

## Review

# Harmaline Tremor: Underlying Mechanisms in a Potential Animal Model of Essential Tremor

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## Abstract

**Background:** Harmaline and harmine are tremorigenic  $\beta$ -carbolines that, on administration to experimental animals, induce an acute postural and kinetic tremor of axial and truncal musculature. This drug-induced action tremor has been proposed as a model of essential tremor. Here we review what is known about harmaline tremor.

**Methods:** Using the terms harmaline and harmine on PubMed, we searched for papers describing the effects of these  $\beta$ -carbolines on mammalian tissue, animals, or humans.

**Results:** Investigations over four decades have shown that harmaline induces rhythmic burst-firing activity in the medial and dorsal accessory inferior olivary nuclei that is transmitted via climbing fibers to Purkinje cells and to the deep cerebellar nuclei, then to brainstem and spinal cord motoneurons. The critical structures required for tremor expression are the inferior olive, climbing fibers, and the deep cerebellar nuclei; Purkinje cells are not required. Enhanced synaptic norepinephrine or blockade of ionic glutamate receptors suppresses tremor, whereas enhanced synaptic serotonin exacerbates tremor. Benzodiazepines and muscimol suppress tremor. Alcohol suppresses harmaline tremor but exacerbates harmaline-associated neural damage. Recent investigations on the mechanism of harmaline tremor have focused on the T-type calcium channel.

**Discussion:** Like essential tremor, harmaline tremor involves the cerebellum, and classic medications for essential tremor have been found to suppress harmaline tremor, leading to utilization of the harmaline model for preclinical testing of antitremor drugs. Limitations are that the model is acute, unlike essential tremor, and only approximately half of the drugs reported to suppress harmaline tremor are subsequently found to suppress tremor in clinical trials.

**Keywords:** Tremor, harmaline, harmine, inferior olive, cerebellum, animal model

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## Introduction

Harmaline induces action tremor in mammals, and as an easily elicited model has attracted increasing interest from workers searching for new therapies for essential tremor (ET). In view of this interest, we review what is known about harmaline's actions. We describe the model and review the anatomy and physiology of the olivocerebellar circuitry underlying harmaline tremor. We consider proposed mechanisms by which harmaline produces tremor and survey the pharmacology of harmaline tremor. We discuss the limitations of the model and consider how well harmaline predicts drug efficacy for ET.

## Methods

We surveyed literature obtained via PubMed using the search words “harmaline” and “harmine”, examining papers describing mechanisms, properties, or tremor. We also consulted related papers on cerebellum physiology and ET. Only a fraction of these publications could be cited.

## Results

### $\beta$ -Carbolines

The basic structure of  $\beta$ -carboline alkaloids is similar to tryptamine, a two-ring indole, but the ethylamine side chain is reconnected to the

indole via a carbon atom, forming a third ring.  $\beta$ -Carbolines differ according to the degree of saturation of the third ring, the third ring side chain, and the side chains on the benzene ring (Figure 1).

Nutritionally the main source of  $\beta$ -carbolines is animal protein, but they are also found in cereals, corn, beverages (wine, whiskey, beer, sake), and in tobacco.<sup>1</sup>  $\beta$ -Carbolines are also formed endogenously from the condensation of tryptophan-derived indolealkylamines with simple aldehydes or with pyruvic acid. Thus some  $\beta$ -carbolines, such as harmane and norharman, are normal constituents of human tissue. Because ethanol is converted to aldehyde in tissues and in the stomach,<sup>2</sup> a question is whether alcohol ingestion elevates  $\beta$ -carboline levels. Rats chronically administered alcohol display elevated plasma and brain norharman,<sup>3</sup> but no change in brain or lung harmane.<sup>4</sup> In humans, elevated plasma norharman is associated with heavy smoking rather than alcohol intake.<sup>5</sup>

The rate of elimination also affects  $\beta$ -carboline levels. In humans, CYP 1A2 and 2D6 are the major cytochrome enzymes metabolizing harmaline and harmine, converting these by O-demethylation to non-tremorigenic harmalolol and harmol.<sup>6</sup> Human Purkinje cells express 2D6, and this expression is upregulated in alcoholics.<sup>7</sup> Smoking also increases 2D6 expression in the human brain, as does nicotine administration in animals.<sup>8</sup> Cytochrome 2D6 may play a role in

defending against  $\beta$ -carboline derivatives that have potential 1-methyl-4-phenylpyridine (MPP)-like neurotoxicity. Endogenous tetrahydro- $\beta$ -carbolines can undergo methylation by *N*-methyltransferases to form *N*(2)-methyl- and *N*(2,9)-dimethyl-tetrahydro- $\beta$ -carbolines, which are similar to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP).<sup>9</sup> Such compounds are found in rat brain and are oxidized by heme oxidases to the corresponding  $\beta$ -carboliniums, structurally analogous to MPP+,<sup>10</sup> and similarly neurotoxic. *N*(2)- and *N*(9)-methylnorharmanium ions induce bradykinesia and dopamine loss in mice.<sup>11</sup> As an alternative to forming  $\beta$ -carbolinium products, *N*-methylated  $\beta$ -carbolines are removed by CYP 2D6, at a rate highly dependent on the 2D6 polymorphism subtype.<sup>12</sup>

Such observations have led to speculation that brain 2D6 may protect against neurotoxins derived from  $\beta$ -carbolines or other toxins, in view of evidence that smoking protects against Parkinson's and ET.<sup>13</sup> However, persons who inherit a 2D6 polymorphism with low activity have an inconsistent or weak increase in risk for Parkinson's disease.<sup>14</sup>

