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The Influence and Mechanism of the Nervous System and Other Factors Inducing Anxiety-like Behavior in Animal Models

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Abstract: Researches on the actions that trigger anxiety is being undertaken gradually, which has shown that anxiety is a common occurrence. In order to gain a comprehensive understanding of the influences and mechanisms of the nervous system and other factors inducing anxiety-like behaviors, this paper employs an animal model as its research topic in order to better understand how the nervous system and other elements impact anxiety and its mechanism. Using optogenetic activation, chemical genetics, electrophysiology, total internal reflection fluorescence (TIRF) imaging, immunoelectron microscopy, viral tracer, and other techniques, this study examines the interaction between the immune and neurological systems in a simulated animal model. The development of the NAc-VTA circuit, the nucleus accumbens, the ventromedial prefrontal cortex's action mechanism, and three other elements, show that the nervous system and related components may induce anxiety-like behavior in a mouse-based animal model and that these factors can also create or obstruct the regulatory pathway for anxiety-like behavior. Human anxiety can be controlled using this knowledge.

Keywords: animal models, anxiety-like behavior, nervous system, optogenetics, chemical genetics

1. Introduction

To comprehend anxiety-like behavior, which is a common occurrence in daily life, people need study the mechanisms of action of the critical neurological system components that create it. The human body is used as the experimental object to thoroughly explore the mechanisms of disease, the research progress is relatively slow, the clinical accumulation experience has time and space limitations, and many experiments also have ethical and methodological restrictions. This necessitates the indirect study of animal models. Animal models are mostly employed in clinical trials, mechanistic studies, and validation. Currently, pigs, mice, and monkeys are some of the animals regularly used in scientific research.

Mice were used as animal models in this work, and the The following studies were conducted on mice: When compared to spleen cells, it was shown that meningeal NK cells and innate lymphocytes (ILC) influence and control certain brain networks, which in turn govern mouse behavior; behavior measurements showed that photoactivation of NG2 glial cells caused anxiety-like behaviors and further led to persistent social stress; In the chronic pain paradigm, the excitatory projection of the

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posterior subregion of the paraventricular thalamic nucleus (PVT) acts as a neuronal input to activate neurons that express vmPFCnNOS. The anxiety signal is then created from the pain signal, and finally NO is created. Mice's ventral tegmental area (VTA) dopaminergic neurons were in charge of regulating impairments in anxiety-like behavior. CES caused chronic emotional stress (CES), which caused VTA dopaminergic neurons to behave anxiously in the naturally anti-anxiety environment, despite chemogenetic inhibition of these neurons helping to strengthen resistance to CES-induced anxiety-like behavior. Furthermore, mice have been shown to activate dopaminergic neurons in the VTA that receive information from the nucleus accumbens (NAc). As a consequence, the NAc-VTA circuit was created, and it proved successful in reducing anxiety-like behavior in mice mediated by CES. Behavioral analysis was also done using optogenetics and chemogenetics methods, electrophysiology, viral tracer, immunoelectron microscopy, and total internal reflection fluorescence (TIRF) imaging in order to observe, research, and comprehend the pertinent nervous system components and their mechanisms. It may establish, using mouse-based animal models, or block the route to govern anxiety-like behavior. It can also stimulate or inhibit the activity of neurons and other factors.

2. Analysis of the Immunological and Neurological System Interactions That Cause Anxiety-like Behavior in Mice

In the mouse brain's diaphragm compartment, innate and adaptive immune cells may continually scan the central nervous system for infections. The contact between the immune system and the nervous system is facilitated by neurotransmitters and cytokines, two soluble molecules [1-3]. T cells are crucial for memory, social behavior, and spatial learning [4-10], and interleukin (IL)-4, -17, and interferon (IFN) have been found to influence GABAergic neurotransmission in mice. Brain microglia and signal neurons are also altered by natural killer (NK) cells. Age-related increases in the number of NK cells in the dentate gyrus of the hippocampus render older neuroblastocytes hazardous, reducing synaptic plasticity and cognitive function. According to research, ILC1 and NK cells alter neuronal activity, modify certain neural networks, and regulate mouse behavior, making them unexpected players in the immune system's management of brain homeostasis. The deletion of NK1.1 cells in mice reduced anxiety-like behavior and may have hampered non-spatial memory through the release of IFN- and acetylcholine (ACh) [11]. For the regulation of the hypothalamic-ventral tegmental region (VTA), the three factors that make up the hippocampus network's effect on anxiety-like behavior are as follows: (1) ACh stimulates dopaminergic receptors in the hippocampus area; (2) dopaminergic neurons in the VTA are activated; and (3) orexin neurons in the hypothalamus are activated.

