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INTRODUCTION

Spinal cord injury (SCI) is a two-phase process, encompassing both primary and secondary injuries. While the term "primary injury" refers to structural damage caused by a mechanical trauma, the term "secondary injury" is used to describe a complex interaction of patho-physiological and biochemical mechanisms initiated by the primary injury (Sekhon and Fehlings, 2001). One of the key processes involved in secondary damage is iNOS (nitric oxide synthase)-mediated cell damage, through the generation of reactive nitrogen species peroxinitrates.

Peroxynitrates results in lipid peroxidation of membranes and cause disturbances in tissue homeostasis that is severe enough to cause cellular damage and eventually cell death (Beattie et al., 2000). Hence, suppression of peroxynitrate formation should decrease lipid peroxidation thereby decreasing damage created by secondary injury within the context of CNS injury. One way to decrease the peroxynitrate formation in the injured spinal cord includes inhibition of iNOS with selective and non-selective antagonists such as Aminoguanidine (AG), and Curcumin (CUR), respectively. Here we investigated the possible neuroprotective effects of these iNOS inhibitors in an established animal model contusive spinal cord injury, and whether they can contribute to functional recovery.

METHODOLOGY
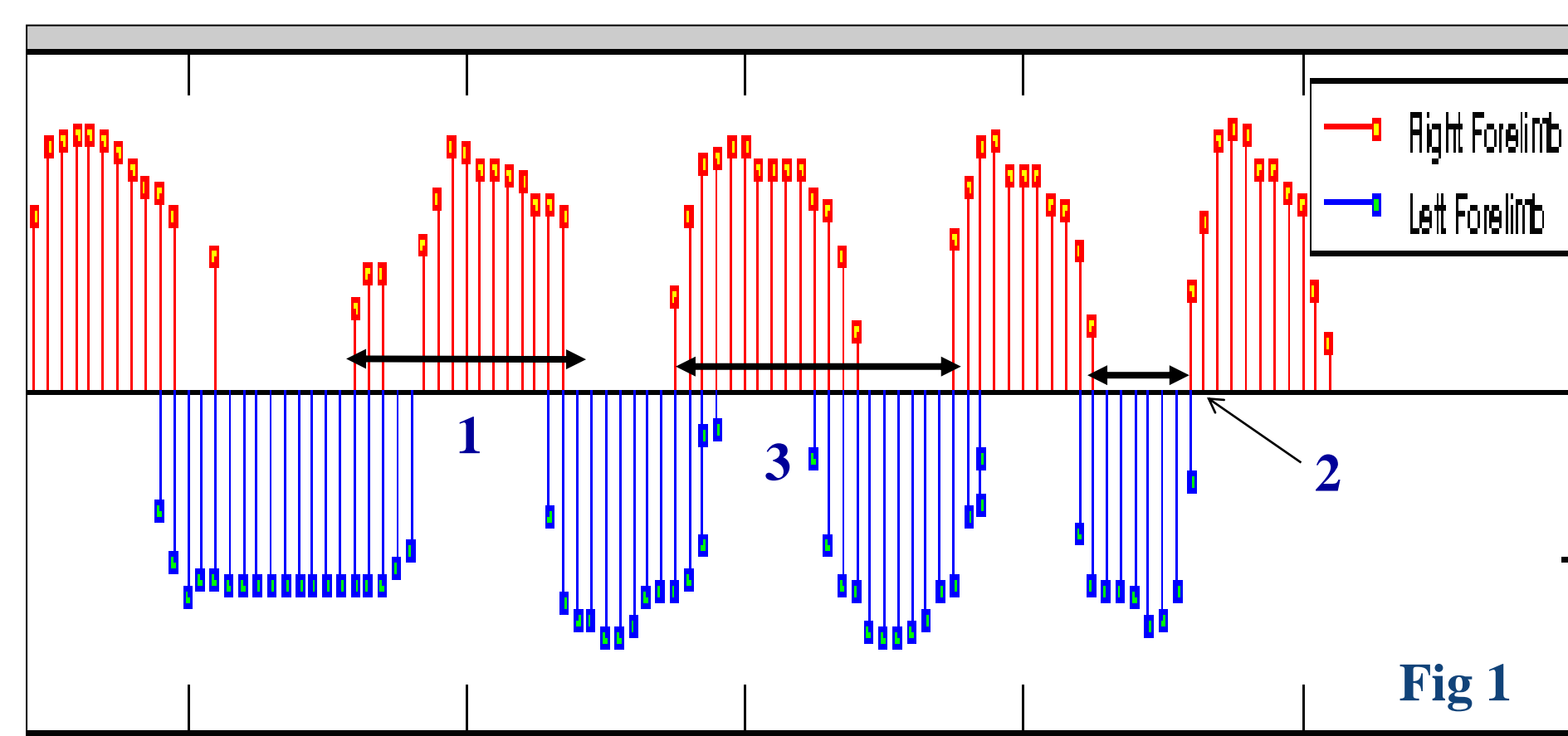
SPINAL CORD INJURY MODEL:
Female SD rats were subjected to a severe thoracic (T8) contusive injury using the Infinite Horizon (IH) device developed by Ohio State University at a force of 200 Kydn.