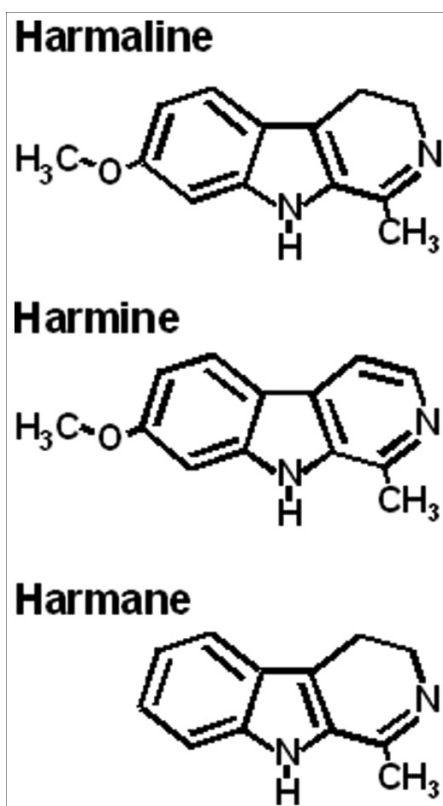
**Do  $\beta$ -carbolines cause ET?** ET patients have higher harmane blood levels, especially in familial cases,<sup>15</sup> which is not due to higher dietary intake.<sup>16</sup> Harmane in high doses may cause tremor,<sup>17</sup> or do so via a metabolite, harmine. A role of harmane could also be from tissue damage that gives rise to tremor. Indeed, among ET patients, blood harmane levels are significantly correlated with the decline in a spectroscopic magnetic resonance imaging measure of viable neurons in the cerebellar cortex.<sup>18</sup>

#### Harmaline tremor: an acute model

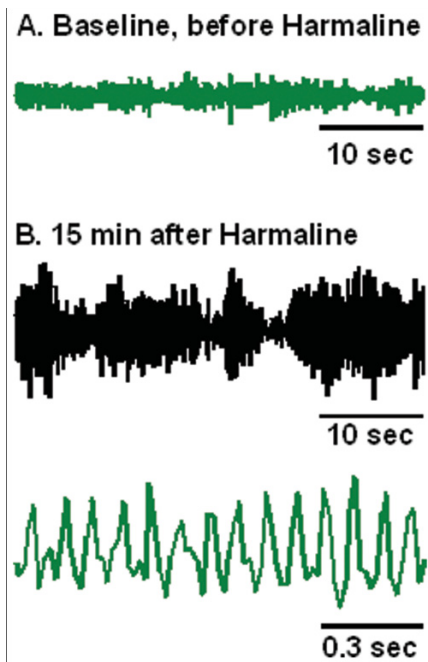
The harmala alkaloids harmine, harmaline, and tetrahydroharmine are especially rich in the seeds of *Peganum harmala* (Syrian Rue) and in the *Banisteriopsis caapi* vine. Extracts from the latter are combined with leaves from *Psychotria viridis*, containing dimethyltryptamine (DMT), to create the ayahuasca sacramental beverage used in shamanic rituals. The principal purpose of the harmala compounds is to inactivate gastrointestinal monoamine oxidase (MAO-A), enabling enough DMT to elude first-pass metabolism so as to produce cognitive/affective effects. The amount of harmala alkaloids absorbed may be small or negligible.<sup>19</sup> Large doses of harmine or preparations of *B. caapi* induce in human volunteers a transient coarse tremor.<sup>20</sup>

Among  $\beta$ -carbolines, ibogaine, harmaline and harmine are especially tremorigenic. Of these, harmaline (7-methoxy-3,4-dihydro- $\beta$ -carboline) has been most frequently utilized experimentally, but harmine acts similarly, and similar doses are employed. Harmaline produces an 8–16 Hz tremor in mice,<sup>21</sup> rats, cats,<sup>22</sup> and monkeys.<sup>23</sup> The tremor involves appendicular and axial musculature during posture and kinesis. In a mouse or rat the tremor visibly involves all four limbs, the tail, trunk, and head, including whiskers. The tremor is particularly visible when the animal ambulates, and is less when it lies down.

The peak tremor frequency varies according to the species, ranging from 8–10 Hz in monkey to 11–14 Hz in mice.<sup>24</sup> After subcutaneous



**Figure 1.  $\beta$ -Carboline Structures.** Harmaline and harmine are used in animal models of tremor. Harmane, found in human tissue, can be converted to harmine.



**Figure 2. Recorded Motion.** Data are shown for example mouse prior to (A), and after intraperitoneal harmaline administration (B). The slow recording speed demonstrates tremor that fluctuates according to general motor activity. The fast recording speed shows that motion is dominated by tremor at 10–16 Hz. Motion between 0 and 34 Hz was detected with a load sensor under a platform and digitized, with filters at 1 and 70 Hz and sampling frequency of 128 Hz.

or intraperitoneal injection, tremor develops within minutes and lasts up to several hours before subsiding. Tremor may be assessed with rating scales,<sup>25</sup> electromyographic recordings, or digitally quantified with systems that detect motion through force or magnetic field transduction.<sup>26,27</sup> Because harmaline-induced tremor is an action tremor, the amount of tremor will vary according to the motor activity level. This source of variation can be greatly reduced by normalizing the data to overall motion.<sup>28</sup>

Whole-body tremor is dose-dependent with harmaline doses at 4 mg and above<sup>26</sup> in rats. Doses of 10–20 mg/kg are frequently employed in rats; mice require approximately twice that. If rats are trained to press a disk connected to a transducer, forelimb tremor can be detected at a dose as low as 1.0 mg/kg.<sup>29</sup>

Repeated daily administration of harmaline to rats, 4–16 mg/kg, results in a loss of the tremor response (tolerance) after three to seven treatments, lasting at least 7–10 days.<sup>26,30</sup> In contrast, tolerance is not observed with four daily doses of a low dose at 1.0 mg/kg,<sup>29</sup> or if tremor is prevented with diazepam or morphine during the initial exposure.<sup>30</sup> Mice also develop harmaline tolerance.<sup>24</sup>

