PsychoGenics

The Role of γ-aminobutyric acid (GABA) and Glutamate in Harmaline-Induced Tremors in Mice

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Introduction

➢Essential Tremor (ET) in one of the most common movement disorders which is thought to be twenty times more common than Parkinson's disease. In general, ET is prevalent in the elderly and does not lead to serious complications. However, it has been shown that ET can be debilitating in a small percentage of patients.

➢Though the causes of ET are unknown, genetics are thought to play a role in about 50% of ET cases. Three gene loci (*ETM1* on 3q13, *ETM2* on 2p24.1 and a locus on 6p23) have been identified in a familial form of ET. In addition, a Ser9Gly variant in the dopamine D_3 receptor gene on 3q13 is thought to be a risk factor. (Deng et al., 2007; Higgins et al., 1999).

➢The neuronal circuitries underlying ET are not well understood, however positron emission topography in brains of ET patients revealed increased activity in the inferior olive, thalamus and cerebellum (Jenkins et al., 1993). In addition, post mortem studies of ET patients revealed higher levels of glutamate and asparate and decreased levels of γ-aminobutyric acid (GABA), serine, and glycine in cerebrospinal fluid. (Mally et al., 1996)

➢Animal models for ET are scarce. Two rodents models that are used to study ET are the harmaline animal model and the $GABA_A$ receptor α_1 subunit knockout mice (Jankovic and Noebels, 2005; Kralic et al., 2005: Miwa H 2007; Lorenz and Deuschl 2007).

➢Dopamine is also implicated in ET. D_2 receptor null mutant mice show increased sensitivity to the tremorigenic effects of harmaline (Fowler et al., 2002), and a serine glycine variant in the dopamine D_3 receptor gene is associated with increased risk to develop ET (Jeanneteau et al., 2006; but see Blair et al., 2008).

➢Harmaline is a beta-carboline derivative that causes generalized tremor in mice with a frequency of 11 – 14Hz (Milner et al., 1995). Harmaline acts on the neurons of the inferior olivary nucleus (ION) to modulate their rhythm-generating ionic currents and thereby resulting in generalized tremor. Harmaline-induced tremors in mice can be attenuated by ethanol and octanol suggesting a GABA-ergic component (Rappaport et al., 1984; Martin et al., 2005) as well as propranolol (Martin et al., 2005) suggesting an adrenergic component.

Hypothesis

GABAergic, glutamatergic and dopaminergic systems underlie some of the mechanisms of harmaline-induced tremors in mice.

Methods

Male ICR mice from Taconic Laboratories (Germantown, NY) were used in this study. Upon receipt, mice were assigned unique identification numbers (tail marked) and group housed in OPTI mice ventilated cages. All animals remained housed in groups of four during the remainder of the study. Mice were maintained on a 12 hr /12 hr light/dark cycle with the light on at 6:00 a.m EST. The room temperature was maintained between 20 and 23ºC with a relative humidity maintained between 30% and 70%. Chow and water were provided *ad libitum* for the duration of the study. Prior to testing, all mice were examined on a regular basis, handled, and weighed to assure adequate health and suitability. In each test, animals were randomly assigned across treatment groups. Animals were not disturbed between test days.

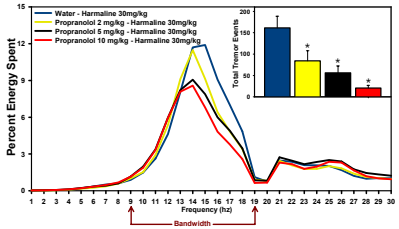
Group housed mice were brought to the experimental room for at least one hour to acclimate prior to testing. Following a 20 min pretreatment with vehicle or test compounds, mice were injected with harmaline (30 mg/kg) and placed inside the Tremor Monitor (San Diego Instruments, SDI) chamber for a 10 minute acclimation period. Following habituation, tremor activity of the mice is measured for approximately 8 min. The recorded frequencies (1-64 hertz) of activity and the number of tremor events are captured electronically.

Data are analyzed by the tremor monitor software (SDI) in a two part process. Using a Fast Fourier Transform (FFT), an output is provided showing the percentage of activity (energy) recorded at each frequency. A center frequency of activity between 14 - 15 Hz is chosen, along with a bandwidth of 10 Hz. Using these parameters, tremor events are tabulated as total tremor events.

Statistical Analysis

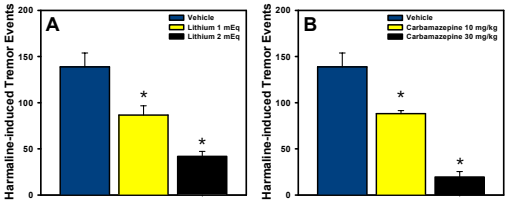
Data were analyzed using analysis of variance (ANOVA) followed by Fisher PLSD post hoc test when appropriate. An effect was considered significant if $p < 0.05$.

Figure 1: Fast fourier transform output for the effects of propranolol on harmaline-induced tremors



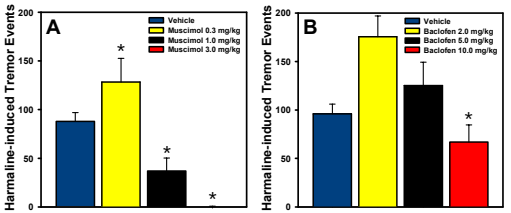
During test sessions, the multiples frequencies (hertz) of activity are recorded. Using a Fast Fourier Transform (FFT), an output is provided showing the percentage of energy (activity) recorded at each frequency. Center frequency (15 hz) and bandwidth (10 hz) are used for analysis of tremor events.

