

# A new simple, automatic and non-invasive method for measurement of chemically induced tremors in rats

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## Introduction:

Essential tremor (ET) is regarded as one of the most common neurological disorders with a prevalence similar to or greater than that of stroke, Alzheimer disease, migraine and lumbosacral pain syndromes and is as much as 20 times more prevalent than Parkinson's disease. Multiple neurotransmitter systems have been implicated in ET, such as elevated levels of glutamate and aspartate and declining in gabaergic activity. Due to lack of understanding of the basic mechanism, origin of tremors and predictive animal models, it is difficult to develop and screen novel pharmacological agents with selective and specific antitremor activity. As an alternative to transgenic mouse models of ET, administration of harmaline causes generalised tremors in rats. These tremors occur with a frequency of 10-14Hz same frequency as ET.

Here a new simple method of quantifying harmaline induced tremors in rats by automated nociception analyser (ANA) is described.

## Advantages:

1. Less labour intensive compared to manual evaluation of tremor activity, it is good for screening new compounds.
2. Data is collected for longer period of time.
3. Animals are not restrained and can freely move unlike the tremor boxes. This is important as harmaline tremors are kinetic tremors.

## Limitations:

1. Only fore limb tremors are counted as opposed to whole body tremors.
2. Grooming and normal movement are also counted as tremor activity.
3. These limitations are the same as for the formalin flinches quantified by ANA method.

## Conclusion:

Harmaline at 10 mg/kg, ip induced significant tremors in rats which were quantified by automatic nociception analyser. Method is validated by clinically used drugs such as propranolol, primidone and diazepam. These drugs dose dependently inhibited the harmaline induced tremors.

The reliability and robustness of the automatic method is confirmed by visual observation method (VAS) as described in the literature. In addition we have shown that riluzole, a neuroprotective, sodium channel stabilizer also inhibited the harmaline tremors.

A new simple automatic and non-invasive method for measurement of chemically induced tremors in rats can be useful for developing and screening novel treatment for human movement disorder.

## Methods

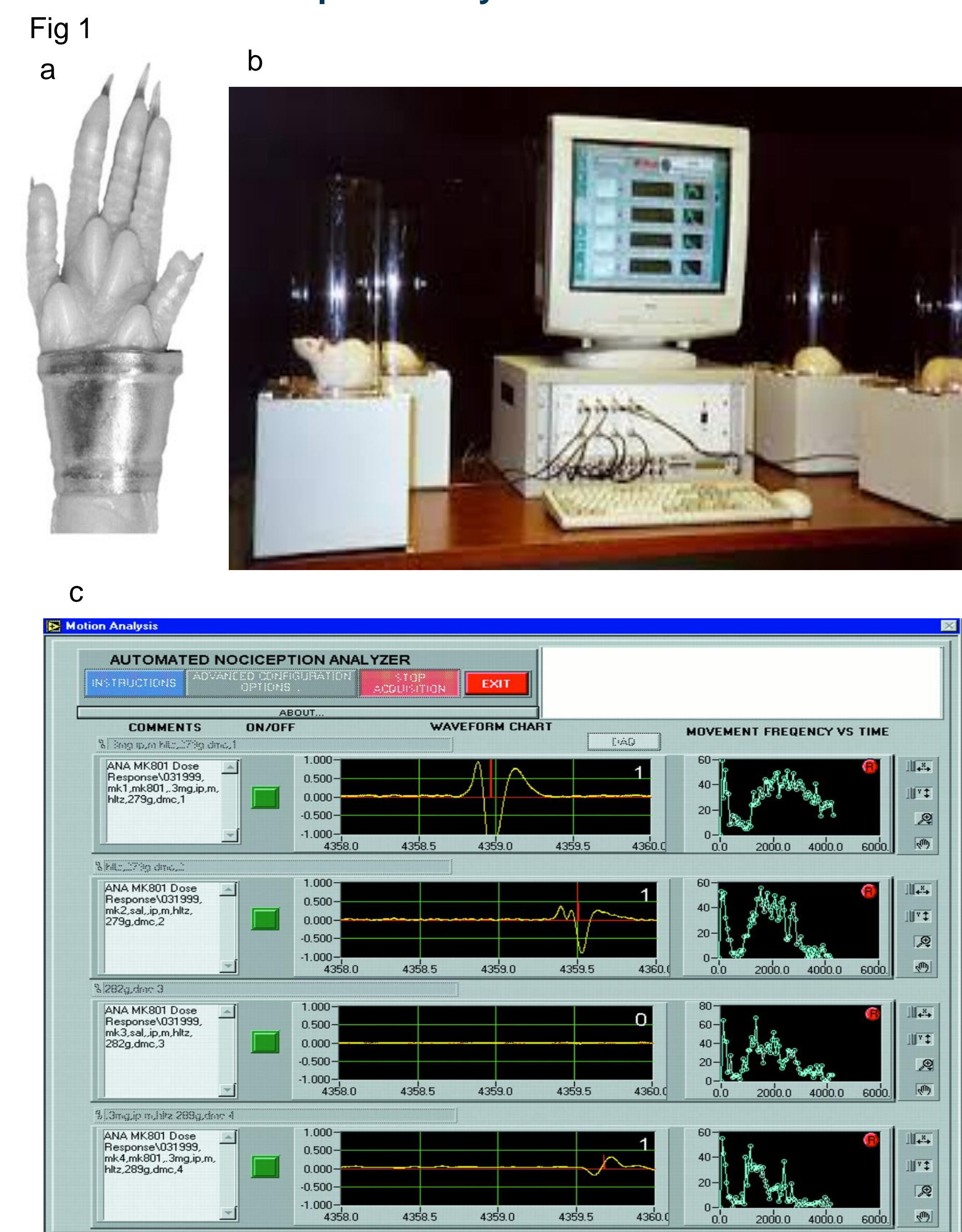
**1) Test compound and harmaline preparation and administration:** Harmaline HCl was dissolved in saline and injected intraperitoneally (ip) in 1ml/kg volume. The test compounds were administered either orally or ip 30-60 minutes before harmaline injections. Tremors were counted immediately after harmaline injection

**2) Acclimatization and counting of tremors:** Animals were acclimatized in the acrylic cylinder with metallic band placed on the right fore limb for 1 hour before administration of harmaline. Tremor activity (shaking of fore paws) was detected by movement of small metal band placed on one of the fore limb of the rats. A signal was generated as the band breaks the electromagnetic field of a loop antenna located under the rat and processed through an algorithm that determines tremor activity using 1) amplitude, 2) zero-voltage crossing, and 3) signal duration. Tremors were summed and stored over a selected collection interval throughout the assay for later analysis as described by Yaksh TL. et al (2000) (fig 1).