Inferior olive (IO) neurons in tolerant rats fail to show harmaline-associated sustained rhythmic activity, and vermal Purkinje neurons do not show expected rhythmic climbing fiber responses, suggesting that tolerance may reflect physiological changes.<sup>31,32</sup>

Another explanation for tolerance may be neuronal damage, but rats and mice show different morphological alterations after

harmaline. In rats, harmaline causes Purkinje neuron loss in narrow parasagittal vermis zones, possibly due to excitotoxic climbing fiber hyperactivity. The IO is unaffected<sup>33,34</sup> In contrast, mice show no cell loss or gliosis in the cerebellar cortex but instead show microgliosis in IO without cell loss, with the medial and dorsal accessory regions most affected.<sup>34</sup>

### The anatomic and physiologic basis of harmaline tremor

Harmaline activates circuits within the olivocerebellar system to produce tremor. Before discussing this circuitry we briefly review selected aspects of olivocerebellar physiology.

#### Normal olivocerebellar system functioning

**Subthreshold oscillation.** IO neurons in brainstem slices normally demonstrate rhythmic membrane voltage subthreshold oscillations (STOs) that involve serial ion conductances. A high threshold calcium spike is followed by a depolarizing shoulder that is terminated by a potassium conductance that leads to an afterhyperpolarization, which in turn deinactivates a low-threshold (T-type) calcium current. That causes a rebound spike which triggers the high-threshold calcium spike, and may or may not be enough to trigger a sodium spike. The oscillation frequency is approximately 10 Hz, whereas individual IO cells fire at 1 Hz.<sup>35–38</sup> Spontaneous STOs are suppressed by apamin, serotonin via 5HT<sub>2a</sub> receptors,<sup>39</sup> NMDA receptor antagonists, and L-, P-, and T-type calcium channel blockers.<sup>40</sup>

STOs are synchronized among ensembles of IO neurons via electrical coupling.<sup>35–37,41</sup> Hundreds of IO neurons oscillate coherently in discrete clusters, with moment-to-moment variation in cluster size. Addition of picrotoxin, a GABA<sub>A</sub> receptor antagonist, induces clusters to merge, forming larger units.<sup>42</sup> IO neurons form dendritic tangles (glomeruli) containing abundant gap junctions.<sup>43</sup> Addition of a gap junction blocker disrupts synchrony.<sup>44</sup> GABAergic afferents terminate near gap junctions, where they can control electrotonic coupling.<sup>36</sup>

**IO–Purkinje ensembles.** IO neurons project climbing fibers to Purkinje cell dendrites, with collaterals to deep cerebellar nuclei (DCN) non-GABAergic and GABAergic neurons.<sup>45</sup> Purkinje cells respond to climbing fibers with complex spikes, whereas parallel fibers from granule cells mediate simple spikes. Because each Purkinje cell receives a single climbing fiber from a dedicated IO neuron, the behavior of multiple IO neurons *in vivo* can be studied by recording complex spikes from many Purkinje cells simultaneously. Such studies reveal that Purkinje cell firing is synchronized at 10 Hz within small vertical cortical bands, indicating that they are controlled by electrically coupled IO ensembles.<sup>46</sup> The intra-band synchrony and band size, and by inference that of the projecting IO ensemble, are not fixed but modulated by afferents to IO. Intra-IO picrotoxin injection or lesions of the dentate nucleus, which projects GABA to the IO, increases Purkinje cell within-band synchrony and synchronous band width.<sup>47,48</sup> Glutamate also modulates IO/Purkinje synchrony. Intra-IO injection of 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide (NBQX), an  $\alpha$ -amino-3-hydroxyl-5-methyl-4-isoxazole-propionic

acid (AMPA) receptor antagonist, reduces synchronous Purkinje cell band width, indicating that glutamate expands synchrony in the IO.<sup>48</sup>

In summary, IO neurons do not fire often, but when they do, the timing is precisely timed according to the depolarization phase of STOs that are tightly synchronized within an IO ensemble. The aggregate firing is thus highly rhythmic. The IO ensembles are not fixed, but sculpted on a moment-to-moment basis.<sup>49</sup> IO gap junctions and the activity of DCN that control IO coupling via GABA projections play critical roles.

**Gap junctions.** IO neurons richly express the gap junction protein connexin 36 (Cx36).<sup>41</sup> Cx36-null mice appear to lack IO gap junctions,<sup>50</sup> and IO slices show rare and weak electrical coupling, so that IO STOs and action potentials are not synchronized.<sup>51</sup> Loss of IO coupling also occurs with transduction of inactive mutant Cx36 and the addition of the gap junction blocker carbenoxolone.<sup>52,53</sup> Purkinje cell complex spike synchrony is lost in mice receiving intra-IO carbenoxolone injection and in Cx36-null mice.<sup>54,55</sup>

**Deep cerebellar nuclei.** DCN neurons send excitatory efferents to extracerebellar structures and a massive GABAergic projection to the IO. Climbing fibers are excitatory and send collaterals to GABAergic DCN neurons, which are coupled with Cx36 gap junctions,<sup>41</sup> and project back to IO, and to non-GABAergic DCN neurons. Climbing fiber-activated Purkinje cells project GABAergic terminals to both DCN non-GABA (glutamate) and GABAergic neurons.<sup>56</sup>

GABA DCN neurons show strong rebound discharges after AHP, due to Cav3.1 T-type channels,<sup>57</sup> compatible with phasic firing, in which timing rather than intensity modulation is important.<sup>58</sup> In contrast, large non-GABA DCN neurons express a linear firing-to-stimulation relationship, functioning as a linear transducer of spike frequency, suitable as output neurons. These neurons express weaker rebound discharges,<sup>58</sup> and may correspond to excitatory neurons observed not to express Cav3.1 T-type channels.<sup>57</sup>