2.1. NG2 Glial Cells and Hippocampus

There have been reports that NG2 glia, also known as OPCs, form NG2 glia to neuronal synaptic complexes in response to specific light stimulation and Selective light stimulation of NG2 glia that express the channel rhodopsin 2 (ChR2), which also enhances adjacent interneurons in the inhibitory synaptic transmission microcircuit, functionally drives the release of GABA. There is proof that NG2 glia cells convey GABA signaling because they produce GAD67 and exocytose vesicles that contain VAMP-2 [12]. Optogenetic activation, NG2 glial selective genetics, transcriptome, electrophysiological, total internal reflection fluorescence (TIRF) imaging, immunoelectron microscopy (EM), and behavioral analysis were the approaches used in this study. According to our research, NG2 glial activation resulted in anxiety-like behavior in a mouse model of chronic social failure stress (CSDS). We first measured Ca^{2+} levels in the CSDS mouse model. The majority of calcium ion fluctuations seen in NG2 glial cells after the mice underwent failed social stress point to

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the activation of NG2 glial cells in CSDS. NG2 glial cell signaling by NG2-CREERTM. Mice were also placed through the elevated maze test (EPM) and the open field test (OFT) in order to examine the behavioral changes in mice before and after CSDS. Because the light stimulation of NG2 glia in the hippocampus had no effect on the CSDS mice's motor activity or social avoidance behavior, the results showed that the activation of NG2 glia directly produces anxiety-like behavior.

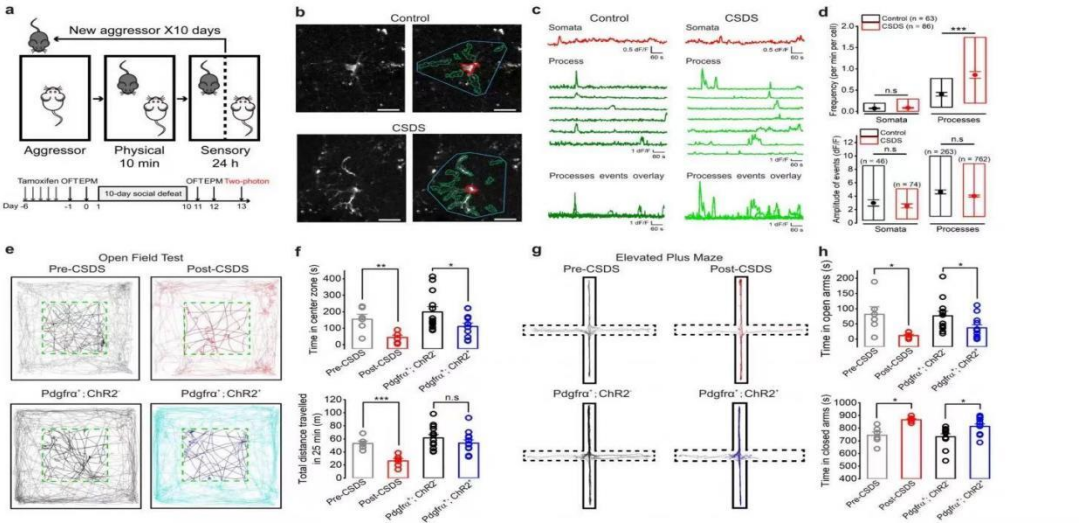

Figure 1: Increased calcium signals in NG2 glia and enhanced anxiety-like behavior in chronic social defeat stress (CSDS) mice [13].