Figure 2: Lithium and Carbamazepine dose-dependently decrease harmaline-induced tremors



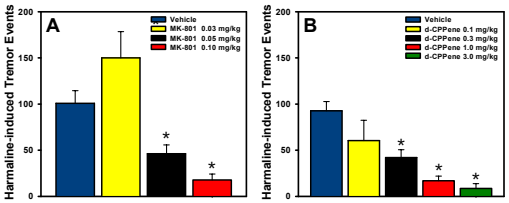
*Mice were injected with vehicle, the mood stabilizer lithium, or the anticonvulsant carbamazepine 20 min prior to harmaline. Data represent mean ± SEM. *significantly different from vehicle (p<0.05)*

Figure 3: $GABA_A$ and $GABA_B$ receptor agonists muscimol and baclofen decrease harmaline-induced tremors



*Mice were injected with vehicle, muscimol, or baclofen (oral) 20 min prior to harmaline injection. Data represent mean ± SEM. *significantly different from vehicle (p<0.05)*

Figure 4: *N*-methyl-D-aspartate (NMDA) receptor antagonists decrease harmaline-induced tremors



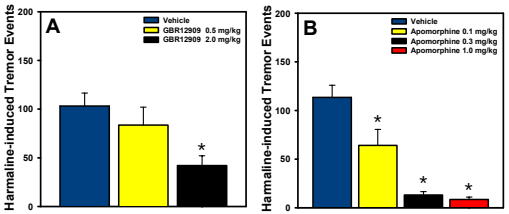
*Mice were injected with vehicle, MK-801 (a non-competitive NMDA receptor antagonist), or d-CPPene (a competitive NMDA receptor antagonist) 20 min prior to harmaline injection. Data represent mean ± SEM. *significantly different from vehicle (p<0.05)*

Table 1: Effects of AMPA and mGlu5 receptor antagonists on harmaline-induced tremors

	n	Harmaline-induced Tremor Events (mean ± SEM)
AMPA Receptor		
Vehicle	7	108.57 ± 16.99
NBQX 1 mg/kg	9	103.22 ± 21.27
NBQX 10 mg/kg	9	57.44 ± 12.52*
NBQX 30 mg/kg	8	23.88 ± 6.20*
mGlu5 Receptor		
Vehicle	12	109.75 ± 25.24
MPEP 10 mg/kg	8	100.5 ± 22.47
MPEP 20 mg/kg	8	134.13 ± 30.44

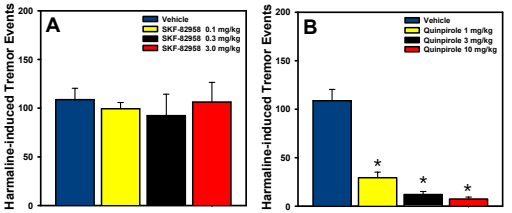
*Mice were injected with water, NBQX (AMPA receptor antagonist), or MPEP (mGlu5 receptor antagonist) 20 min prior to harmaline injection. *significantly different from vehicle (p<0.05)*

Figure 5: The dopamine agonist apomorphine decreases harmaline-induced tremors



*Mice were injected with water, the DA transporter inhibitor, GBR12909, or apomorphine (s.c.) 20 min prior to harmaline injection. Data represent mean ± SEM. *significantly different from vehicle (p<0.05)*

Figure 6: Dopamine D_2, but not D_1 receptor stimulation decreases harmaline-induced tremors



*Mice were injected with water, SKF82958, or quinpirole 20 min prior to harmaline injection. Data represent mean ± SEM. *significantly different from vehicle (p<0.05)*

Summary

➢In ICR mice, injection of harmaline produces tremors that can be quantified based on the magnitude of energy spent.

➢The mood stabilizer lithium and the anticonvulsant carbamazepine showed a dose-dependent attenuation of harmaline-induced tremors.

➢ The GABAergic involvement in harmaline-induced tremors is supported by our findings showing attenuation of harmaline-induced tremors by $GABA_A$ and $GABA_B$ receptor agonists.

➢These data also support the possibility of glutamaterigc mechanisms underlying harmaline-induced tremors. Both competitive and non-competitive NMDA receptor antagonists d-CPPene and MK-801 blocked harmaline-induced tremors, respectively. In addition to NMDA-receptors, inhibition of ionotropic AMPA receptors with NBQX also attenuated harmaline-induced tremors. On the other hand, we found no effect for metabotropic glutamate receptors on this measure, at least with the mGluR I receptor family. Further investigation is needed to determine whether other mGluR receptors are involved in harmaline-induced tremors.

➢Since PCP and MK-801 can increase dopaminergic tone and we have previously shown that the dopamine transporter inhibitor GBR12909 decreased harmaline-induced tremors, we investigated some of the dopaminergic systems involved. The data presented show that the dopamine agonist apomorphine attenuated harmaline-induced tremors. This effect could be primarily on activation if D_2 receptors since quinpirole, a D_2/D_3 receptor agonist, significantly decreased harmaline-induced tremor at all doses tested. Interestingly, DA D_1 receptors do not appear to play a role since the DA D_1 receptor agonist SKF82958 had no effect on harmaline-induced tremor.

➢The harmaline-induced tremor model in mice continues to provide a valuable and functional model for screening novel compounds for treatment of ET.

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