## References:

1. Yaksh TL. et al. (2001). J App. Physiol 90, 2386-2402.
2. Shourmasti, F.R. et al. (2014). Basic and clinical Neuroscience 5, 138-143.
3. Martin, F.C. et al. (2005). Movement Disorder 20, 298-305.
4. Handforth Adrian. (2012). Tremor Other Hyperkinet Mov. 2, 1-12.

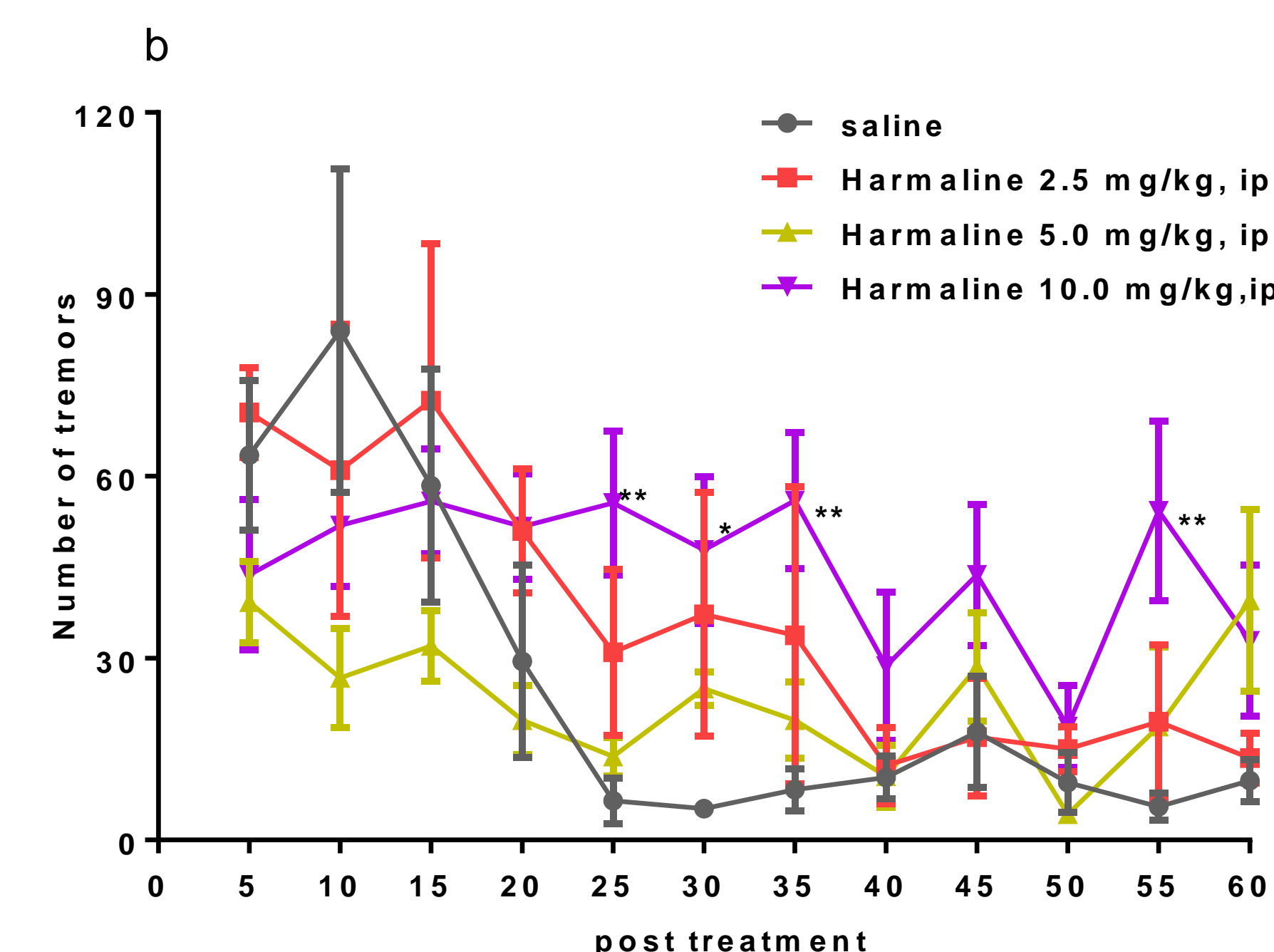
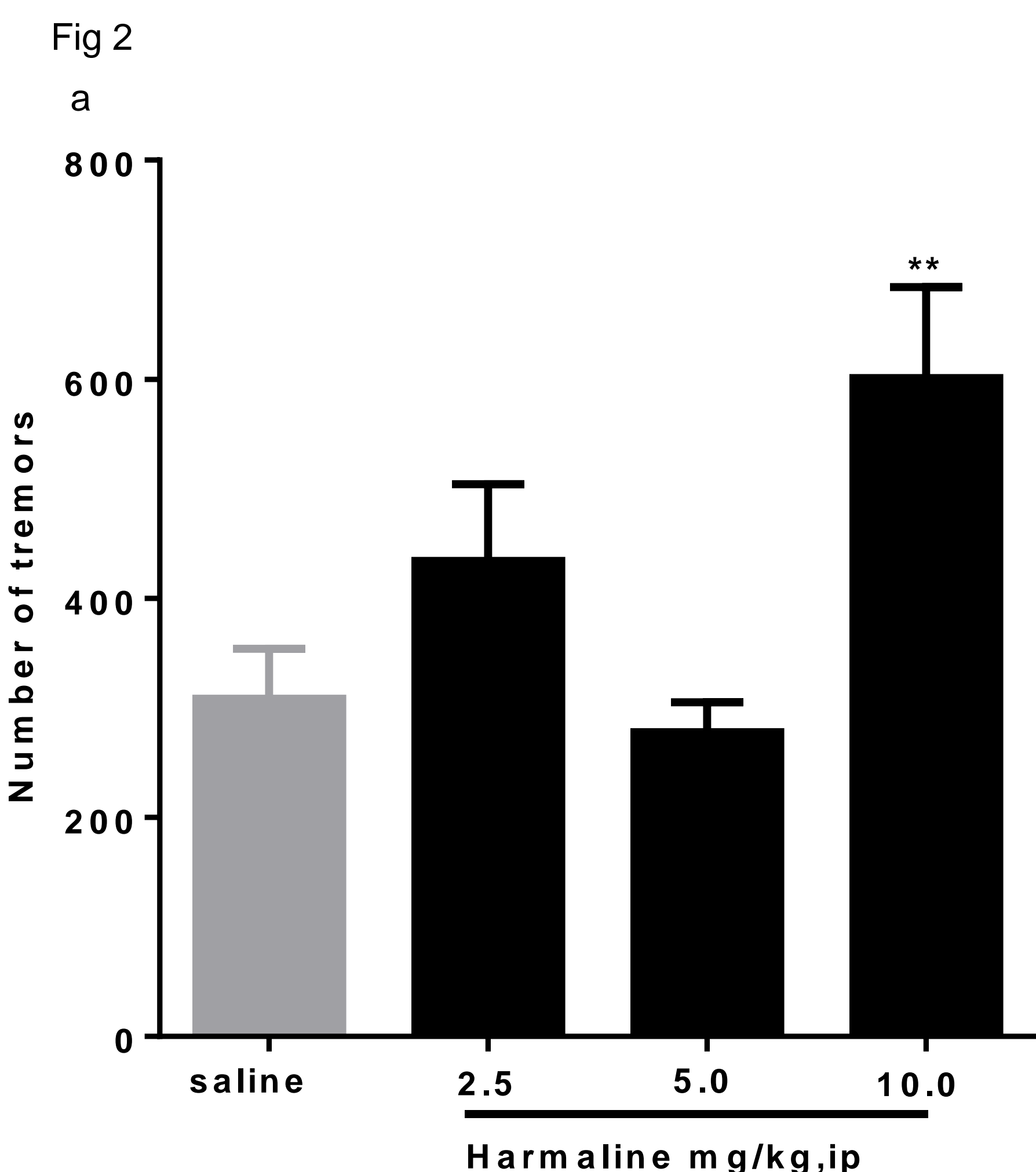
## Automated Nociception analyser to count fore limb tremors