BEHAVIOR ANALYSIS:
Beginning at 1 week after injury, animals were behaviorally tested using the Basso Beattie and Bresnahan (BBB) scoring system, weekly for 6 weeks. At 6 weeks, foot print analysis was done to assess correct spatial placement of the hind paw. The hind paw recordings of the footprints were analyzed for 1) *base of support-* distance between contralateral paws 2) *stride length-* distance between two consecutive prints of the same paw 3) *limb rotation-* the angle that the central digit deviates from the direction of walking. Grid walk test was carried out to assess deficits in motor control by allowing rats to walk on irregular grid and no of foot-falls were counted.

NEUROCUBE ™ ANALYSIS:
The NeuroCube™ was used to detect changes in the Gait. Rats were allowed to move freely in the NeuroCube™. Gait geometry and dynamics were analyzed and discrimination probability was recorded. Animals were tested once a week for 4 weeks after surgery. The following gait measures were measured and analyzed (Fig.1): Stance duration (1) Swing duration (2) Stride duration (3) .

Fig 1

AMINOGUANIDINE

OPEN FIELD BBB SCORES

FOOT PRINT ANALYSIS
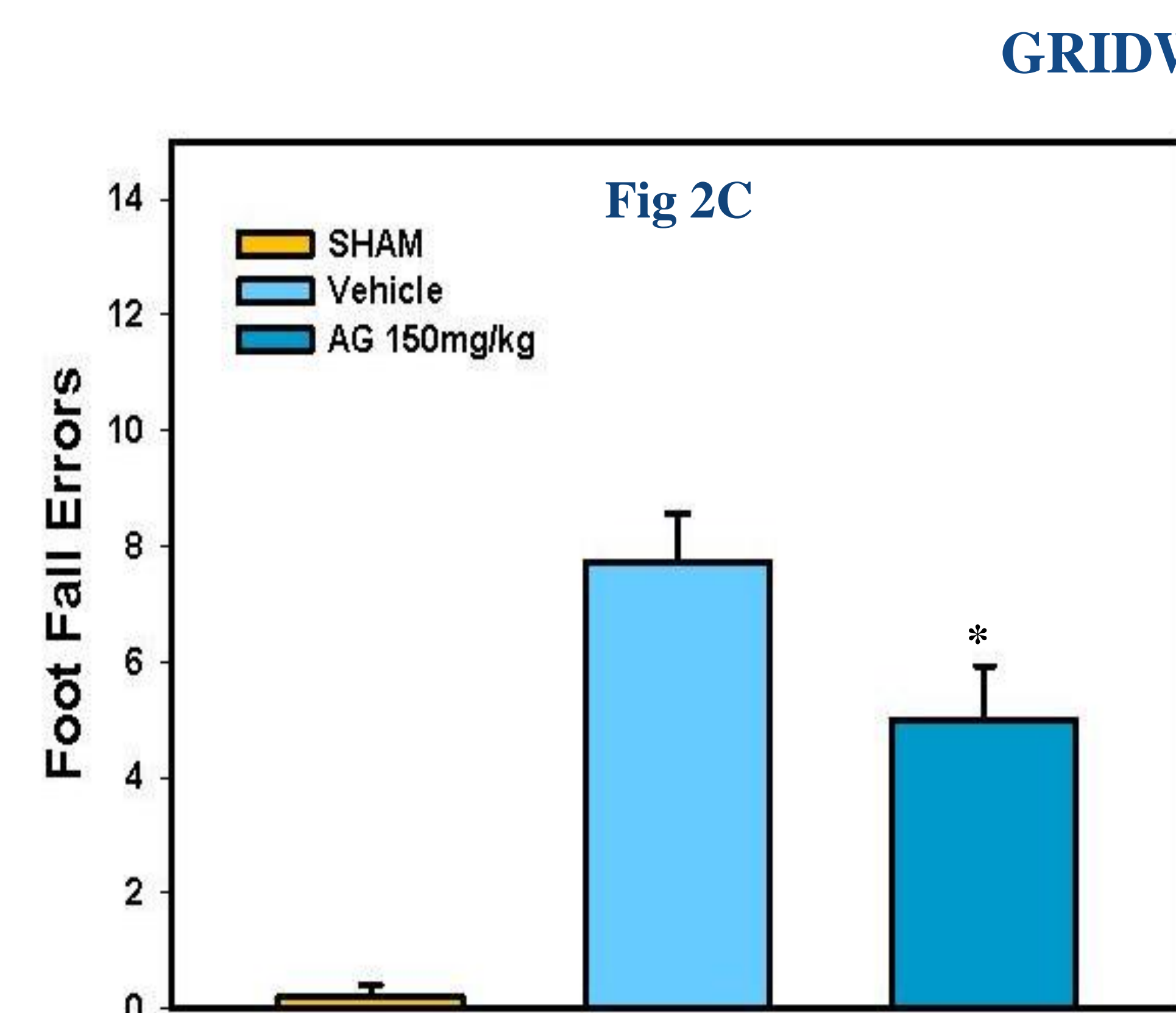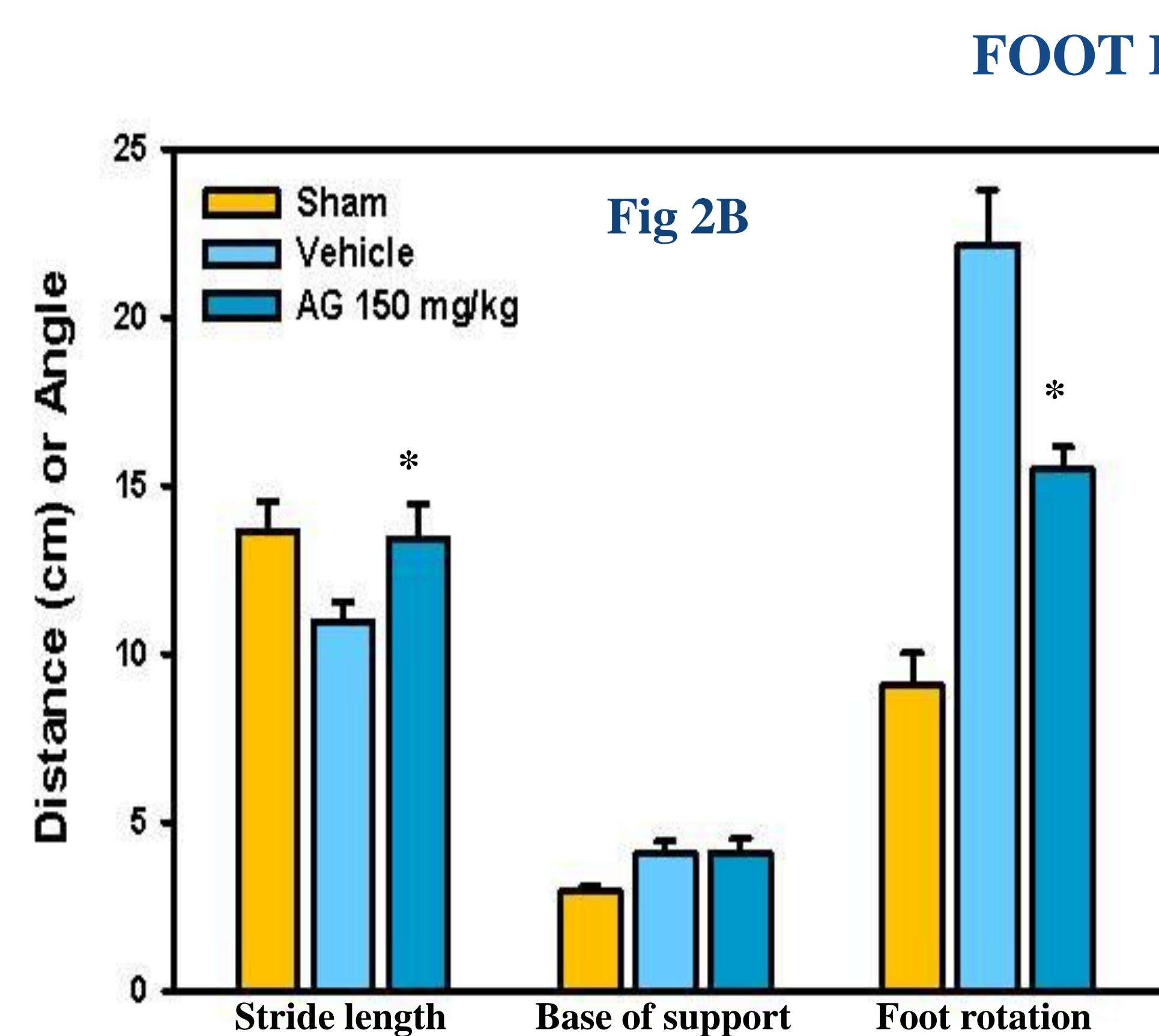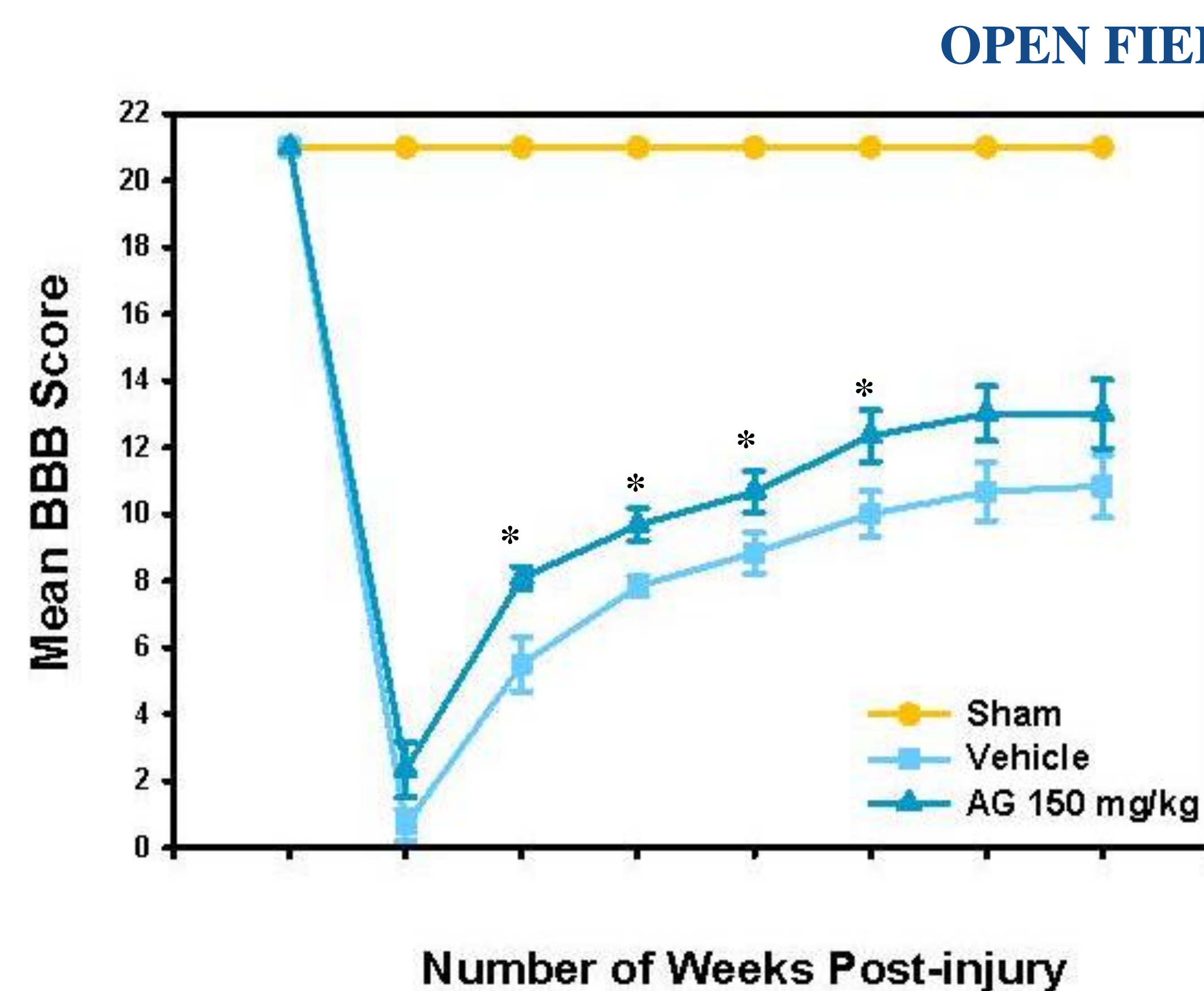

