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AN ELECTROPHYSIOLOGICAL MODEL OF SPINAL TRANSMISSION DEFICITS IN MOUSE EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

Susan E. Hanak, Erin M. Reilly, Jill Wotanis, Bin Zhu, Claudine Pulicicchio, Kathleen McMonagle-Strucko, Joseph G. Wettstein, and Mark D. Black

CNS Pharmacology, Aventis Pharmaceuticals, Inc., Bridgewater, New Jersey, USA.

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Electrophysiological Mouse EAE Spinal Transmission Deficits

Susan E. Hanak, Ph.D.

CNS Pharmacology

Aventis Pharmaceuticals, Inc.; PO Box 6800

Building: JR2-3204D; mailstop: JR2-303A

Bridgewater, New Jersey 08807

Telephone/Fax: (908) 231-2283; Email: Susan.Hanak@aventis.com

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Non-standard abbreviations: EAE: Experimental Autoimmune Encephalomyelitis; PLP: myelin proteolipid protein; MT: Mycobacterium tuberculis; CFA: complete Freund's adjuvant; AMPA: (+/-)-α-Amino-3-hydroxy-5-methylisoxazole-4-propionic acid; MS: Multiple sclerosis; CNS: Central Nervous System; CAP: Compound Action Potential; PI: post inoculation; ACSF: artificial cerebral spinal fluid

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ABSTRACT

Chronic relapsing/remitting Experimental Autoimmune Encephalomyelitis (EAE) can be induced in 8 week old female, SJL/J(H-2) mice via inoculation with the p139-151 peptide of myelin proteolipid protein (PLP), *Mycobacterium tuberculosis* (MT), complete Freund's adjuvant (CFA), and *Bordatella pertussis.* EAE is a relevant preclinical model of MS that incorporates several aspects of the clinical disease. Chief among these are the inflammatory mediated neurological deficits. While the impact of localized spinal cord demyelination on neurotransmission has been modeled successfully, relatively little work has been done with spinal cord from animals with EAE. The goal of this study was to assess the utility of a grease-gap tissue bath methodology in the detection of transmission deficits in EAE spinal cord tissue. Spinal cords removed from EAE mice at different phases of the neurological deficit were assessed for their response to both lumbar and sacral application of one of several depolarizing agents (veratridine, potassium chloride [KCl], (+/-)-α-Amino-3-hydroxy-5-methylisoxazole-4-propionic acid [AMPA]). The main finding of this study is that transmission deficits were detected in EAE mice at the onset of the neurological deficits. They were sustained for a period of approximately 2-3 weeks post disease onset followed by a gradual recovery of group function. The other finding is that there is a decrease in the latency to achieve AMPA-mediated depolarization in sacral spinal cord that is independent of the magnitude of the depolarization response. These results suggest that this methodology can be utilized to assess sensory and motor deficits in spinal cord from EAE animals.

Multiple sclerosis (MS) is a chronic, progressively debilitating disease presenting with neurological symptoms resulting from degenerative changes in the Central Nervous System (CNS). Axonal demyelination induced by autoimmune-triggered neuroinflammation has been reported to be at the core of the CNS lesions that characterize MS (Waxman,1998; Waxman, 2000). Recently, postmortum MS studies have correlated the degree of inflammation at the lesion site with the incidence of axonal transection (Trapp et al., 1998). Active demyelination has been correlated with a high incidence of axonal damage, as compared to less damage in lesions with inactive centers, and still less damage in remyelinated plaques (Kornek et al., 2000). These findings suggest that neuroinflammatory events ultimately lead to the axonal degeneration which figures prominently in the progressive forms of MS (Trapp et al., 1998).

While the precise physiological event by which the disease is triggered and progresses is unknown, both MS patients and pre-clinical EAE mice exhibit changes in CNS glutamate levels (Hardin-Pouzet et al, 1997; Stover et al, 1997). The pre-clinical findings suggest that glutamate excitotoxicity impacts upon the myelinating function of oligodendrocytes. Several investigators have reported that the AMPA/kainate antagonist NBQX improves the neurological symptoms of EAE, increasing oligodendrocyte survival and decreasing axonal damage without affecting CNS neuroinflammation (Pitt et al., 2000; Smith, 2000; Matute, 2001). Both oligodendrocytes and spinal motor neurons exhibit AMPA-mediated excitotoxicity (Ikonomidou and Turski, 1996; Yoshioka et al, 1996; McDonald et al, 1998). Therefore rigorous examination of AMPA-mediated transmission in the

mouse EAE model of demyelination may aid us in understanding the contribution of the glutamate pathway to the physiological progression of demyelinating disease.