Automatic nociception analyser used for counting tremors: a) Metal band was placed on the right fore limb of the rat to detect the fore limb tremor. b) Four detection units in which rats were placed for tremor activity and analog input and signal processing modul along with the computer display. c) The screen present during the current data collection in 4 rats. The view from each animal from left to right is 1) study and animal 2) Small green on/off switch that initiates or stops data collection 3) Smooted range waveform signal 4) Minute by minute tremor count sums.

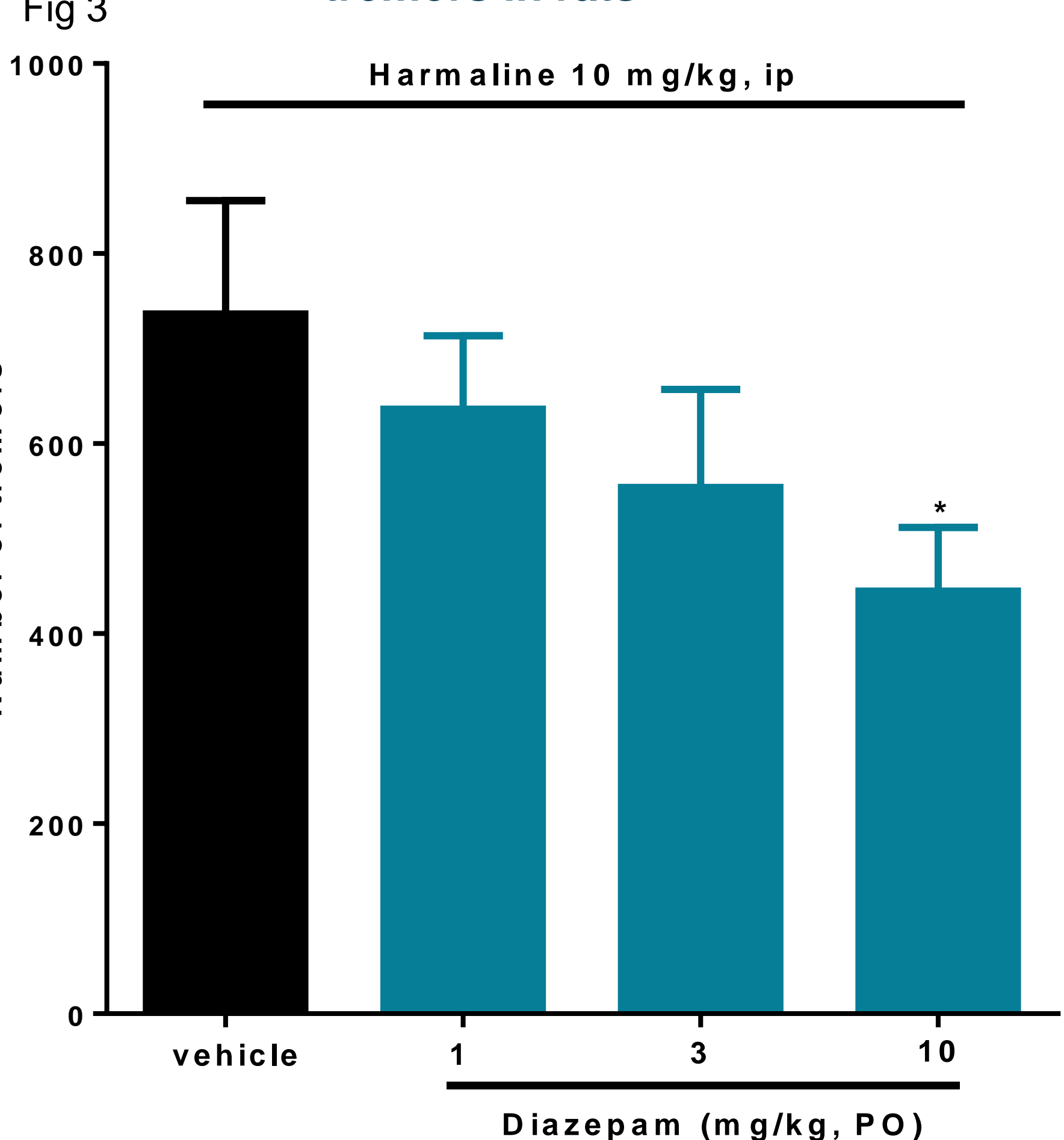
