PsychoGenics

# Pharmacological Characterization of 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 time 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.
- Although the causes of ET are not known, 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 this 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 either but 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 revealed higher levels of glutamate and asparate and decreased levels of γ-aminobutyric acid (GABA), serine and glycine in the 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 GABAA receptor alpha1 subunit knockout mice (Jankovic and Noebels, 2005; Kralic et al., 2005: Miwa H 2007; Lorenz and Deuschl 2007).
- 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). Propranolol is also reported to inhibit harmaline-induced tremors in mice and rats (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

### Animals

Male ICR mice from Taconic Laboratories (Germantown, NY) were used in this study. Upon receipt, mice were assigned unique identification numbers (tail marked) and were 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.

### Harmaline-induced tremors

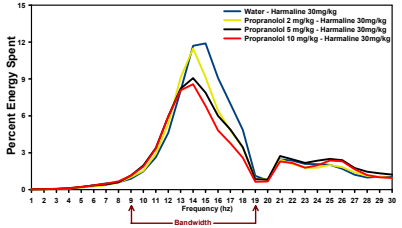
Group housed mice were brought to the experimental room for at least one hour to acclimate prior to testing. Following a 20 min pretreatment with vehicle or test compounds, mice were injected with harmaline (30 mg/kg) and placed inside the Tremor Monitor (San Diego Instruments, SDI) chamber for a 10 minute acclimation period. Following the habituation period, 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 (San Diego Instruments) 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 short, long, and total events. A short event was an event that lasted 0.3 – 0.5 seconds and a long event lasted more than 0.5 seconds.

### Statistical analysis

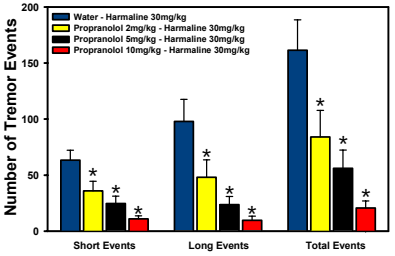
Data were analyzed using analysis of variance (ANOVA) followed by Fisher PLSD post hoc test when appropriate. An effect was considered significant if $p < 0.05$.

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



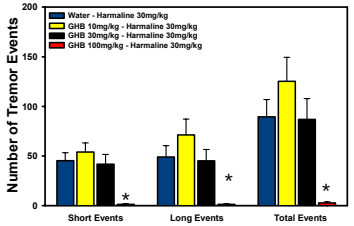

*During test sessions, the multiples frequencies (hertz) of activity are recorded. Using a Fast Fourier Transform (FFT), an output is provided showing the percentage of energy (activity) recorded at each frequency. Center frequency (15 hz) and bandwidth (10 hz) are used for analysis of tremor events.*

## Figure 2: Propranolol decreases harmaline-induced tremors



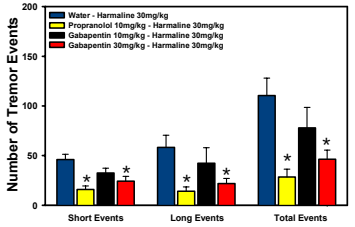

*Mice were injected with water or propranolol (i.p) 20 min prior to harmaline injection. Data represent mean ± SEM.*

## Figure 3: γ-hydroxybutyrate decreases harmaline-induced tremors



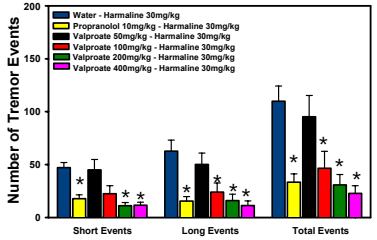

*Mice were injected with water or GHB (i.p) 20 min prior to harmaline injection. Data represent mean ± SEM.*

## Figure 4: Gabapentin decreases harmaline-induced tremors



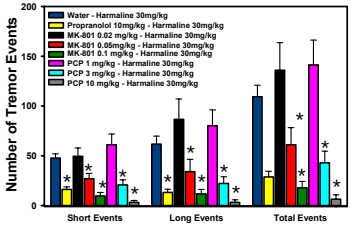

*Mice were injected with water, propranolol or gabapentin (i.p) 20 min prior to harmaline injection. Data represent mean ± SEM.*

## Figure 5: Valproate decreases harmaline-induced tremors



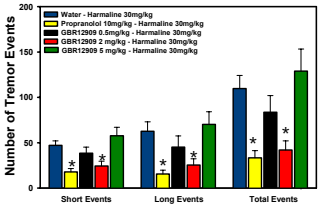

*Mice were injected with water, propranolol or valproate (i.p) 20 min prior to harmaline injection. Data represent mean ± SEM.*

## Figure 6: *N*-methyl-D-aspartate receptor antagonists decrease harmaline-induced tremors in mice




*Mice were injected with water, propranolol, phencyclidine (PCP) or MK-801 (i.p) 20 min prior to harmaline injection. Data represent mean ± SEM.*

## Figure 7: The dopamine uptake blocker GBR12909 decrease harmaline-induced tremors in mice only at 2 mg/kg




*Mice were injected with water, propranolol or GBR 12909 (i.p) 20 min prior to harmaline injection. Data represent mean ± SEM.*

## Summary

- In ICR mice, injection of harmaline produces tremors that are can be quantitated based on the magnitude of energy spent.
- We confirmed previously reported data showing the ability of propranolol to attenuate harmaline-induced tremors.
- Pharmacological validation of this model suggests the involvement of GABAergic pathways since GHB blocked the tremors induced by harmaline. These data support the findings of Kralic et al., (2005) showing similar effects of GHB in the $GABA_A$ KO mice.
- Glutamatergic pathways might underlie harmaline-induced tremors in mice. Non-competitive NMDA receptor antagonists MK-801 and PCP blocked harmaline-induced tremors. However, since both compounds can increase dopaminergic tone either directly or indirectly, we tested the dopamine uptake blocker GBR12909 and found that it also attenuated harmaline-induced tremors at 2 mg/kg only.
- The anticonvulsant agents gabapentin and valproate showed a dose-dependent attenuation of harmaline-induced tremors.
- The data suggest the involvement of multiple neurotransmitter systems in harmaline-induced tremors in mice. Further validation is needed to support a dopaminergic role in this test.

Nonetheless, harmaline-induced tremors in mice provide could be used as a model for screening novel therapeutic compounds for ET.

## References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