**What happens to this system when harmaline is administered?** When harmaline is added to the brainstem slice, IO neurons exhibit increased rebound low threshold (T-type) calcium spikes, so that each rebound is now associated with bursts of sodium action potentials. Thus the IO neurons are made more excitable by harmaline and convert from STO to rhythmic 9–12 Hz burst-firing.<sup>36,37</sup> In animals, IO harmaline microinjection elicits rhythmic local bursting only when the medial accessory olive (MAO) and dorsal accessory olive (DAO) are injected.<sup>59</sup> Intravenous harmaline in cats causes IO neurons confined to MAO and DAO to fire rhythmically and synchronously at 6–12 Hz, generating rhythmic Purkinje cell complex spikes, whereas simple spikes are suppressed.<sup>22</sup> In addition, neurons in DCN, lateral reticular nucleus, red nucleus, nucleus reticularis tegmenti pontis, spinal cord interneurons, and motoneurons also fire at the tremor frequency.<sup>60,61</sup> In cat, harmaline increases glucose utilization in the MAO, caudolateral DAO, the molecular layer of the vermis and paravermis cerebellar cortex, and the same three brainstem nuclei shown to fire at the tremor frequency.<sup>62</sup> Fos

mapping demonstrates IO activation 15 minutes after harmaline administration, followed by DCN at 30 minutes, cerebellar cortex at 1 hour, and vertical bands of vermal Purkinje cells at 2–6 hours.<sup>63</sup> The delay in DCN recruitment has also been found with field potential recordings.<sup>64</sup>

The harmaline-responsive Purkinje cells are mainly found in the vermis and paravermis regions in the rat and cat,<sup>65</sup> to which MAO and DAO project. These cortical regions project to the fastigial and interpositus DCN, which also receive climbing fiber collaterals from MAO and DAO. The DCN send reciprocating GABAergic projections back to the IO subnuclei. These connections are highly organized with somatotopic precision.<sup>66,67</sup>

**Climbing fibers are required for tremor expression.** The destruction of IO by systemic 3-acetylpyridine injection eliminates the tremor response.<sup>68</sup> If the cerebellar peduncles are cut to sever climbing fibers, the MAO and DAO still show bursting and increased glucose utilization by harmaline, whereas Purkinje cell and fastigial nucleus bursting is abolished, and metabolic activation of other medulla structures fails to occur.<sup>22,61</sup> The importance of climbing fibers is also illustrated in genetically dystonic rats, which do not show normal climbing fiber-induced complex spike responses.<sup>69</sup> On harmaline administration, IO neurons but not Purkinje cells, fire rhythmically, and tremor does not occur.<sup>32,70</sup>

**Are Purkinje cells required for tremor?** Cooling of the cerebellar cortex does not abolish harmaline-induced motoneuron firing, suggesting that the olivo–DCN loop may be sufficient for tremor.<sup>61</sup> Mice with Purkinje cell degeneration (*pcd*) still manifest harmaline tremor despite the complete absence of Purkinje cells, although the tremor is of lower frequency and amplitude than controls. Lurcher mice also have no Purkinje neurons, but mount no harmaline tremor. The difference between these two strains of mice is that *pcd* mice have intact climbing fibers capable of contacting DCN neurons, whereas lurcher mice appear not to.<sup>71</sup>

**What is the role of DCN in tremor?** DCN neurons, as the sole output of the cerebellum, are required for the expression of harmaline tremor. On the other hand, lesions of the dentate nucleus or DCN outflow pathways are well known to induce action tremor in humans,<sup>72</sup> and lesions or inhibitory injections of the interpositus nucleus or combined lesions of interpositus and dentate nucleus can induce action tremor in monkeys.<sup>73–75</sup> Thus DCN paradoxically can express and suppress tremor. On adding harmaline to a guinea pig cerebellum–brainstem *in vitro* preparation, one group of DCN neurons responds with an excitatory post-synaptic potential, an inhibitory post-synaptic potential (IPSP), then a rebound discharge, whereas another group responds with an initial IPSP followed by a rebound discharge. These responses have been interpreted to indicate that harmaline induces phasic rhythmic activity in excitatory DCN output neurons, thereby expressing tremor, and in inhibitory nucleo-olivary neurons, thereby modulating rhythmicity and synchronicity.<sup>76</sup>

**Are the brainstem and spinal cord sufficient for harmaline tremor?** Cooling of the motor cerebral cortex and lesions of the ventrolateral thalamus or globus pallidus reportedly do not affect harmaline tremor in intact monkeys.<sup>77,78</sup> Not even intercollicular decerebration abolishes tremor in cats or monkeys.<sup>78,79</sup> These observations suggest that the brainstem and spinal cord are sufficient to express harmaline tremor, even in primates. On the other hand, high-frequency stimulation simulating deep brain stimulation (DBS) of the ventrolateral thalamus, and intrathalamic infusion of muscimol or an adenosine A1 receptor agonist suppress harmaline tremor in mice,<sup>80</sup> indicating a potential role of the thalamus in modulating harmaline tremor.

**Summary.** Harmaline induces rhythmic bursting in accessory IO neurons that then recruits medial regions of cerebellar cortex and DCN. The end result is rhythmic activation of the spinal gamma and alpha motoneurons and tremor.

### Proposed mechanisms of harmaline tremor

Here we consider the question how harmaline acts at the cellular level to induce tremor.