## Results

### Harmaline induced tremors in SD male rats



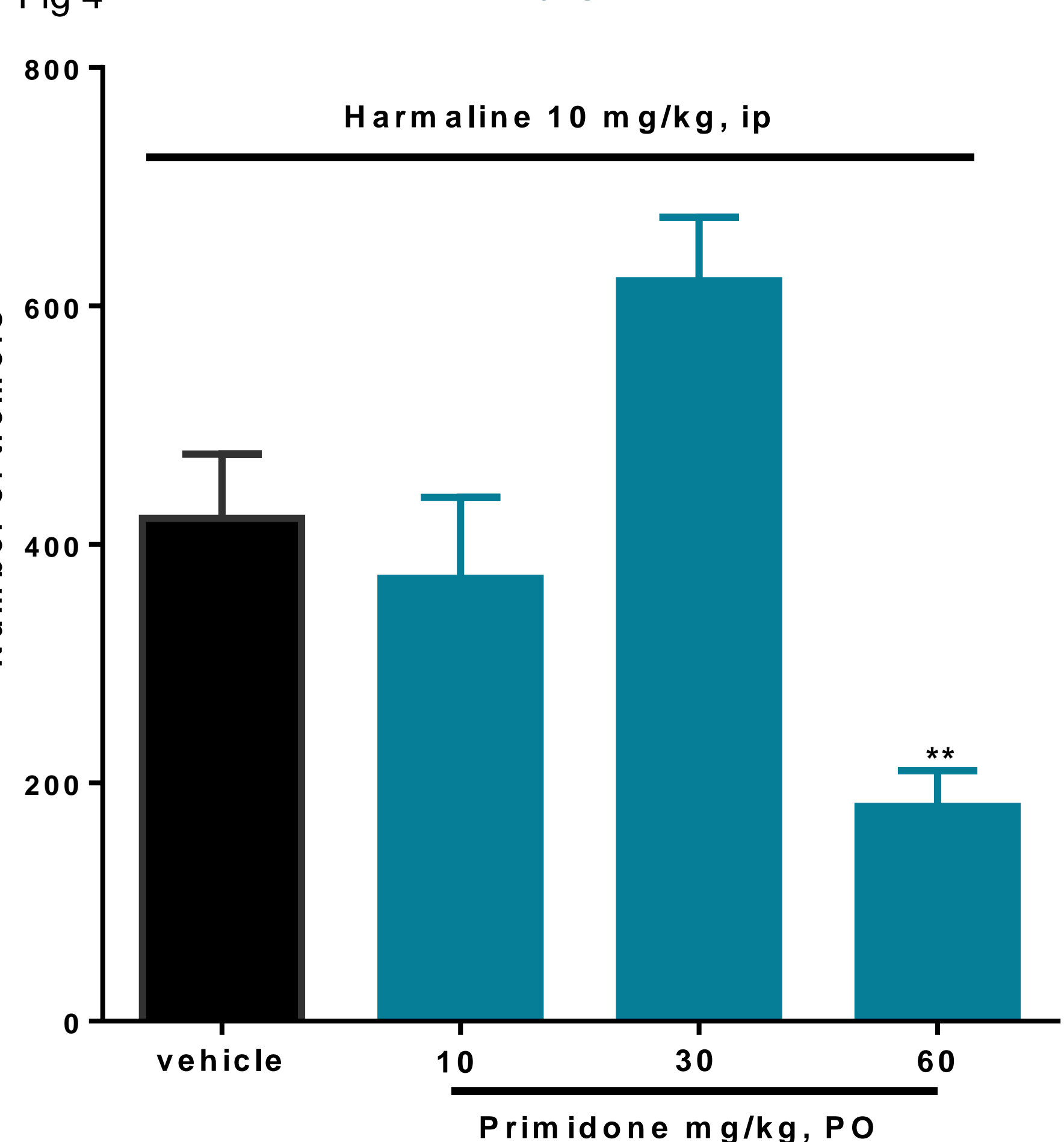
a) Cumulative tremor activity induced by saline and harmaline. \*\* $p < 0.01$  vs saline, one way ANOVA, Bonferroni post test, b) A Cumulative sum of tremor behaviour during five minute time period. \*\* $p < 0.01$ , \* $p < 0.05$  vs saline, Two way ANOVA, Bonferroni post test, n=4-6

### Effect of Diazepam on harmaline induced tremors in rats



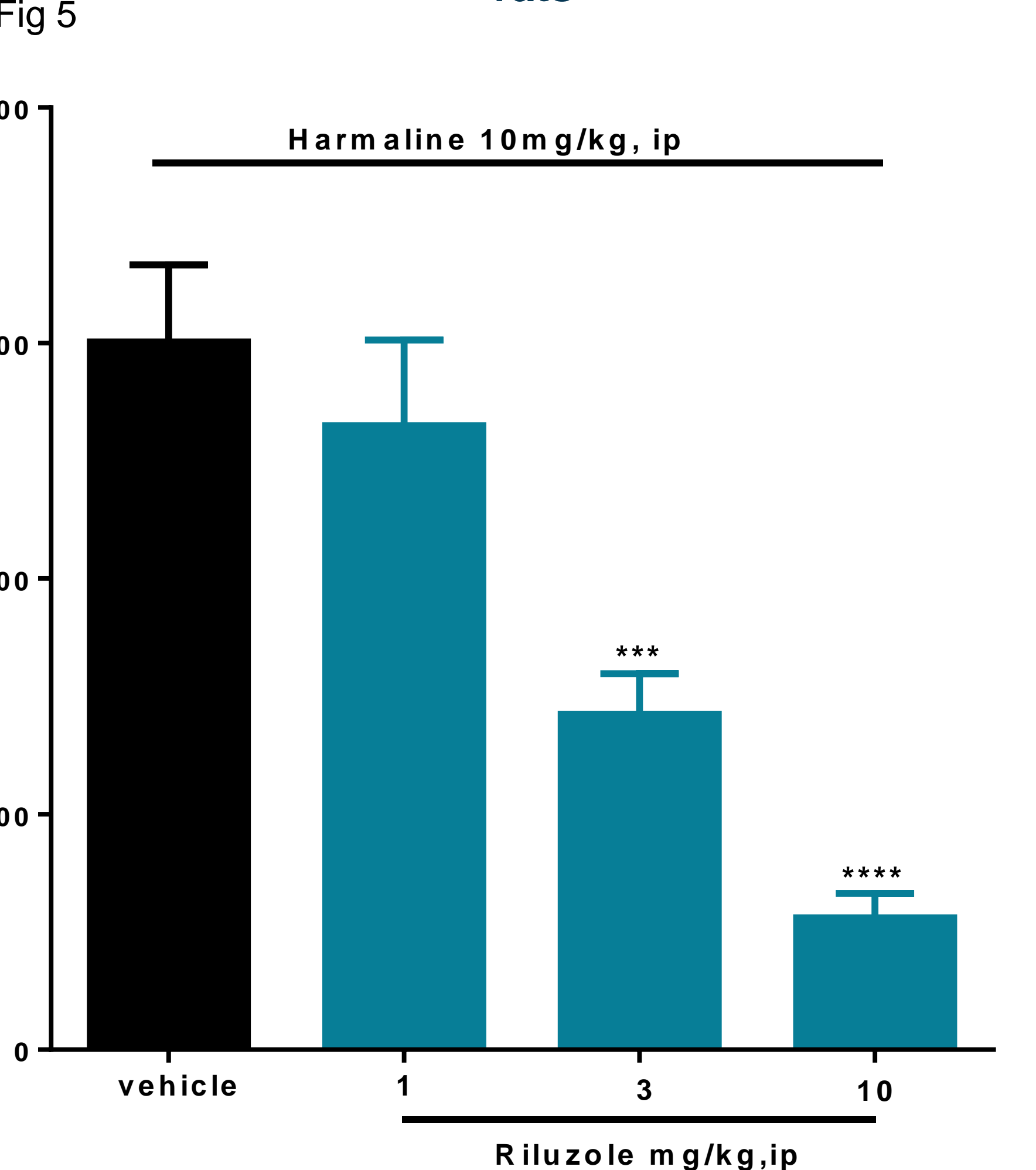
Diazepam significantly reduced the harmaline induced tremors in rats \*  $p < 0.05$  vs vehicle, One-way ANOVA Fisher's LSD test, n=7