The intracellular Ca^{2+} of mouse cells was measured in a CSDS mouse model, and NG2 glial cells were using NG2-CREER to signal (Figure 1a). Experimental results showed that hippocampal NG2 glial cells started to fluctuate freely but gradually, in contrast to normal animals. (Figure1b-d) Figure 1(b&c) displays hippocampal NG2 glial cells measured 2-8 weeks after birth in control and CSDS mice. Somatic waves (red) and process waves (green) are displayed in the CSDS. Ca^{2+} waves are seen during social failure stress in mice exposed to NG2 glial cells. The wave features of CSDS mice (three animals per group) and the intrinsic activation of NG2 glial cells are compared in Figure 1d with the average Ca^{2+} data. Mice were subjected to elevated plus maze and field tests to compare their behavior before and after CSDS. Animals exhibit more anxiety-like behavior when social failure is present. Figure 1e shows how visual stimulation affects mouse and control movement patterns in an open environment. Figure 1f shows the core region of the two groups of mice in the open field throughout a 25-minute period in terms of time and distance. The EPM test showed that mice spent less time in the middle of the open field and less time with their arms extended (Figures 1e and 1f). Activity traces showing how CSDS mice and their control interact in the raised plus maze group. Further evidence that activation of NG2 glial cells directly results in anxiety-like behavior in mice was provided by the fact that anxiety-like behavior persisted in CSDS mice after in vivo application of simple light activation of hippocampal NG2 glial cells without affecting motor activity or social avoidance behavior (Figure 1h).

Among other serious neurological conditions, disruptions in the excite-inhibitory (E-I) balance or dysregulation of the GABAergic system in the hippocampus can contribute to stress and mood issues [14-16]. In response to NG2 glial cell photo-stimulation, the AP firing rate of pyramidal neurons increased but the neuronal resting membrane potential remained constant, upsetting the E-I balance in the hippocampal circuit. As was previously mentioned, NG2 glial cells regulate postsynaptic inhibitory activity by encouraging the release of the GABA neurotransmitter through release by

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selective optogenetic stimulation. This, in turn, causes E-I balance disruption, which eventually causes anxious-like behaviors and may be related to CSDS [12].

2.2. Mechanism of Action of VMPFC Ventromedial Prefrontal Cortex Mediating Anxiety-like Behavior

It has been found that anxiety induced by pain depends on nNOS-expressing neurons in the ventromedial prefrontal cortex (vmPFC). As a consequence, a mouse model of chronic pain was studied using optogenetics and chemogenetic methods. When vmPFC NNOS-expressing neurons convert pain impulses into anxiety signals, nitric oxide (NO) is released [17]. Previous studies have demonstrated that nitric oxide synthase (nNOS) and its product, nitric oxide (NO), can modify mouse anxiety-related behaviour.Numerous neurons in the neocortex, including those in the vmPFC, express nNOS [18]. Increased nNOS enzyme activity and NO production in the vmPFC are associated with long-term antianxiety-like effects caused by predator exposure and acute constraint stress [19, 20]. NO is a key neurotransmitter in the perception of pain from injuries. Neuropathic pain can be lessened by inhibiting nNOS [21, 22]. Therefore, it may be hypothesized that NNOS-NO signaling and neurons in the vmPFC that produce nNOS are implicated in anxiety brought on by chronic pain [17]. Three days after CFA injection in mice, nNOS protein levels and enzyme activity were assessed, and it was shown that anxiety-like behaviors brought on by chronic pain were obvious. Enzyme activity dramatically increased while nNOS protein levels remained unchanged (Figure 2a & 2b). When vmPFCnNOS expressing neurons were stimulated by chemogenetics, nNOS enzyme activity increased in comparison to animals that had received NS injections [17]. As a result, the presence of dispersed NO modulates the anxiety brought on by chronic pain.The neuronal population is stimulated by the glutamergic pPVT-vmPFC input to create anxiety signals, which in turn causes the creation of pain signals. AMPARs are then transported to the plasma membrane and interact with the protein S-nitrosoylation by diffuse NO, which increases their capacity to cause anxiety in those who are suffering from chronic pain.