Undoubtedly demyelination is a prominent causative factor in the pathology of the axonal transmission deficits associated with EAE (Chalk et al, 1994; Chalk et al., 1995). Both conduction block and "ectopic" firing patterns have been observed. While demyelinated central axons have been shown to conduct over large areas (2500 μm) they exhibit a decrease in conduction velocity and an increase in the refractory period of transmission (Felts et al., 1997). While seminal electrophysiological studies (Hodgkin and Katz, 1949; Hodgkin and Huxley, 1952) have clearly defined the role of sodium and potassium channels in the generation of the action potential the impact that demyelination has on the relationship between these ion channels and conduction is still evolving. The picture that is emerging suggests that demyelination influences both the location of the ion channels on the axons as well as which subtypes are expressed. Increases in the concentration of sodium channels along the demyelinated regions of the axon may account for the reversal of conduction block (Foster et al., 1980). Transient redistribution of peripheral nerve potassium channels (Kv1.1) from the juxtaparanodal to the nodal region following demyelination translates into increases in both the amplitude and duration of the Compound Action Potential (CAP) elicited by the potassium channel blocker, 4-aminopyridine (Rasband et al., 1998) as well as the incidence of "ectopic" action potentials (Felts et al., 1995; Kapoor et al., 1997). The sodium channel blocker TTX has been shown to block both the "ectopic" action potentials induced in demyelinated axons by 4-AP as well as the oscillations in the membrane potential

recorded from sites near the lesion (Kapoor et al., 1997) suggesting that both the sodium and potassium current contribute to the "ectopic" discharge. Expression of sodium channel SNS mRNA in cerebellar Purkinjie cells of both EAE mice and human patients with MS but not normal patients (Black et al., 2000a) may be responsible for the ataxia observed in clinical MS (Waxman, 2000; Waxman, 2001b). Therefore examining the impact of EAE on spinal cord depolarization mediated via either the sodium channel opener, veratridine or elevated levels of potassium may help elucidate the physiopathology of demyelinating disease.

While there is extensive preclinical data on the behavioral expression and histopathology of EAE less attention has been focused on analyzing the impact to spinal neurotransmission. Isolating the spinal cord of both EAE and untreated animals allows one to investigate the pharmacology of its sensory and motor components post-EAE insult. Therefore a series of studies were designed to characterize spinal cord conduction in EAE and naïve animals using a grease-gap bath methodology. The overall goal of the study was to determine whether this method could be used to detect changes in spinal cord neurotransmission mediated by EAE and whether there was a correlation between the neurological deficit phase of EAE and spinal cord transmission.

MATERIALS AND METHODS

1. Preparation of EAE mice

EAE was induced in 8 week old female, SJL/J(H-2) mice (Jackson Laboratories, USA) using a protocol developed from referenced publications (Tuohy et al., 1989; Kuchroo et al., 1991; Kuchroo et al., 1994). Briefly, on Post Inoculation Day 0 (PI) subjects received both a subcutaneous inoculation of a combination of PLP 139-151 (75 μg) (Bachem, Bioscience, Inc., King of Prussia, PA) and MT (400 μg) (Difco, USA) in CFA H37 Ra (1 mg/ml *Mycobacterium Tuberculosis* H37 Ra) and an intravenous inoculation of *Bordatella pertussis* (35 ng, List Biological Laboratories, USA). A second inoculation of *Bordatella pertussis* (50 ng) was administered on PI day 3. Sham controls (all inoculation components except PLP 139-151) were utilized in the veratridine studies. Naïve controls (no injection) were utilized in both the KCl and AMPA studies.

Beginning on 10 days PI subjects were scored daily for the onset and progression of the behavioral symptoms of motoric impairment associated with the induction of EAE in the scientific literature. The scoring scale was from 0 to 5: 0 = normal; 0.5 = partial limp tail; 1.0 = complete limp tail; 2.0 = impaired righting reflex; 2.5 = delayed righting reflex; 3.0 = partial hind limb paralysis; 3.5 = one leg completely paralyzed, one leg partially paralyzed; 4.0 = complete hind limb paralysis; 4.5 = legs are completely paralyzed and moribund; 5.0 = death due to EAE. The subjects were scored over approximately a 2-5 week time period during which their progression from the acute attack, to first remission, relapse and second remission was tracked. The acute attack was defined as the first clinical episode with a score greater then 1.0 for a period of two

consecutive days. Remission was defined as a phase of clinical improvement following an attack where the clinical score was decreased by at least one point for a period of two consecutive days. Relapse was defined as the period following remission where the clinical score rose by at least one point for a period of two consecutive days. Spinal cord transmission was assessed at different phases of the illness and is presented in relationship to the number of weeks PI.