Fig 2B

GRIDWALK ANALYSIS

Fig 2C

Fig. 2A. Open field locomotor testing using the BBB score shows that AG displayed a faster recovery of function compared to controls and they are significantly better from weeks 2 to 4 (*p<0.05). At later time points no persistent improvement in the BBB score was observed. (B) AG treatment significantly improved stride length and foot rotation in foot print analysis. (C) There was significant decrease in the foot fall errors with treatment of AG as compared to vehicle controls
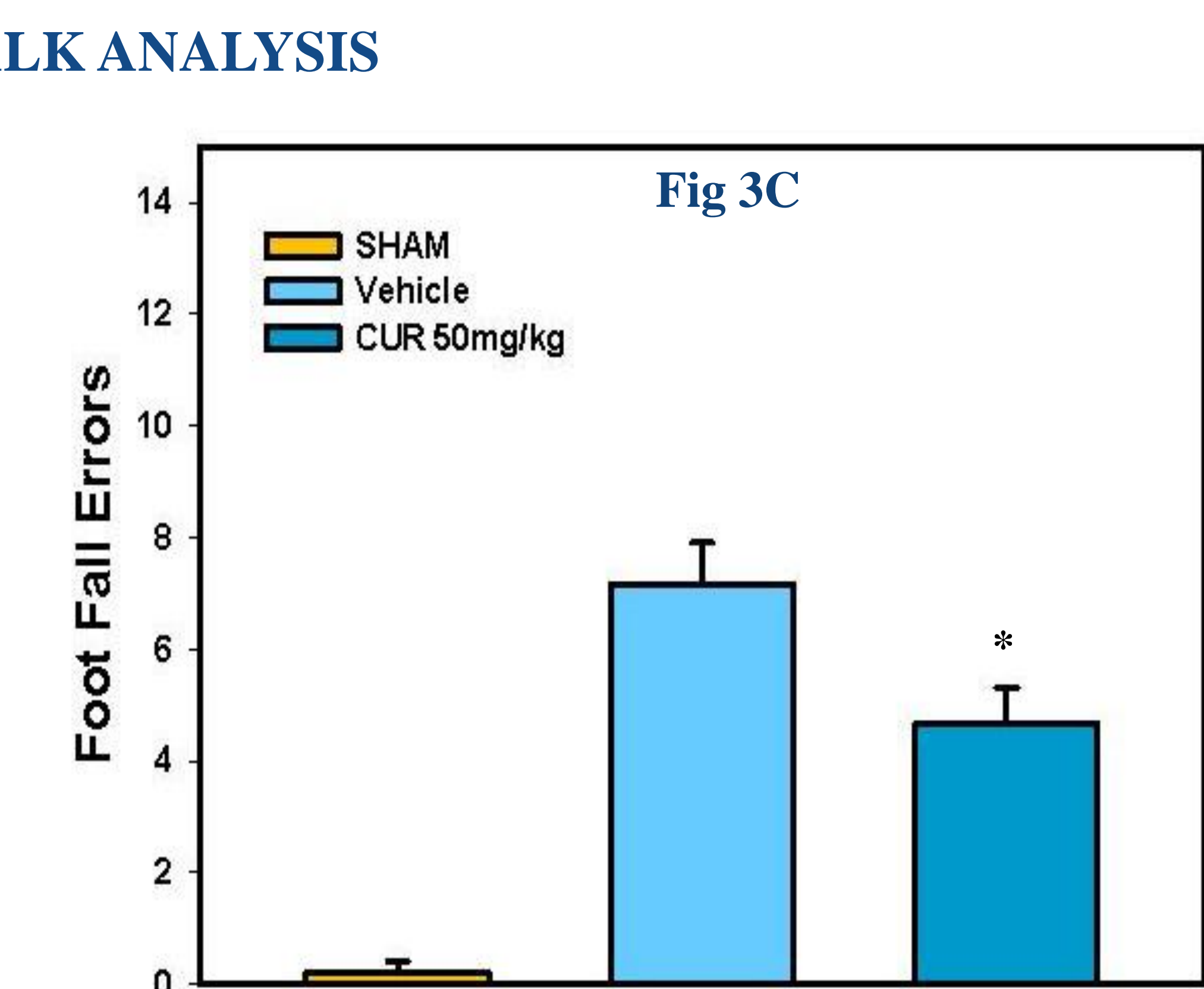
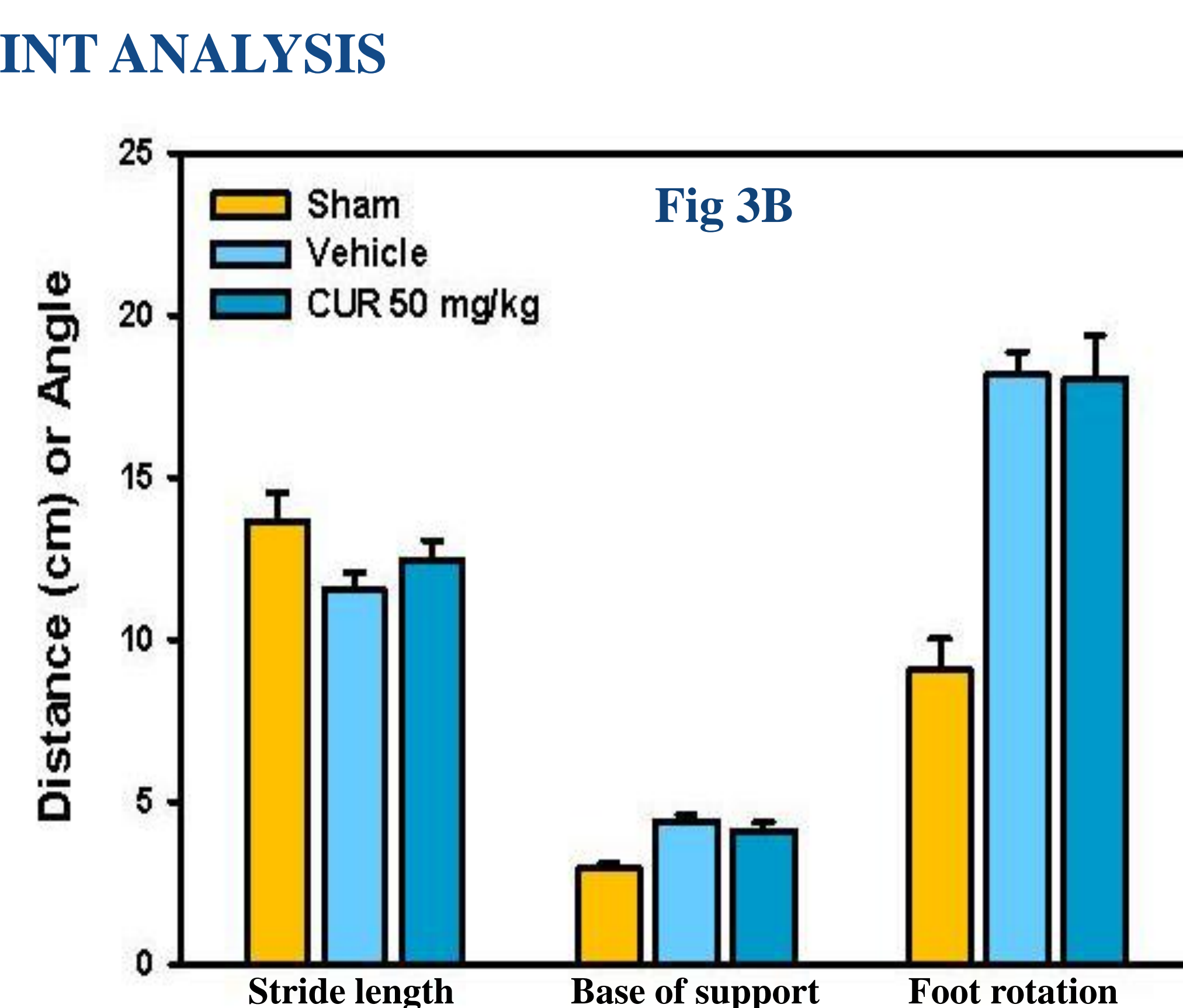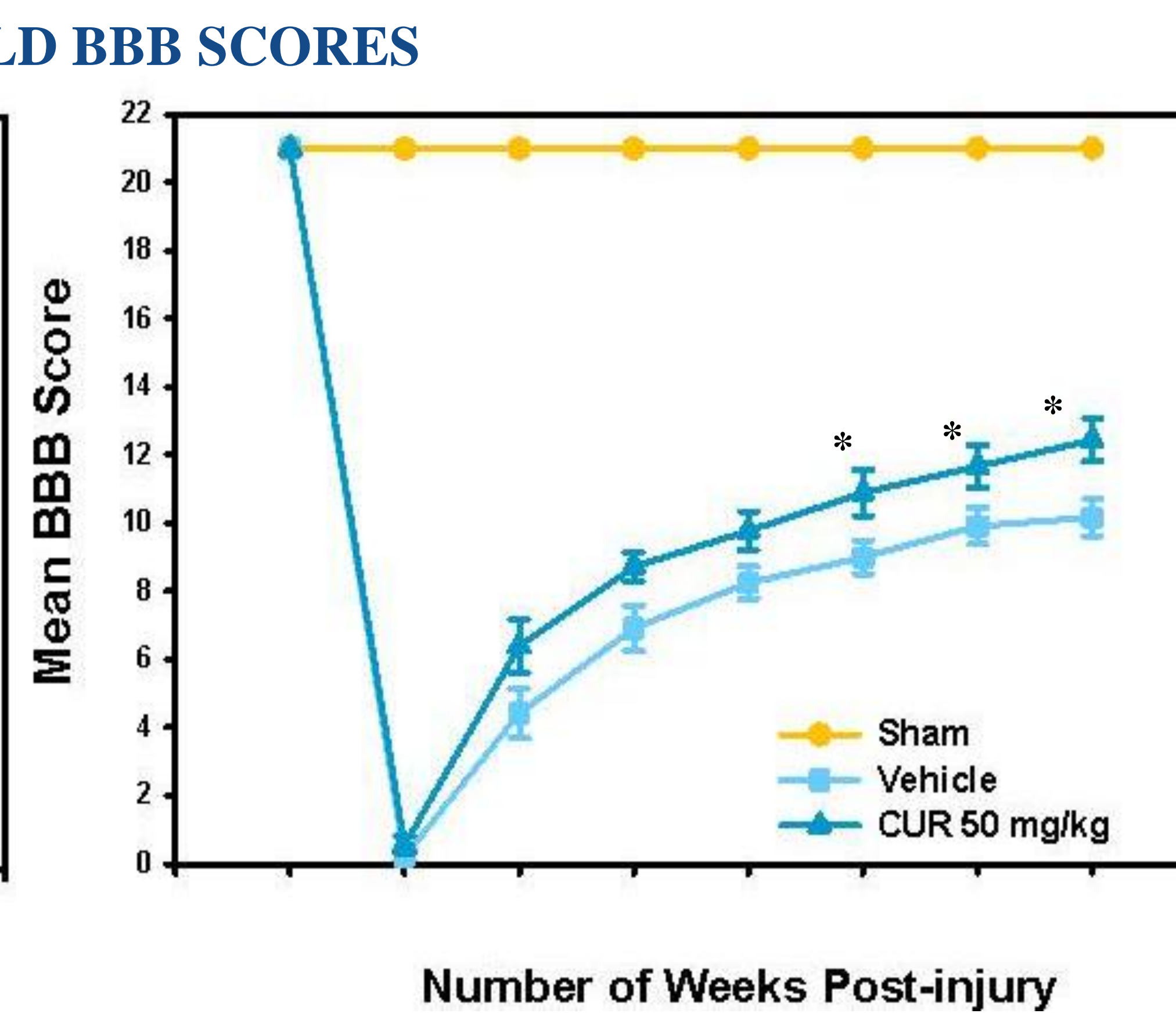## CURCUMIN