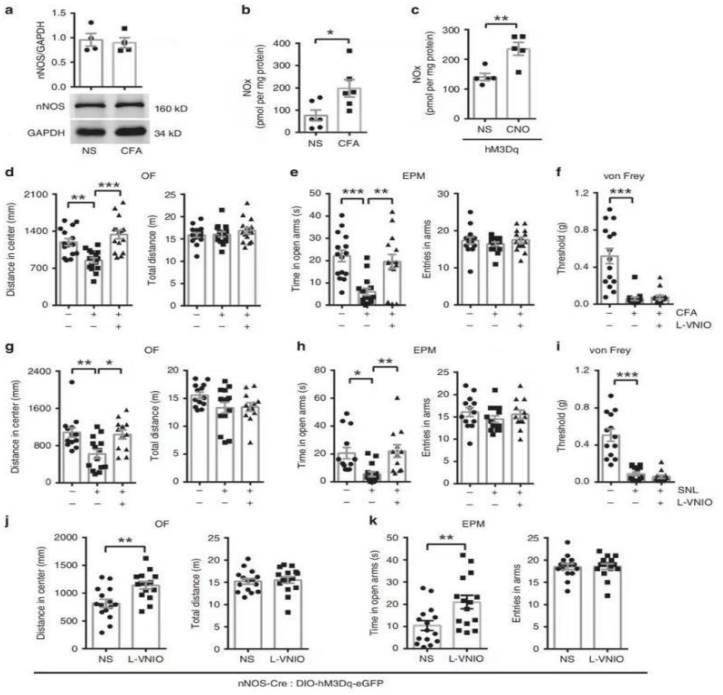

Figure 2: n-NOS-expressing neurons in the vmPFC mediate chronic pain-induced anxiety through diffusing NO.a,b [23].

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The NO donor used in the experiment was 3,3-bis(aminoethyl)-1-hydroxy-2-oxo-1-triazene (DETANOate), while the Nos-specific inhibitor used was N5-(1-imino-3-butenyl)-L-Ornithine (L-VNIO). Topical l-VNIO administration to the vmPFC did not significantly reduce anxiety in normal mice, but 3 days after CFA injection, it reversed anxiety-like behavior brought on by chronic pain. However, it had no impact on mouse movement. In the SNL-induced chronic neuropathic pain study, inhibition of nNOS enzyme activity was revealed to have the same antianxiety effect and had no influence on motor activities. NOS-CRE animals (n=3) were injected with vmPFC 30 days after CNO (0.5 mM, 1 L) or 21 minutes after NS administration, respectively, or the vmPFC's nNOS enzyme activity. Von Frey test (f) in L-VN1O (1.5mM, 1pl) or NS (1l) withdrawal threshold of the posterior posterior paw after 3 minutes after microinjection into vm PFC CFA or NS on day 15 after injection. D-f in the OF test for total distance from height (d, right) and distance from center (d, left) n=30. G-i uses the open arm time (h, left) and the total arm entry (h, right) of the EPM test, as well as the center distance (g, left) and total distance (g, right) of the OF test, and the exit threshold (i) of the posterior posterior paw in the vonFrey test on day 1 following SNL or sham surgery in the vmPFC L-VNIO (5.1mM, 1l) or NS (7l) 13 minutes after microinjection in VMPFC. n=14, 12, and 30. j, k center distance (j, left) and total distance (j, right) with the OF test.

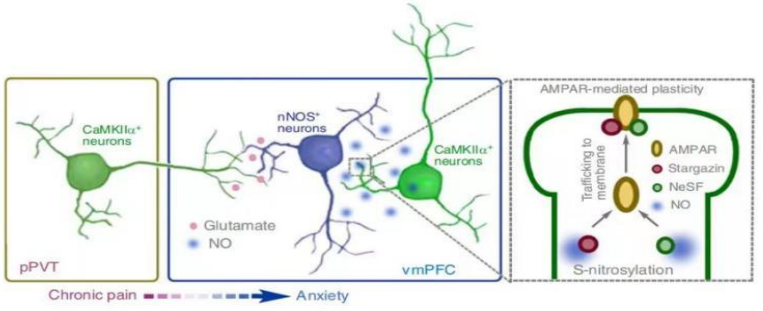

Figure 3: A model diagram illustrating the role of vmPFC nNOS-expressing neurons in chronic pain-induced anxiety [24].