### Effect of Primidone on harmaline induced tremors in rats



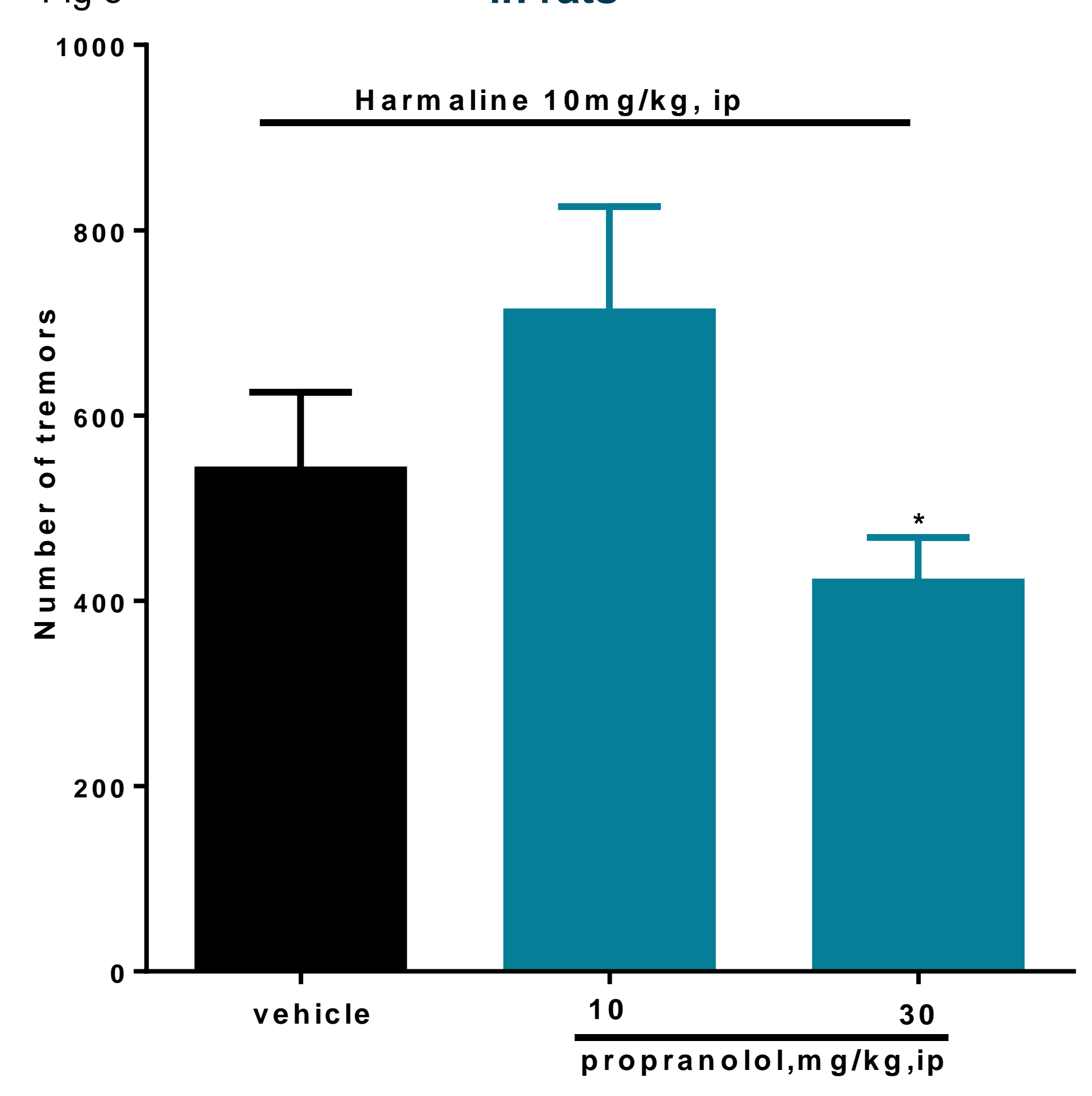
Primidone significantly reduced the harmaline induced tremors in rats \*\*  $p < 0.01$ , vs vehicle, One-way ANOVA Fisher's LSD test, n=7-8.

