mice triggers antidepressant effects by enhancing VTA^{DA} activity. Further investigation is required to identify the specific brain regions where the heightened VTA^{DA} activity induced by taVNS results in increased DA release and whether this contributes to the antidepressant effect. Furthermore, preclinical validation in animal models of depression is essential to confirm whether taVNS is effective for clinical application based on the mechanisms we have elucidated.

Abbreviations

AAV	Adeno-associated virus
CSDS	Chronic social defeat stress
DA	Dopamine
FST	Forced swim test
MDD	Major depressive disorder
SI	Social interaction
SIT	Social interaction test
taVNS	Transcutaneous auricular vagus nerve stimulation
TH	Tyrosine hydroxylase
VNS	Vagus nerve stimulation
VTA	Ventral tegmental area

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13041-024-01162-x>.

Additional file 1.

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Author contributions

TYC and JWK designed and supervised the entire study. TYC and JK performed all experiments and data analysis. TYC wrote the manuscript. All authors edited the manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding authors on reasonable request.

Declarations

Ethics approval and consent to participate

All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) at Korea Brain Research Institute (IACUC-22-00050 and IACUC-24-00008).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

1. Belujon P, Grace AA. Dopamine system dysregulation in major depressive disorders. *Int J Neuropsychopharmacol*. 2017;20(12):1036–46. <https://doi.org/10.1093/ijnp/pyx056>.
2. Ungless MA, Magill PJ, Bolam JP. Uniform inhibition of dopamine neurons in the ventral tegmental area by aversive stimuli. *Science*. 2004;303(5666):2040–2. <https://doi.org/10.1126/science.1093360>.
3. Tye KM, Mirzabekov JJ, Warden MR, Ferenczi EA, Tsai HC, Finkelstein J, et al. Dopamine neurons modulate neural encoding and expression of depression-related behaviour. *Nature*. 2013;493(7433):537–41. <https://doi.org/10.1038/nature11740>.
4. Carreno FR, Frazer A. Vagal nerve stimulation for treatment-resistant depression. *Neurotherapeutics*. 2017;14(3):716–27. <https://doi.org/10.1007/s13311-017-0537-8>.
5. Tan C, Qiao M, Ma Y, Luo Y, Fang J, Yang Y. The efficacy and safety of transcutaneous auricular vagus nerve stimulation in the treatment of depressive disorder: a systematic review and meta-analysis of randomized controlled trials. *J Affect Disord*. 2023;337:37–49. <https://doi.org/10.1016/j.jad.2023.05.048>.

6. Slattery DA, Cryan JF. Using the rat forced swim test to assess antidepressant-like activity in rodents. *Nat Protoc.* 2012;7(6):1009–14. <https://doi.org/10.1038/nprot.2012.044>.
7. Krishnan V, Han MH, Graham DL, Berton O, Renthal W, Russo SJ, et al. Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. *Cell.* 2007;131(2):391–404. <https://doi.org/10.1016/j.cell.2007.09.018>.
8. Kim J, Kang S, Choi TY, Chang KA, Koo JW. Metabotropic glutamate receptor 5 in amygdala target neurons regulates susceptibility to chronic social stress. *Biol Psychiatry.* 2022;92(2):104–15. <https://doi.org/10.1016/j.biopsych.2022.01.006>.
9. Parker KE, Pedersen CE, Gomez AM, Spangler SM, Walicki MC, Feng SY, et al. A paraventricular VTA nociception circuit that constrains motivation for reward. *Cell.* 2019;178(3):653–671.e19. <https://doi.org/10.1016/j.cell.2019.06.034>.
10. Xue M, Atallah BV, Scanziani M. Equalizing excitation-inhibition ratios across visual cortical neurons. *Nature.* 2014;511(7511):596–600. <https://doi.org/10.1038/nature13321>.
11. Zhang Y, Rozsa M, Liang Y, Bushey D, Wei Z, Zheng J, et al. Fast and sensitive GCaMP calcium indicators for imaging neural populations. *Nature.* 2023;615(7954):884–91. <https://doi.org/10.1038/s41586-023-05828-9>.
12. Breit S, Kupferberg A, Rogler G, Hasler G. Vagus nerve as modulator of the brain-gut axis in psychiatric and inflammatory disorders. *Front Psychiatry.* 2018;9:44. <https://doi.org/10.3389/fpsyt.2018.00044>.
13. Han W, Tellez LA, Perkins MH, Perez IO, Qu T, Ferreira J, et al. A Neural Circuit for Gut-Induced Reward. *Cell.* 2018;175(3):665–678.e23. <https://doi.org/10.1016/j.cell.2018.08.049>.
14. Grimonprez A, Raedt R, Portelli J, Dauwe I, Larsen LE, Bouckaert C, et al. The antidepressant-like effect of vagus nerve stimulation is mediated through the locus coeruleus. *J Psychiatr Res.* 2015;68:1–7. <https://doi.org/10.1016/j.jpsychires.2015.05.002>.
15. Rush AJ, Sackeim HA, Marangell LB, George MS, Brannan SK, Davis SM, et al. Effects of 12 months of vagus nerve stimulation in treatment-resistant depression: a naturalistic study. *Biol Psychiatry.* 2005;58(5):355–63. <https://doi.org/10.1016/j.biopsych.2005.05.024>.
16. Brougher J, Aziz U, Adari N, Chaturvedi M, Jules A, Shah I, et al. Self-administration of right vagus nerve stimulation activates midbrain dopaminergic nuclei. *Front Neurosci.* 2021;15:782786. <https://doi.org/10.3389/fnins.2021.782786>.
17. Kraus C, Quach D, Sholtès DM, Kavakbasi E, De Zwaef R, Dibué M, et al. Setting up a successful vagus nerve stimulation service for patients with difficult-to-treat depression. *Neuromodulation.* 2022;25(3):316–26. <https://doi.org/10.1016/j.neurom.2021.12.010>.
18. Kim AY, Marduy A, de Melo PS, Gianlorenco AC, Kim CK, Choi H, et al. Safety of transcutaneous auricular vagus nerve stimulation (taVNS): a systematic review and meta-analysis. *Sci Rep.* 2022;12(1):22055. <https://doi.org/10.1038/s41598-022-25864-1>.

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