2. Electrophysiology

After neurological assessment the test subjects (2-5 weeks PI) were anesthetized with urethane (Sigma; 0.5 ml of a 1.12 M solution), decapitated and their spinal columns were exposed. The spinal column and surrounding viscera was dissected so that the portion remaining was comprised of mainly the lumbar and sacral portions. A 26-G 3/8-A needle attached to a 5 cc syringe filled with the artificial cerebral spinal fluid (ACSF) and 0.10 M urethane was inserted into the sacral portion of the vertebral column and the spinal cord was pressure ejected into a bath of cold, oxygenated ACSF containing urethane (0.10 M). The ACSF was composed of the following (mM): NaCl 118 mM; $NaHCO_3$ 25 mM; KCl 4.7 mM; KH_2PO_4 1.2 mM; $MgSO_4$ 2.0 mM; Glucose 11mM; $CaCl_2$ 2.0 mM; H_2O_2 0.003% (pH 7.3-7.4).

The tissue was further dissected so that the remaining block consisted of all of the tissue below the lumbar enlargement and approximately 5 mm of tissue above the lumbar enlargement. The cords were then transferred to one of eight, 2-compartment grease-gap

baths filled with cold, oxygenated ACSF/urethane solution. The baths were constructed so that there was a greased slot in the central barrier separating the two compartments (Hayes and Simmonds, 1978; Harrison and Simmonds, 1985; Black et al., 2000b). The cords were orientated in the baths so that the barrier was inserted across the spinal cord at the level of the beginning of the lumbar enlargement. Once all eight spinal cords were loaded into the baths, the baths were perfused with ACSF at a rate of 2 ml/minute.

The tissues were allowed to equilibrate in the baths for approximately 60 minutes prior to the initial drug application. The direct current (DC) potential between the two compartments was measured using silver/silver chloride electrodes. Three studies were conducted. In each case increasing concentrations of the selected drugs were applied sequentially to the lumbar portion of the cord followed by the sacral portion of the cord. For the purposes of this study we have defined motor transmission as potential differences resulting from drug application to the lumbar portion of the spinal cord (corticospinal pathway). Sensory transmission is defined as potential differences resulting from drug application to the sacral portion of the cord (mechanosensory pathway). In the first study AMPA (Sigma) was assessed at concentrations of 1.0, 10.0 and 30.0 μM. In the second study, potassium was assessed at concentrations of 0.30, 1.0, 3.0, 10.0 μM. In the third study, veratridine (Sigma) was assessed at concentrations of 10.0, 30.0, and 100.0 μM.

The standard drug application time was two minutes in length. The signals were captured, amplified and filtered using specialty hardware and software (Spike2, Cambridge Electronics Design Limited, AxonInstrument, CyberAmp 380, Programmable Signal Conditioner; Axoclamp filter).

3. Data Analysis

A Spike2 subprogram (min/max for peaks) was used to analyze the data in terms of the peak potential change (mV) caused by each drug application. Statistical comparison of the concentration-depolarization response curves elicited by drug application to EAE subjects at either 2, 3, 4 or 5 weeks PI or to their age-matched controls was accomplished via a repeated measures ANOVA (Statistica 5.0, Statsoft, Inc.).

The latency to the peak level of depolarization (time required to reach peak depolarization from the point of drug application) achieved by the highest concentration of each drug tested was also calculated. The values calculated for EAE subjects at either 2, 3, 4 or 5 weeks PI were compared to their age-matched controls using an ANOVA (GraphPad Prism 2.0).

RESULTS

Neurological deficit assessment in SJL/PLP EAE mice. Inoculation with PLP results in a clearly defined acute attack in approximately 90% of the subjects. While it appears that all of the subjects go into remission there are essentially two subsets within that group; those in chronic remission (approximately 60% of the inoculated subjects) and those that relapse and experience subsequent remissions (approximately 30% of the inoculated subjects). Owing to the fact that the group's behavior is the most synchronized at the acute attack and the first remission those time points are also the most prominent features of the neurological deficit graph (Fig. 1). Subsequent relapse and remission phases demonstrate the least separation due to the effect of averaging the two subgroups.