**Serotonin.** Initial ligand binding studies indicated that harmaline does not bind significantly to 5-hydroxytryptamine (HT)<sub>1a-d</sub>, 5-HT<sub>2</sub> or 5-HT<sub>3</sub> receptors ( $K_i > 100 \mu\text{M}$ ).<sup>81</sup> Subsequently harmaline was found to have affinity to the 5-HT<sub>2a</sub> ( $K_i = 7.8, 42.5 \mu\text{M}$ ) and 5-HT<sub>2c</sub> ( $K_i = 9.4 \mu\text{M}$ ) receptors,<sup>82,83</sup> comparable to the cerebellar harmaline level of 18.2  $\mu\text{M}$  after 15 mg/kg in mice.<sup>84</sup> Acting through 5-HT<sub>2a</sub> receptors, intra-IO 5-HT injection increases IO neuronal firing rates and improves coherence, while increasing intra-band Purkinje cell synchrony, similar to harmaline, but harmaline's action is not blocked by a 5-HT<sub>2a</sub> antagonist.<sup>85</sup>

**NMDA receptor channel.** Harmaline competitively displaces tritiated MK801 (dizocilpine) from the NMDA receptor in rabbit IO fractions (IC<sub>50</sub> 60  $\mu\text{M}$ ),<sup>86</sup> leading to the suggestion that harmaline induces tremor by acting as an NMDA receptor inverse agonist. However this action would produce depolarization, whereas harmaline hyperpolarizes IO neurons.

**Benzodiazepine receptor.** Harmaline displaces tritiated flunitrazepam from brain tissue only at high IC<sub>50</sub> concentrations: 126–600  $\mu\text{M}$ .<sup>84,87</sup> The benzodiazepine antagonists flumazenil and CGS8216 do not affect harmaline tremor in mice.<sup>88</sup> Moreover, binding of <sup>3</sup>H-flunitrazepam in IO of adult rodents is very sparse.<sup>89</sup> Harmaline is thus not likely to induce tremor via benzodiazepine receptors.

**Sodium and high-voltage calcium conductances.** Harmaline does not significantly displace ligands at adrenergic, dopamine, opiate, muscarinic, nicotinic, GABA receptors or the chloride channel. An affinity for the voltage-gated sodium channel, ( $K_i = 13.9 \mu\text{M}$ ), suggested this as a potential mechanism of tremor.<sup>81</sup> However, very high levels of harmaline are needed to affect the action potential (>0.5 mM).<sup>90</sup> At 100  $\mu\text{M}$ , harmaline inhibits sodium conductance by 23% in dorsal root ganglia neurons. High-voltage calcium channels (L- and N-type)

are more sensitive, with an IC<sub>50</sub> by harmaline of 100  $\mu\text{M}$ . However harmaline, which is less tremorigenic, is more potent (IC<sub>50</sub> = 76  $\mu\text{M}$ ),<sup>91</sup> thus this channel is not likely to mediate tremor.

**The Cav3.1 T-type calcium channel.** IO slices from Cav3.1-null mice fail to show STOs or low-threshold calcium spikes. Harmaline fails to produce rhythmic firing in IO slices from Cav3.1 null mice.<sup>64</sup> Park et al.<sup>64</sup> studied harmaline effects on Cav3.1 currents *in vitro* and found a complex set of actions that in combination leads to enhanced rebound spikes. They postulate that harmaline engenders tremor by effects on the Cav3.1 channel.<sup>64</sup> Effects on Cav3.2 or Cav3.3 channels were not studied.

**Other actions.** Harmaline inhibits sodium-dependent transport of substances into various tissues, such as choline into striatal synaptosomes ( $K_i = 36 \mu\text{M}$ ),<sup>92</sup> and gamma-hydroxybutyrate into whole-brain synaptosomes ( $K_i = 94 \mu\text{M}$ ).<sup>93</sup> Harmaline is a potent inhibitor of MAO-A, with IC<sub>50</sub> values as low as 4–8 nM. Activity against MAO-B is negligible.<sup>94</sup> Harmaline inhibits synaptosomal GABA uptake (IC<sub>50</sub> 47  $\mu\text{M}$ )<sup>95</sup> and dopamine uptake (IC<sub>50</sub> = 8.1  $\mu\text{M}$ ),<sup>96</sup> and increases dopamine release from striatal slices at 6  $\mu\text{M}$ .<sup>94</sup> Low doses enhance levodopa-induced stereotypy in mice.<sup>97</sup> These observations are compatible with reports that Parkinson's motor symptoms are ameliorated by extracts of *B. caapi*.<sup>94</sup>

Harmaline also potently inhibits cerebral histamine *N*-methyltransferase (IC<sub>50</sub> = 4.4  $\mu\text{M}$ ), which may raise histamine levels,<sup>98</sup> and displaces tritiated tryptamine in brain tissue (IC<sub>50</sub> = 25 nM).<sup>99</sup> Harmaline potently binds the imidazoline 2B receptor ( $K_i = 177 \text{ nM}$ ).<sup>100</sup>

**Summary.** Several potential mechanisms by which harmaline could produce tremor have been investigated. At present the most likely mechanism appears to be modulation of T-type calcium channels. It is not clear whether this action is restricted to Cav3.1 channels or also involves Cav3.2 and Cav3.3. Harmaline is a potent inhibitor of MAO-A, and has significant effects on dopamine and histamine processing.

### The pharmacology of harmaline tremor

**Serotonin (5-HT).** Serotonergic fiber innervation in IO is highest in caudal MAO and caudolateral DAO, correlating with high sensitivity to harmaline-induced rhythmicity.<sup>101,102</sup> Lesions of 5-HT fibers to the IO, or of the medial and dorsal raphe nuclei, reduce the harmaline tremor response.<sup>101,103</sup> The genetically epilepsy-prone rat (GEPR) has reduced serotonergic IO innervation, and manifests poor harmaline tremor.<sup>104</sup> Harmaline tremor is exacerbated by the 5-HT precursor 5-hydroxytryptophan,<sup>103</sup> an effect reduced by raphe lesions.<sup>103</sup> Conversely, the broad-spectrum 5-HT antagonist methysergide and the 5-HT synthesis inhibitor para-chlorophenylalanine reduce harmaline tremor.<sup>105,106</sup> The serotonin uptake inhibitor citalopram (10–40 mg/kg) enhances harmaline tremor in rats,<sup>107</sup> as does imipramine.<sup>106</sup>

