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COMMENTARY

$GABA_A$ Receptor Subtype Specificity in Essential Tremor

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Essential tremor (ET) is a very common neurological disorder, yet it can be highly disabling. People with ET have prominent hand action tremors, causing difficulty with performing activities of daily living such as drinking, eating, handwriting, and typing. Tremor can also involve head and voice in ET. Since ET is an age-related disorder, the prevalence of ET is expected to continue to increase as the worldwide population ages [1]. Therefore, novel therapies for ET are urgently needed.

One interesting clinical observation is that about two-thirds of ET patients experience less tremor after drinking alcohol (i.e., ethanol) [2]. However, how ethanol suppresses tremor in ET remains a mystery, which may have implications for developing new therapies. Ethanol can potentiate γ-aminobutyric acid type A ($GABA_A$) receptors [3]; thus, insufficient GABA neurotransmission is considered to be strongly linked to the pathophysiology of ET [4]. This concept is further corroborated by the fact that tremor in some ET patients responds to primidone [5], a form of barbiturate that can increase the duration of $GABA_A$ receptor opening [6]. It is important to point out that not all ET patients have ethanol or primidone responsiveness, indicating heterogeneity of ET patients.

The key brain areas underlying ET are the cerebellum and the downstream cerebello-thalamo-cortical loop based on ample evidence from neuroimaging [7], neuropathology [8], and animal model studies [9]. There are many different types of synaptic connections in the cerebellum that utilize $GABA_A$ receptors for inhibitory neurotransmission: (1) Purkinje cells to deep cerebellar nuclear neurons; (2) interneurons, such as basket cells and stellate cells, to Purkinje cells; and (3) deep cerebellar nuclear neurons to inferior olivary neurons. These locations have been postulated to be sites of ethanol action to suppress tremor in ET [10]. However, $GABA_A$ receptors are widely expressed throughout the central nervous system; therefore, $GABA_A$ receptor manipulations are likely to have cognitive side effects such as sedation. Interestingly, ET patients who drink alcohol usually have tremor suppression before they become drunk. Therefore, the tremor-suppressing and sedative effects of ethanol may be via different $GABA_A$ receptor subtypes at different synaptic or extra-synaptic locations. Understanding $GABA_A$ receptor subtype specificity in ET will serve as the basis to develop effective therapies for tremor and to minimize side effects.

$GABA_A$ receptors have complex subtypes, depending on the subunit composition. Each $GABA_A$ receptor is pentameric containing at least one α and one β subunits. The common arrangement of $GABA_A$ receptors is two α and two β subunits with one γ subunit [11]. Extra-synaptic $GABA_A$ receptors contain δ instead of γ subunit and are particularly enriched in the cerebellum [12]. Granule neurons are the most abundant types of neurons in the cerebellum and express distinctive types of $GABA_A$ receptors. Specifically, α6βγ2 $GABA_A$ receptors are located at Golgi cell to granule cell synapses, whereas α6βδ $GABA_A$ receptors are situated at the extra-synaptic site [13]. As more small molecules that can target specific subtypes of $GABA_A$ receptors are being developed, understanding $GABA_A$ receptor subtype specificity in tremor has become the central focus of ET therapeutic development.

In this issue of Neurotherapeutics, Huang et al. [14] studied the role of the α6 subunit-containing $GABA_A$ receptors in tremor. They used the harmaline-induced tremor model in mice. Harmaline can induce action tremor in mice at a frequency between 10 and 16 Hz [15] that is responsive to ethanol and primidone [16], similar to ET patients. They found that a positive allosteric modulator of α6$GABA_A$