Neurological deficits and electrophysiological assessment. In a satellite study we observed that the subjects in chronic remission that were assessed between 39 and 41 days PI had significantly better spinal cord conduction then those that relapsed and remitted (Fig. 2). Clearly that finding would impact the ability to correlate overall group spinal cord transmission with the neurological deficit phase after the acute attack given that you can't predict a priori which subgroup a subject would ultimately belong to at first remission. In fact preliminary analysis of spinal transmission in relationship to the phase of the neurological deficit (acute attack, first remission, relapse, second remission) did not demonstrate a clear correlation between the cyclic oscillations in the neurological deficit and the ability to depolarize spinal cord tissue (data analysis not presented). Therefore, for the purpose of developing a viable model for pharmaceutical development,

overall spinal cord transmission for each group was assessed in relationship to the number of weeks PI. The concentration/response curves for AMPA, KCl, and veratridine are presented graphically in figures 3, 4, and 5 respectively. The latency to achieve the peak level of depolarization elicited by the highest concentration of each drug tested is presented in Tables I., II. and III. For AMPA (30.0 μM), KCl (10.0 mM), and veratridine (100.0 μM) respectively.

Both sham (all inoculation components except PLP) and naïve controls (age-matched for EAE subjects at 2-5 weeks PI) were generated for the veratridine studies. Initial comparison of the sham and naïve animals (age-matched for EAE subjects at 2-5 weeks PI) response to veratridine did not reveal any discernable differences between the treatment groups (Table III.)

Electrophysiological assessment of AMPA-mediated depolarization in EAE and naïve age-matched controls. AMPA mediated motor transmission was suppressed at both 2 and 3 weeks PI. Recovery of function was observed by 4 weeks PI (Fig. 3A). While sensory transmission was decreased by approximately 25% from 2-4 weeks PI (30.0 μM concentration) the effect was not statistically significant (Fig. 3B). EAE subjects exhibited a decrease in the latency to achieve sensory depolarization at 3 weeks PI (30.0 μM AMPA) (Table I). Induction of EAE did not affect the latency to motor depolarization.

Electrophysiological assessment of KCl-mediated depolarization in EAE and naïve age-matched controls. KCl-mediated motor transmission (10.0 mM) was decreased by between 10% and 40% at 2-3 weeks PI but the effect was not statistically significant (Fig. 4A). There was however, a significant decrease in KCl-mediated sensory transmission in the EAE group at both 2 and 3 weeks PI (Fig. 4B). Induction of EAE did not affect the latency to depolarize at any PI interval (KCl 10.0 mM, Table II).

Electrophysiological assessment of Veratridine-mediated depolarization in EAE and sham age-matched controls.

Lumbar application of veratridine in the EAE group demonstrated that motor transmission was suppressed at both 2 and 3 weeks PI (Fig. 5A.). Recovery was noted by 4-5 weeks PI. Induction of EAE resulted in suppression of sensory transmission at 2 through 5 weeks PI (Fig. 5B). There was significant recovery between 2 weeks and 5 weeks PI since spinal transmission was significantly better at 5 weeks PI then 2 weeks PI. Induction of EAE did not alter the latency to depolarize at any PI interval (veratridine 100.0 μM, Table III.).

Discussion

The primary finding from this study is that PLP-mediated EAE resulted in deficits in spinal cord transmission that were quantifiable using a grease-gap bath methodology. In general, spinal cord tissues extracted from subjects expressing EAE at 2 to 4 weeks after inoculation were less responsive to local application of the depolarizing agents AMPA, KCl, and veratridine. Sensory, more so then motor transmission deficits appear to be a common, consistent feature of EAE animals.

Glutamate excitotoxicity has been hypothesized to be a central feature of the cycle of inflammation, demyelination, and axonal damage that occurs in both MS and EAE. CSF glutamate metabolite levels are altered in neuroinflammatory diseases and chronic treatment with AMPA/kainate antagonists has been reported to improve the neurological outcome of EAE (Pitt et al., 2000; Smith et al., 2000). One phase of the current study was designed to assess whether this glutamatergic pathophysiology was reflected in our model. In fact AMPA-mediated transmission deficits were observed. When veratridine and KCl were utilized as general mediators of spinal cord depolarization they also revealed EAE-mediated transmission deficits.

The second major finding was that the cyclical pattern of neurological remissions and relapses was not mirrored in the spinal cord transmission studies. In general, transmission deficits appear to be sustained for a period of approximately 2 weeks (2-4 weeks PI). Signs of recovery as measured by spinal cord depolarization were observed

at approximately 4-5 weeks after inoculation, corresponding with the period where there was a substantial decrease in the average neurological deficit score.