**Norepinephrine.** When norepinephrine is added to guinea pig brainstem slices, harmaline-induced rhythmic IO oscillations ceases.<sup>37</sup>

Systemic injection of the norepinephrine precursor L-threo-3,4-dihydroxyphenylserine (L-threo-DOPS, 50–200 mg/kg) suppresses harmaline tremor in rats.<sup>108</sup> Intraventricular L-threo-DOPS also suppresses harmaline tremor, as does electrical stimulation of locus ceruleus.<sup>109</sup> In contrast, the tyrosine hydroxylase inhibitor alpha-methyl-p-tyrosine, 200 mg/kg; 6-hydroxydopamine injections that reduce cerebellar norepinephrine; and locus ceruleus destruction each exacerbate harmaline tremor.<sup>108–110</sup>

Beta-adrenergic blockers such as propranolol suppress harmaline and harmine tremor in rodents.<sup>111,112</sup> Selective beta<sub>1</sub>- and beta<sub>2</sub>-adrenergic antagonists can each suppress harmaline tremor in rats, but beta<sub>2</sub>-blockade may be more effective.<sup>113</sup> Although propranolol may act in part peripherally,<sup>113</sup> it also acts directly by antagonizing the electrophysiological effects of harmaline on IO neurons in brainstem slices.<sup>114</sup> In contrast, the alpha-adrenergic antagonist phenoxybenzamine does not suppress harmine tremor,<sup>115</sup> and is ineffective for ET.<sup>116</sup>

**Glutamate.** Intracisternal 2-amino-5-phosphonovalerate (2-APV), an NMDA receptor antagonist, suppresses harmaline tremor in mice.<sup>115</sup> Similarly the competitive NMDA antagonist d-CPPene suppresses harmaline tremor in mice and rats.<sup>117,118</sup> The non-competitive NMDA antagonist dizocilpine (MK-801) potently suppresses harmaline tremor in mice and rabbits,<sup>118,119</sup> as does phencyclidine in mice.<sup>118</sup> Memantine has only a weak antitremor effect in rats,<sup>120</sup> comparable to a weak or non-existent effect on ET.<sup>121</sup> However, memantine confers striking protection against harmaline-induced cell loss in the cerebellum and IO.<sup>120</sup>

The AMPA receptor antagonist RPR117824 suppresses harmaline tremor,<sup>122</sup> as does NBQX disodium salt.<sup>118</sup>

The mGluR1 antagonist JNJ 16259685-a strongly enhances harmaline tremor in rats, suggesting an agonist at this receptor should suppress tremor.<sup>123</sup> In contrast, the mGluR5 receptor antagonist 6-methyl-2-(phenylethynyl)pyridine (MPEP) has no effect in mice.<sup>118</sup>

**GABA.** The benzodiazepine diazepam, 1.5–5 mg/kg, suppresses harmaline tremor in rodents.<sup>88,124</sup> The GABA<sub>A</sub> receptor agonist muscimol also suppresses harmaline tremor in mice.<sup>118</sup> The GABA<sub>B</sub> receptor agonist baclofen, 2.5–10 mg/kg, dose-dependently suppresses harmaline tremor in rats,<sup>125</sup> and reduces harmine tremor in alcohol-withdrawing rats.<sup>126</sup> However Paterson et al.<sup>118</sup> did not find tremor suppression by baclofen in mice.

**Dopamine (DA).** Harmine tremor is reduced by levodopa and by the agonists apomorphine and pibedil in rats and mice.<sup>103,118</sup> Similarly the dopamine uptake inhibitor GBR12909 reduces tremor.<sup>118</sup> Apomorphine is a D<sub>1</sub>/D<sub>2</sub> agonist. Tremor is not reduced by the D<sub>1</sub> agonist SKF82958, but is by the D<sub>2</sub>/D<sub>3</sub> agonist quinpirole.<sup>118</sup>

**Alcohol.** Suppression of harmaline tremor by ethanol has been well replicated,<sup>28,127</sup> but the site of action and mechanism remain unclear. Ethanol reduces harmaline tremor in mice at low doses that do not suppress harmaline-induced cerebellar cyclic GMP elevations.<sup>127</sup> This

observed dissociation between ethanol's climbing fiber-mediated and behavioral effects raised the possibility of an extra-olivary localization of the antitremor action. In rats anesthetized with agents other than urethane, or immobilized and given local anesthesia, ethanol increases IO firing rates.<sup>128</sup> A moderate ethanol dose (1 g/kg) increases vermal Purkinje cell complex spike rhythmicity and synchrony in ketamine-anesthetized rats, and by inference IO ensemble rhythmicity and synchrony. Moreover ethanol fails to affect the rhythmicity of harmaline-induced complex spike activity.<sup>129</sup> These observations suggest that alcohol does not suppress but instead increases IO firing with potentially excitotoxic effects. This inference is supported by the finding that although ethanol, 1.5 g/kg, effectively suppresses tremor in rats, histology 24 hours later reveals that ethanol-treated rats display an exacerbation of the harmaline-associated vermal and paravermal Purkinje and granule cell loss, and more IO neuronal loss.<sup>120</sup> Conceivably alcohol's antitremor efficacy may lure ET patients to alcohol-exacerbated cerebellar damage, to which they may be more vulnerable.