### Effect of Riluzole on harmaline induced tremors in rats



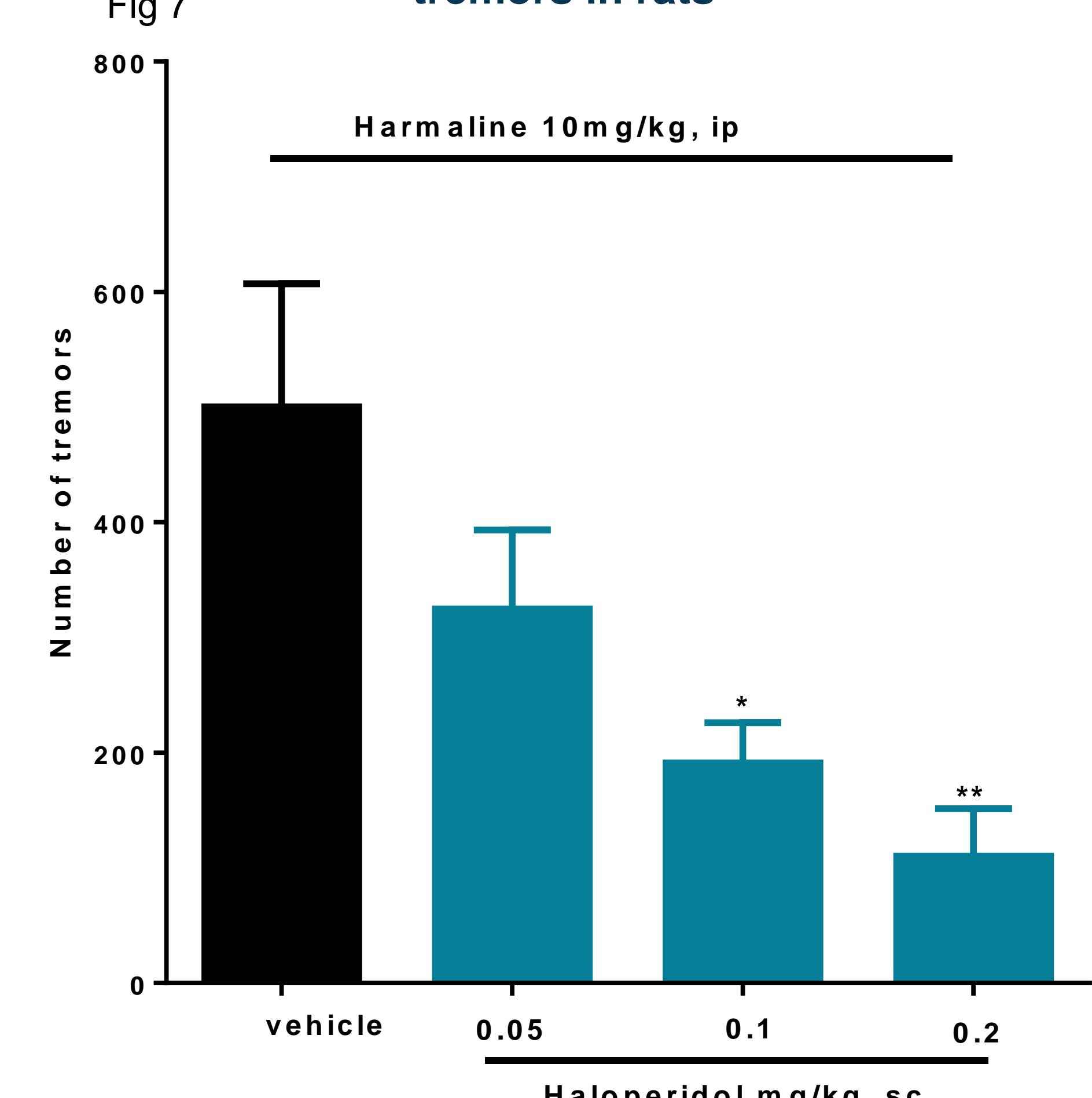
Riluzole dose dependently reduced the harmaline induced tremors in rats \*\*\*\* $p < 0.0001$  \*\*\*  $p < 0.01$ , vs vehicle, One-way ANOVA Bonferroni test, n=8.

### Effect of Propranolol on harmaline induced tremors in rats



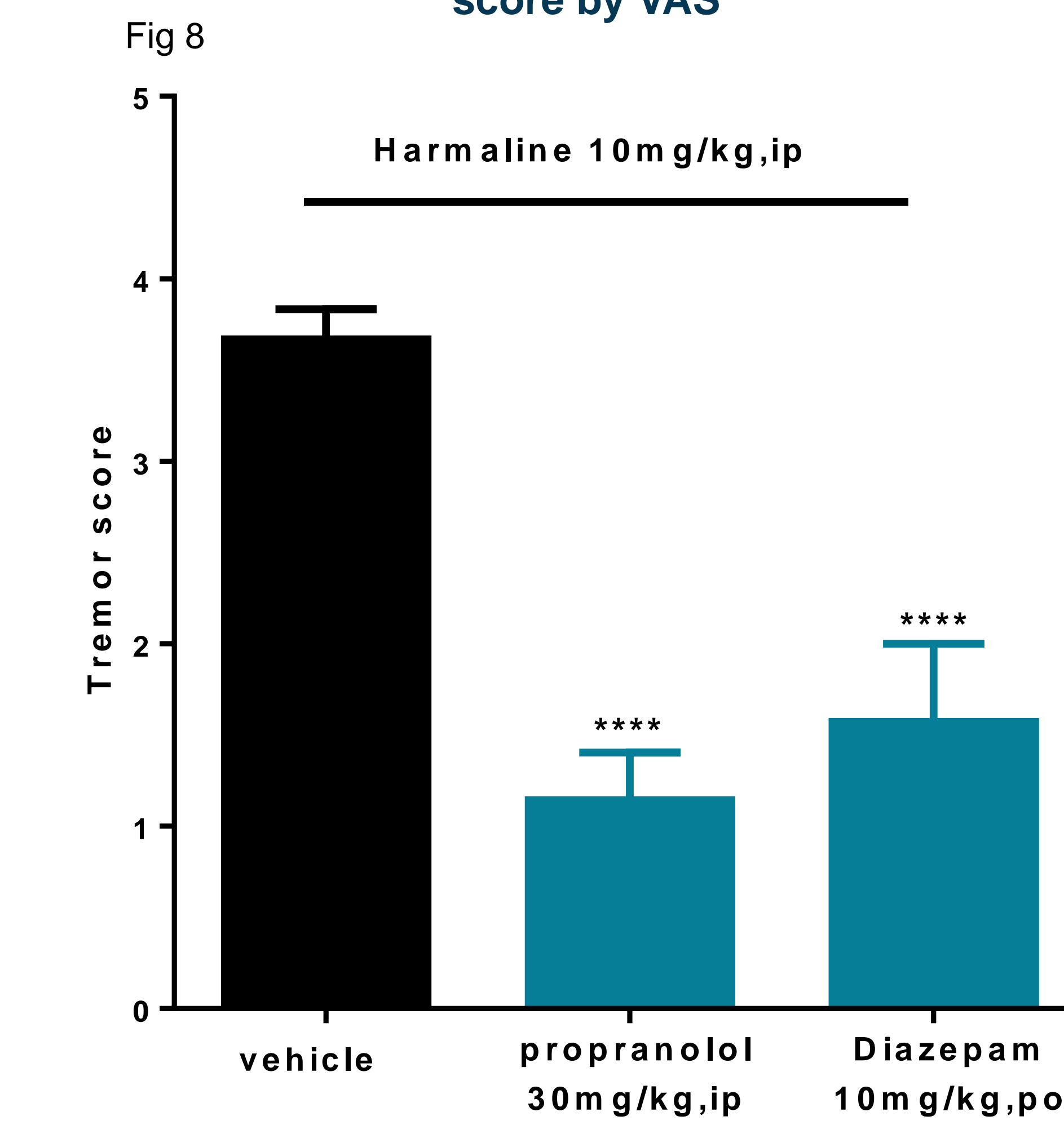
Propranolol significantly reduced the harmaline induced tremors in rats \*  $p < 0.05$ , vs vehicle, One-way ANOVA Bonferroni test, n=7-10.