Figure 3 shows that during chronic pain, vmPFCnNOS-expressing neurons were activated by glutamatergic input from pPVT CaMKII alpha pyramidal neurons. S-nitrosylation of AMPAR interacting proteins in CaMKII alpha pyramidal neurons, which was brought on by the enhanced expression of nNOS neurons, promoted AMPAR trafficking to the plasma membrane. As a result, enhanced AMPAR activity in vmPFC CaMKII alpha pyramidal neurons contributes to anxiety-like behavior.

2.3. Mechanism of Anxiety-like Behavior Induced by Nucleus Accumbens (NAC) Related Factors

The circuit mechanism underlying anxiety-like behavior is examined in the next section. Both chronic emotional stress (CES) and chemogenetic activation of dopaminergic neurons in the ventral tegmental area (VTA) have been shown to directly cause anxiety-like behaviors. Here is a diagram of a three-compartment mouse replacement. It is demonstrated using the 3C-VSDS model of social failure stress that mice subjected to chronic emotional stress (CES) exhibit anxiety-like behavior and temporary changes in social interaction. We found that the nucleus accumbens (NAc) of CES mice contains active VTA dopaminergic neurons. To mimic or counteract the effects of anxiety-inducing CES, the NAc VTA circuit can be manipulated in both directions [25]. One of the crucial regions in the adaptive behavioral coping circuit is the ventromedial prefrontal cortex (vmPFC), is also responsible for controlling anxiety. It was proven in the 3C-VSDS model that VTADA neurons govern anxiety-like

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behavior in CES using a combination of viral tracer, in vivo electrophysiological, calcium ion monitoring, chemical genetics, and optogenetics approaches [25]. During the course of the investigation, it was proposed that the NAC-VTA circuit generates and mediates the anxiety-like behavior of CES by examining the functional role of the nucleus accumbens (NAc) on the VTA circuit.

3. Conclusion

In conclusion, the results demonstrate that: (1) the nervous and immune systems can cooperate to regulate anxiety-like behavior in mice; (2) photoactivation of NG2 glial cells directly causes anxiety-like behavior; (3) NNOS-expressing neurons in the vmPFC and the nNOS-NO signaling pathway directly cause anxiety-like behaviors induced by chronic pain; and (4) bidirectional modulation of the NAc-VTA circuit mimics or inhibits CES-induced anxiety-like behavior. The study attempts to manipulate these pathways to promote anxiety-like behavior in animal models where neurological and other related factors contribute to anxiety-like behavior. There are also certain time and space restrictions, as well as a small number of animal models used in this study, that must be taken into consideration. The previous research described in section 2.3 indicate that there are not many reports between anxiety and chronic pain. Both chronic pain and anxiety have similarities and differences.

These problems with this paper must be resolved: (1) NG2 glial cells, as opposed to other glial cells, may be able to directly sense the synaptic input of neurons, although further research is needed to determine the physiological importance of this synaptic activity. Furthermore, it is still unclear how NG2 glial cells communicate with neurons and affect the synaptic structure of the neural network. (2) Psychology has historically been the main area of research for anxiety, but little is understood about the brain circuits and chemical signals involved in the anxiety-like behaviors caused by chronic pain, making management and relief of these behaviors difficult. This paper is intended to inspire people to do study and develop strategies for lessening anxiety-like behaviors. (3) Although the biological information and brain regions linked to emotional stress have been discovered in the early phases of the investigation, the neurological mechanism of the circuit is still unclear. (4) Since the anxiety stress experiment is challenging and there are few animal models of pure emotional stress, the development of ideal animal models can enhance the experiment. There are no better tools for studying the CES process in mice directly at the level of individual circuits and neurons, hence the 3C-VSDS model was employed in this work. If the circumstances are good, more research should be done to look into these limitations in the future.

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