**Antiepileptic drugs.** De Ryck et al.<sup>130</sup> found antitremor effects for primidone, clonazepam, gabapentin, and carbamazepine. Whereas levetiracetam had minimal effect on tremor, the derivative brivaracetam was effective. Similarly, Paterson et al.<sup>118</sup> found that primidone, gabapentin, and carbamazepine suppress harmaline tremor in mice, but also reported that valproate does as well. Zonisamide suppresses harmaline tremor in mice, with 50 mg/kg more effective than 5 mg/kg.<sup>25</sup> Zonisamide has shown efficacy for ET.<sup>131</sup> Lacosamide, 0.3–30 mg/kg, suppresses harmaline tremor in rats;<sup>132</sup> however, a clinical trial did not show efficacy for ET.<sup>133</sup> Similarly, carisbamate suppressed harmaline tremor in preclinical testing, but demonstrated no antitremor efficacy in an ET trial.<sup>134</sup>

**Gap junction blockers.** Contrary to expectation, Cx36-null mice or mice with IO transduction of inactive mutant Cx36 mount a vigorous tremor response to harmaline.<sup>51,52</sup> On the other hand, harmaline tremor is suppressed by the broad-spectrum gap junction blocker carbenoxolone and by more specific mefloquine.<sup>135</sup> It may be conjectured that other gap junctions also play a role in harmaline tremor, such as connexin 57.<sup>136</sup> More research on this topic is warranted.

**T-type calcium channels.** Isomers of octanol suppress harmaline tremor in the rat.<sup>137</sup> Because octanol blocks T-type calcium channels it was predicted that antagonists of these channels could be effective for tremor. However, octanol exerts multiple actions. We showed that each of five drugs that block T-type calcium channels suppress tremor in the harmaline model and in the GABA<sub>A</sub>  $\alpha$ 1-null genetic mouse tremor model.<sup>138</sup> The best agent appeared to be NNC55-0396.<sup>139,140</sup>

Of the three subtypes of T-type calcium channels, Cav3.1 is expressed in IO, Purkinje cells and some DCN neurons. Park et al.<sup>64</sup> reported that Cav3.1-null mice generated in the laboratory of HS Shin are impaired in manifesting harmaline tremor. This was demonstrated with a low harmaline dose that in wild-type mice induced tremor for only 15 minutes. Tremor was greatly reduced in Cav3.1-null mice, but

not abolished.<sup>64</sup> Although these authors postulate Cav3.1 is the “pacemaker” for tremor, they do not explain how harmaline is able to induce any tremor in Cav3.1-null mice. Our laboratory found that a routine dose of harmaline that results in longer tremor produces as much tremor in Cav3.1-null mice generated by K Sakimura as in wild-type mice. Moreover, NNC55-0396 suppresses tremor in the Cav3.1-null mice.<sup>138</sup> As NNC55-0396 may act on Cav3.2 and/or Cav3.3 channels as well as Cav3.1, it is possible harmaline has an effect on these subtypes, although such a role in tremor remains unexplored.

**Adenosine.** Harmaline tremor is enhanced by the adenosine receptor antagonist caffeine, 50–150 mg/kg, in rats,<sup>141</sup> and by the A1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), 4 mg/kg, in mice.<sup>80</sup> Intrathalamic infusion of adenosine or the A1 receptor agonist 2-chloro-*N*-cyclopentyladenosine reduces harmaline tremor. Infusion of DPCPX or the use of A1 receptor null mice reduces the threshold for DBS-induced involuntary movements (related to glutamate release), so that tremor cannot be suppressed by DBS. Thalamic DBS is suggested to cause local adenosine release that limits glutamate effects, enabling tremor to be suppressed at current levels below those associated with involuntary movements.<sup>80</sup>

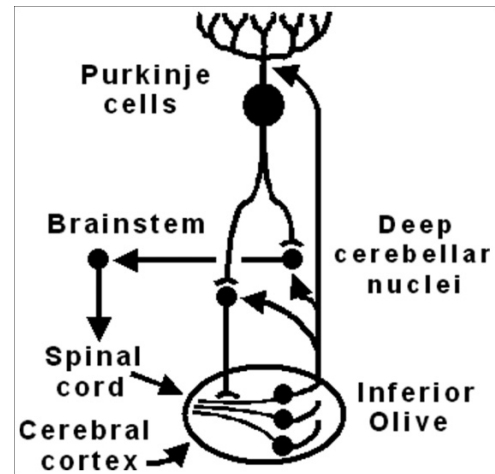
**Other drugs.** Anticholinergics do not reduce harmaline tremor in rodents.<sup>111</sup> Harmaline tremor is suppressed by systemic lidocaine (12.5–50 mg/kg);<sup>142</sup> dantrolene, 10 mg/kg;<sup>124</sup> lithium, 2 mEq,<sup>118</sup> in mice; and 16-methyl-prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), 25–50 µg/kg, in cats.<sup>143</sup> Conversely, harmaline tremor is enhanced by cyclosporin, 25–50 mg/kg, in mice.<sup>144</sup> MK-0249, a histamine-3 receptor inverse agonist, suppresses harmaline tremor, but does not suppress ET tremor.<sup>145</sup>

**Summary.** Knowledge of the physiology and the basic circuit of harmaline tremor (Figure 3) suggests that drugs affecting specific ion conductances, such as the T-type calcium channel, gap junctions, glutamate, and GABA receptors should affect tremor; notions that have received support. In addition, the tremorigenic circuit is susceptible to influence by various neurotransmitter systems, including norepinephrine, 5-HT and DA. Further research is likely to reveal more neuromodulators of tremor.

### Harmaline tremor as a preclinical model of ET

**Comparison with ET.** Harmaline-induced tremor has been suggested as useful for preclinical screening of potential ET therapies. ET and the harmaline animal model differ in a number of respects, however, and the harmaline model possesses limitations.