### Effect of Haloperidol on harmaline induced tremors in rats



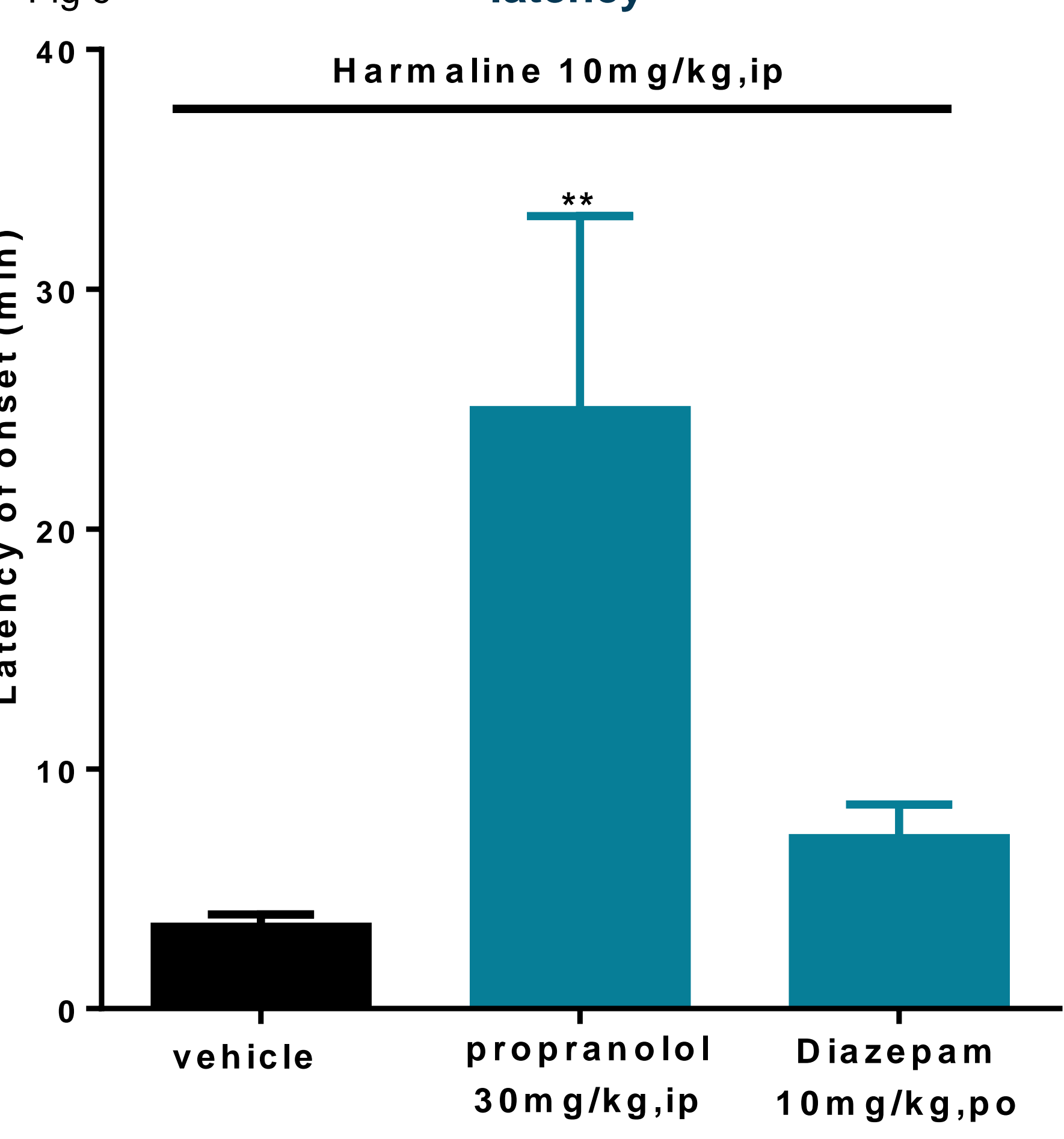
Haloperidol dose dependently decreased harmaline induced tremors rats \*\* $p < 0.01$ , \* $p < 0.05$ , vs vehicle, One-way ANOVA Bonferroni post test, n=7-8

### Effect of propranolol and diazepam on tremor score by VAS



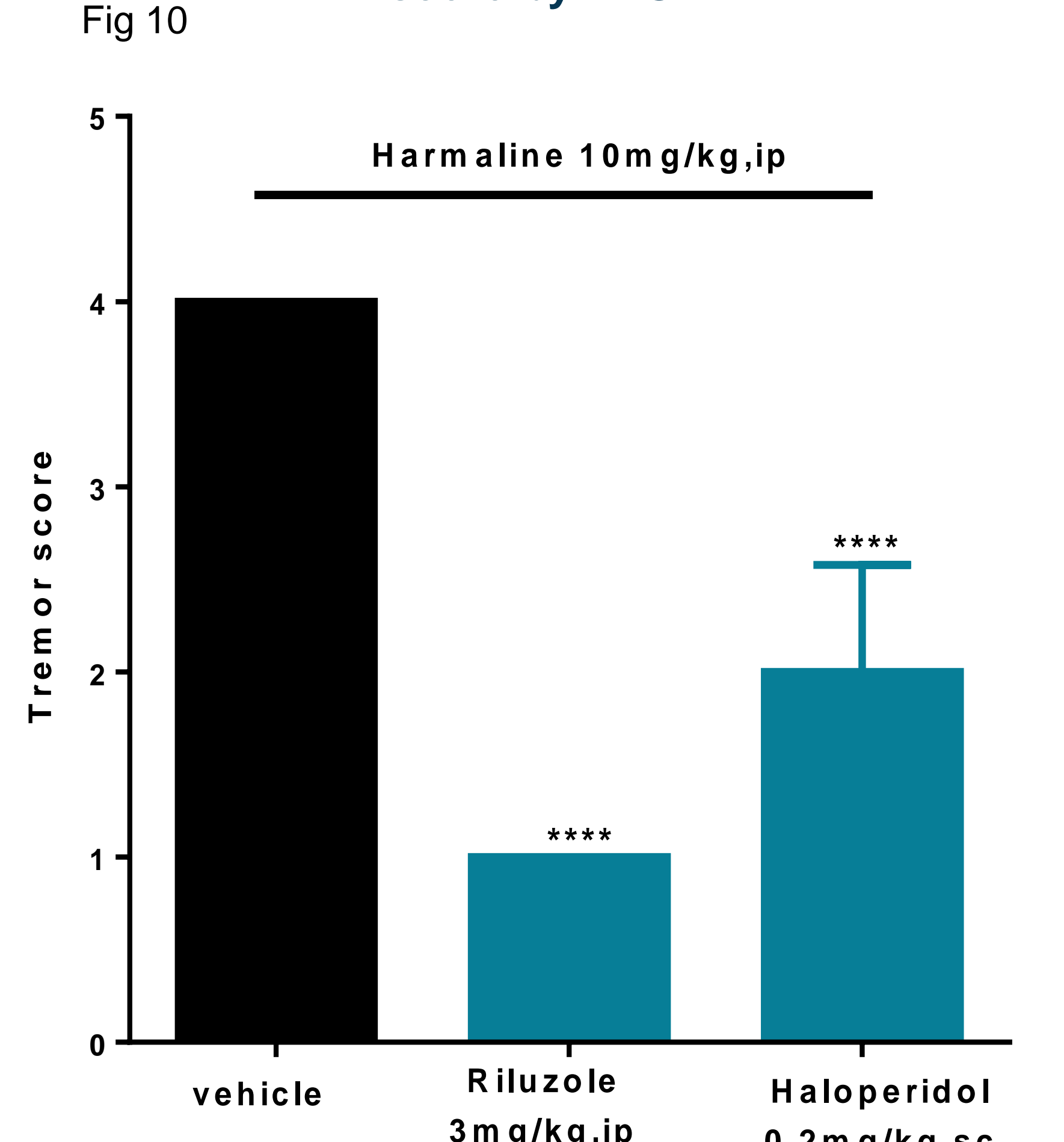
Propranolol and diazepam significantly reduced the harmaline tremor intensity in rats \*\*\*\* $p < 0.0001$ , vs vehicle, One-way ANOVA Bonferroni post test, n=7-9