Fig 3B

Fig 3C

Fig. 3A. Open field locomotor testing using the BBB score shows that CUR displayed a slower recovery of function compared to controls and they are significantly better * (p<0.05) at later time points, week 4 to 6. (B) CUR treatment failed to improve foot rotation and stride length in foot print analysis. (C) There was significant decrease in the foot fall errors with treatment of CUR as compared to vehicle controls

NEUROCUBE ™ ANALYSIS

GAIT ANALYSIS
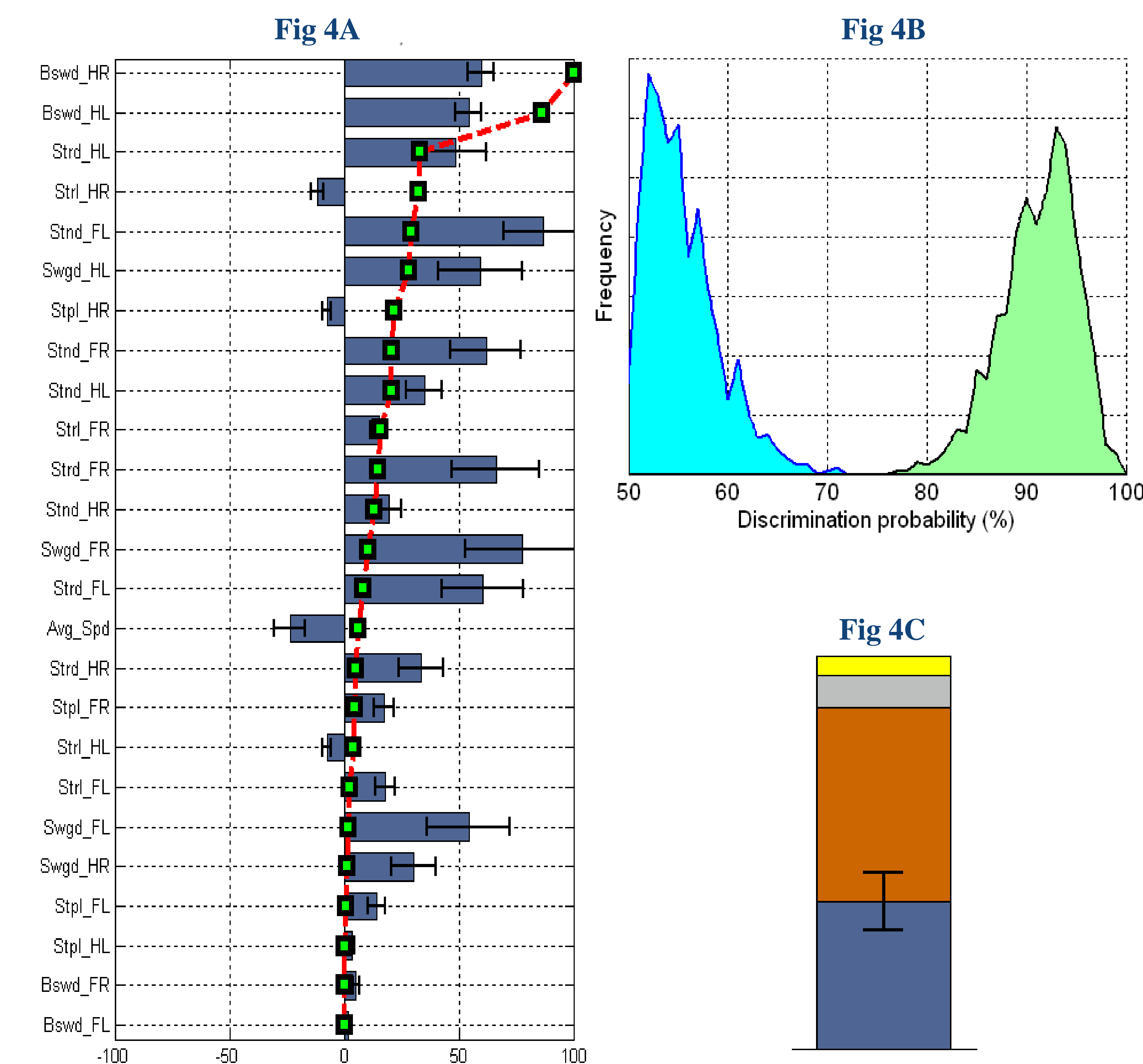
Fig 4A

Fig 4B

Fig 4C

SCI: 4 weeks after Injury	
Fig. 4A. Rank ordered features	--
Fig.4B. Discrimination probability	91%, p<0.0001
Fig. 4C. Recovery with 150 mg/kg AG	43%, p<0.0001

SUMMARY

Results of the present study shows an early significant neuroprotective effect of specific iNOS inhibitor aminoguanidine versus late neuroprotective effects of curcumin a non specific iNOS inhibitor in open field tests In foot print analysis : aminoguanidine revealed better effects in terms of walking stability, body support as compared to curcumin treated rats

Both treatments significantly reduced the foot fall errors in grid walk tests after SCI.

Neurocube™ analysis revealed a significant deficits in a variety of features but the deficit in gait analysis was the most predominant. aminoguanidine treatment showed a 43% recovery in the gait features

Our study further strengthens the significant role of iNOS in the pathology of spinal cord injury.