Harmaline tremor is an acute state induced pharmacologically, following which animals are resistant to further doses. In contrast, ET develops gradually, and is chronic, without remission. Based on associations of lower risk for ET with smoking and a Mediterranean diet,<sup>13,146</sup> and findings of Purkinje cell loss with cerebellar cortical gliosis or locus ceruleus depletion and/or Lewy bodies, it appears likely that at least in some cases ET is a neurodegenerative disorder.<sup>147</sup> Harmaline acts on the IO to produce tremor. In ET, the role of IO



**Figure 3: The Olivocerebellar Circuit Implicated in the Generation and Expression of Harmaline Tremor.** Synaptic bar endings connote inhibition, while arrowhead endings connote excitation. Harmaline elicits rhythmic bursting in clusters of inferior olivary neurons, synchronized via dendritic glomeruli. Climbing fibers excite complex spikes in Purkinje cells, which project GABA to both GABAergic and excitatory output deep cerebellar nucleus cells. These cells also receive collaterals from climbing fibers. The deep cerebellar nuclei project GABA to inferior olivary glomeruli, opposing synchrony. Glutamate receptor activation in the inferior olive promotes synchrony. The deep cerebellar nuclei and inferior olive are required for harmaline tremor expression; the absence of Purkinje cells reduces but does not abolish tremor. The deep cerebellar outflow to the brainstem and spinal cord is sufficient for harmaline tremor expression without the need for supratentorial structures.

is less certain. In ET subjects, eye-blink conditioning, which depends on the olivocerebellar pathway, is impaired.<sup>148</sup> One imaging study found increased glucose utilization in the IO region in ET,<sup>149</sup> but another study found no change in IO blood flow.<sup>150</sup> Cerebellar cortical hypermetabolism, known to depend in the harmaline model on climbing fiber activation, also occurs in ET.<sup>149–151</sup> Interestingly, alcohol administration suppresses cerebellar hypermetabolism in ET subjects, and increases IO blood flow, which does not happen in controls, suggesting that IO physiology differs in ET.<sup>151</sup>

Each pharmacotherapy suppresses tremor in only a fraction of ET patients. Given ET’s heterogeneity, it is uncertain to what extent harmaline or any other animal model can offer predictive success. Another caveat in comparing drug efficacy for harmaline vs. ET is that, based on published reports, it is difficult to assess to what extent suppression of harmaline tremor is a non-specific effect of sedation or reductions in motor activity. Potential approaches are to employ tremor measures that are insensitive to locomotor activity levels, and to select doses shown in independent behavioral tests not to affect motor activity.

**How accurate is the harmaline model in predicting efficacy for ET?** An initial prediction was encouraging. Sinton et al.<sup>137</sup> reported in 1989 that isomers of octanol suppress harmaline tremor in the rat, subsequently replicated in mice.<sup>135</sup> Early-stage clinical trials later demonstrated that 1-octanol reduces tremor in ET.<sup>152</sup> In humans,

**Table 1.** Comparison of Harmaline Rodent Tremor Model and Essential Tremor

Feature	Harmaline Tremor	Essential Tremor
<i>Clinical</i>		
Action tremor	Yes	Yes
Time course	Acute	Chronic
Inducing agent	Pharmacologic	Probably neurodegenerative
Role of inferior olive	Definite	Uncertain
Cerebellar hypermetabolism	Yes	Yes
<i>Response to drugs</i>		
Caffeine	Worsens	Worsens in some
Citalopram, imipramine	Worsens	Worsens in some
Phenoxybenzamine	Does not suppress	Does not suppress
Anticholinergics	Do not suppress	Do not suppress
Levetiracetam	Does not suppress	Does not suppress
Primidone	Suppresses	Suppresses in some
Clonazepam, diazepam	Suppresses	Suppresses in some
Gabapentin	Suppresses	Suppresses in some
Carbamazepine	Suppresses	Does not suppress
Valproate	Suppresses	Does not suppress/worsens
Zonisamide	Suppresses	Suppresses in some
Lacosamide	Suppresses	Does not suppress
Carisbamate	Suppresses	Does not suppress
Propranolol	Suppresses	Suppresses in some
L-dopa, DA agonists	Suppresses	Do not suppress
Ethanol	Suppresses	Suppresses in some
Gamma-hydroxybutyrate	Suppresses	Suppresses in some
Lithium	Suppresses	Does not suppress/worsens
1-Octanol	Suppresses	Suppresses in some
Memantine	Weakly suppresses	Weak or no suppression
MK-0249	Suppresses	Does not suppress

1-octanol is rapidly converted to octanoic acid, which may be the active antitremor agent.<sup>153</sup>

Citalopram, imipramine and caffeine worsen both harmaline<sup>107,143</sup> and ET tremor (Table 1). We do not know of any agent that fails to suppress harmaline tremor yet is effective for ET. Phenoxybenzamine,

levetiracetam, and anticholinergics do not suppress harmaline tremor, and do not usually suppress tremor in ET.

Of 16 agents reported to suppress harmaline tremor, including weakly effective memantine, seven fail to suppress ET tremor, including several anti-epileptic drugs,<sup>133,134,154</sup> levodopa and dopamine agonists,<sup>103</sup>



lithium,<sup>118</sup> and MK-0249. Matches between positive results in the harmaline model and efficacy in ET trials occurred in 9 out of 16 agents or a 56% concordance rate, and include propranolol, several anti-epileptic drugs, alcohol, memantine, and gamma-hydroxybutyrate.<sup>118,155</sup> Because some of these agents were tested in the model after having been found effective in ET, the true predictive success rate for the model may be lower than 56%.

**Summary.** As is often the case with neurological disease models, the harmaline model is prone to false positives. Because the harmaline state is poorly compatible with sensitive tests of drug intoxication, independent tests of behavioral tolerability in non-harmaline control subjects may be appropriate.

### Conclusion

Like ET, harmaline induces an action tremor that involves cerebellar circuitry, and responds to drugs that suppress clinical tremor. Unlike ET, harmaline tremor is acute and temporary. Harmaline converts IO STOs to rhythmic burst firing that is propagated through the cerebellum, then ultimately activates spinal motoneurons to express tremor. The minimum cerebellar circuit required includes the IO, climbing fibers and DCN. Pharmacologic studies indicate that harmaline tremor severity is influenced by several glutamate receptors, GABA A and B receptors, serotonin receptors, norepinephrine receptors, dopamine receptors, gap junctions, T-type calcium channels, alcohol, and anti-epileptic drugs. Of drugs that suppress harmaline tremor, approximately half suppress ET tremor.

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