### Effect of propranolol and diazepam on tremor latency



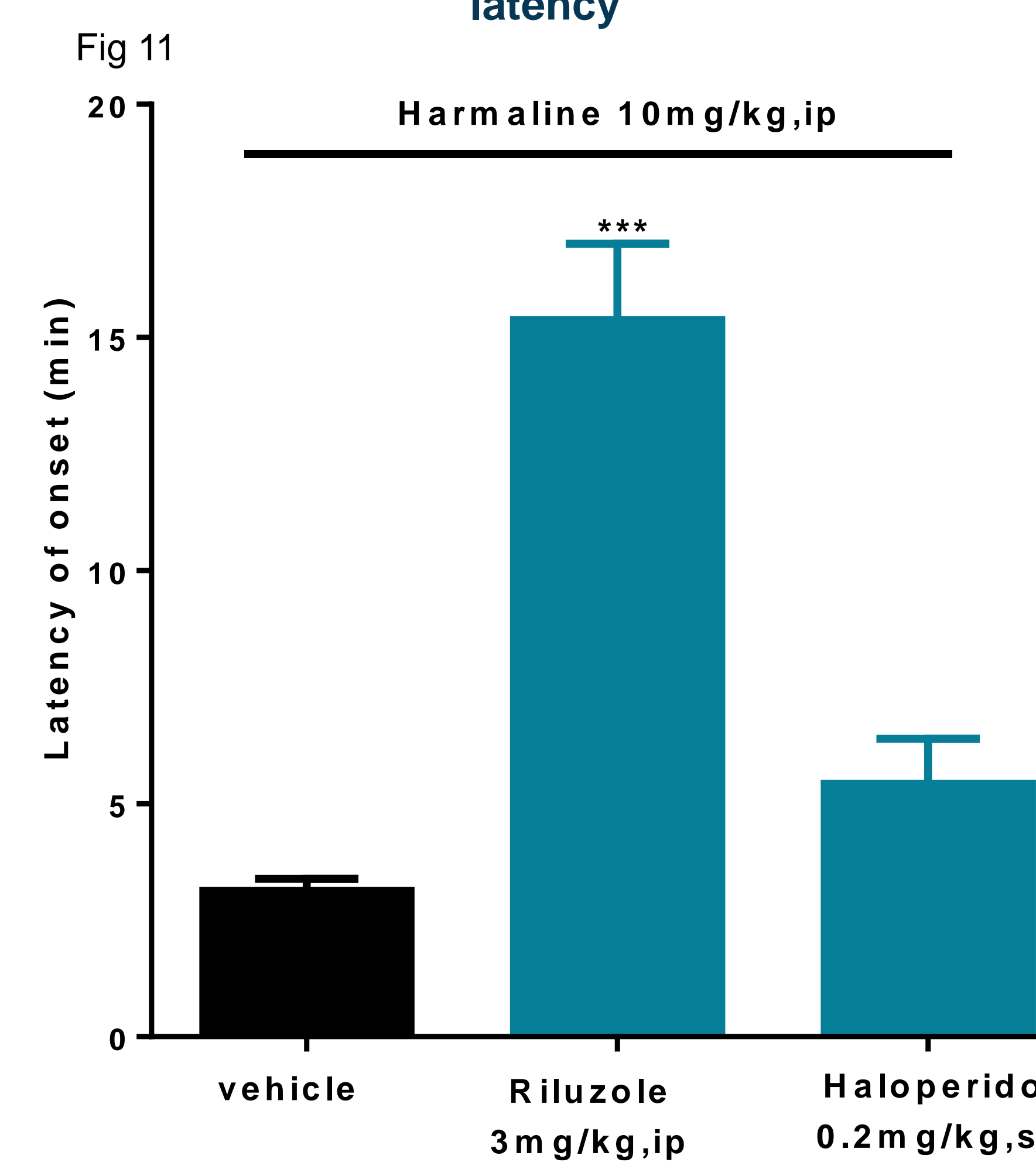
Propranolol significantly increased the onset of harmaline induced tremor latency in rats \*\* $p < 0.01$ , vs vehicle, One-way ANOVA Bonferroni post test, n=7-9

### Effect of riluzole and haloperidol on tremor score by VAS



Riluzole and haloperidol significantly reduced the tremor score \*\*\*\* $p < 0.0001$ , vs vehicle, One-way ANOVA Fisher's LSD test, n=3-6

### Effect of Riluzole and haloperidol on tremor latency



Riluzole significantly increased the onset of the tremor latency in rats \*\*\* $p < 0.001$ , vs vehicle, One-way ANOVA Fisher's LSD test, n=3-6