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receptors, called compound 6, or ethanol can effectively suppress harmaline-induced tremor in mice. Interestingly, intracerebellar microinfusion of furosemide ($\alpha6GABA_A$ receptor antagonist, which is clinically used as a diuretic because of its additional function in inhibiting sodium and potassium-2 chloride co-transporters) could partially reverse the effects of ethanol or compound 6 in harmaline-induced tremor. Finally, the authors demonstrated that compound 6 and ethanol synergistically suppress harmaline-induced tremor. These findings are important to help us understand the brain circuitry for tremor. First, activating the α6 subunit-containing $GABA_A$ receptors suppresses tremor, which helps us to zoom in on the subtype specificity of $GABA_A$ receptors. Second, cerebellar microinfusion of a $\alpha6GABA_A$ receptor antagonist can partially reverse the suppressive effects of ethanol and compound 6 in harmaline-induced tremor, which further indicates that $\alpha6GABA_A$ receptors in the cerebellum play a role in tremor. Within the cerebellum, granule cells are likely to be responsible for such effects because $\alpha6GABA_A$ receptors are particularly enriched in these neurons [13]. And finally, since ethanol and compound 6 do not counteract each other's effects on tremor suppression, it is likely that other mechanisms also exist, in addition to $\alpha6GABA_A$ receptor activation. Collectively, these experiments further help us to understand the specific $GABA_A$ receptor subtypes involved in tremor and have therapeutic implications for ET.

Several limitations and future perspectives should be considered in the context of the current study. First, all the experiments are based on the responses of pharmacological agents (harmaline, ethanol, compound 6, and furosemide); thus, the specificity will need to be further elucidated in the future with genetic approaches. In addition, the pharmacokinetics and pharmacodynamics of each agent are different, which adds to the complexity of the interpretation. Second, while the evidence suggests that granule cells are involved based on the expression of $\alpha6GABA_A$ receptors [13], cell-type-specific manipulations will be needed in the future to confirm this claim. Third, the effects of a $\alpha6GABA_A$ receptor antagonist in blocking the tremor-suppressing effects of compound 6 and ethanol are modest, suggesting that other mechanisms could also be important. And fourth, ET is likely to be a family of diseases with different pathophysiologies [17]. Studying other mouse models of tremor [18, 19] may shed light on the generalizability of the current findings.

It is noteworthy that another $GABA_A$ receptor subunit has been proposed in the pathophysiology of ET, namely the δ subunit. As mentioned above, $\delta GABA_A$ receptors are also enriched in the granule cells of the cerebellum, and α6 and δ subunits are often co-expressed as extrasynaptic $GABA_A$ receptors at the Golgi cell to granule cell synapses [20]. In a harmaline-induced tremor mouse model, activating these extra-synaptic $GABA_A$ receptors with gaboxadol suppresses tremor [21]. In addition, gaboxadol failed to suppress tremors in δ subunit knockout mice treated with harmaline [21]. These data suggest that the δ subunit of $GABA_A$ receptors can also be a target for ET therapeutic development.

The identification of $\alpha6GABA_A$ receptor involvement in tremor paves the way to further study GABA mechanisms in ET. However, $\alpha6GABA_A$ receptors do not appear to be directly involved in tremor generation, because furosemide microinfusion into the cerebellum does *not* exacerbate harmaline-induced tremor. Rather, $\alpha6GABA_A$ receptors may be an intrinsic circuit mechanism that ethanol or compound 6 can harness to suppress tremors. This is further corroborated by clinical observations that furosemide, when taken as a diuretic by patients, does not appear to worsen or induce tremors. In addition, $\alpha6GABA_A$ receptor knockout mice do not appear to develop tremors either [22]. A similar situation also occurs with δ subunit-containing $GABA_A$ receptors. Genetic deletion of $\delta GABA_A$ receptors in mice does not appear to impact harmaline-induced tremor but merely changes the tremor responsiveness to gaboxadol [21]. Of note, $\delta GABA_A$ receptor deletion in the cerebellum also does not appear to produce tremor [12].

The harmaline-induced tremor comes from the enhanced coupling of inferior olivary neurons, which entrains the downstream Purkinje cells to fire synchronously and rhythmically via climbing fiber innervation [9]. Much of the literature demonstrates the importance of the inferior olive-Purkinje cell-deep cerebellar nucleus loop in harmaline-induced tremor [10]. The current study by Huang et al. [14] suggests that the granule cells-parallel fiber system can modulate the brain circuitry of tremor. Further exploration of the detailed mechanism of the interactions of climbing fiber and parallel fiber systems, integrated at the Purkinje cells, is likely to yield a more complete picture of ET pathophysiology.

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