The failure to detect a cyclical pattern of spinal neurotransmission deficit and recovery for the group underscores the fact that there are multiple factors influencing the translation of physiological function into behavioral expression. One caveat to these findings is the inherent difficulty in categorizing the phase of EAE for each individual animal in order to assign them to test groups. Like others (Yu et al., 1996) we have observed a certain degree of intersubject variation in terms of the onset, duration and relative magnitude of the acute attack, remission and relapse phases. Clearly discernable transitions between the phases are less obvious after the 1st remission phase. This can be seen clearly in Figure 1. which graphs the typical progression of neurological symptoms in a representative study. Only approximately 30% of the group expresses what is considered to be a behaviorally defined relapse. The majority of the group (approximately 60%) goes into chronic remission. Therefore the gradual attenuation of the group's transmission deficits may well be a mathmatical artifact of the heterogeneity of the group

Similar distribution patterns have been reported by other groups (Yu et al., 1996). For their SJL/PLP EAE study they reported a 42% mortality rate. Of the 14 surviving animals with EAE approximately 36% went into chronic remission, while the remainder displayed either 1 relapse (36%) or 2 relapses (28%) in the 56 days following onset. The satellite study we reported on confirms that there is a physiological basis for the

heterogeneity in the group's behavioral phenotype. Both motor and sensory spinal transmission was significantly better in the subjects in chronic remission as compared to those that relapsed and remitted subsequent to the acute attack. .

Therefore, similar to MS, EAE is a phenotypically heterogeneous and evolving disease state. If you analyze the group data from the perspective of weeks after inoculation rather then by the neurological deficit phase the picture that emerges suggests a gradual recovery of physiological function. It seems unlikely that the cycle of transmission deficit and recovery is age-dependent since developmental differences were not observed in the age-matched controls. Therefore, it's possible that the time-dependent, gradual attenuation of the robust transmission deficits that we observed during early EAE is in reality a reflection of the fact that the groups physiological and behavioral state are most synchronized during the initial inflammatory phase of EAE which occurs during the acute attack.

In fact qualitative histopathological analysis of EAE lumbar spinal cord tissue generated in a separate in-house study (N = 5 mice / PI interval [collected 2x/week]; period = from PI 0- PI 56) indicates that there is a large time lag between peak leukocyte infiltration and demyelination following inoculation with PLP (personal communication). Based on the percentage of subjects affected as well as their overall infiltrate score, leukocyte infiltration appears to peak at 14-21 days post inoculation. Demyelination was detected at approximately 56 days post inoculation. Samples were not assessed beyond PI 56 so the time course for further changes is unclear beyond that point. Therefore it appears

that the transmission deficits that we observed for the whole group correspond with the time period when neuroinflammation was at its peak.

Overall these results suggest that inflammatory products produced during the initial phases of EAE are able to induce conduction block prior to the expression of neurodegenerative changes. Using a similar inoculation protocol Sobel et al. (1990) found that clinical disease onset occurred at between 10 and 15 days PI. Inflammatory infiltrates in CNS parenchyma occurred at between 7 and 10 days PI, and plateau by 14 days PI. The areas of parenchymal demyelination correlated with the neurological score at the time of death. Other investigators have demonstrated that during the acute attack neurological disability is tied to inflammatory changes while chronic disability is dependent upon axon loss (Wujek et al., 2002; Bjartmar et al., 2003).

The third major finding was that there was a decrease in the latency to AMPA-mediated sensory depolarization at 3 weeks post-inoculation. It appears that the latency to depolarize and the magnitude of depolarization are independent measures since the magnitude of motor but not sensory depolarization was decreased in EAE.

It may be possible to interpret the magnitude of depolarization as a reflection of the overall integrity of the conduction pathway and the latency to depolarize as the qualitative sum of the physiological attributes of conduction. Demyelinated axons have been reported to exhibit changes in both the distribution and the expression pattern of both sodium and potassium ion channels (England et al., 1991; England et al., 1996;

Rasband et al., 1998; Waxman, 2001a). These changes could impact conduction parameters (Waxman, 2000; Waxman, 2001a; Waxman, 2001b). The fact that alterations in EAE spinal cord depolarization latencies were detected by AMPA alone may be a function of KCl and veratridine's lack of selectivity for a specific transmitter pathway.

The overall aim of this study was to determine whether the neurological deficits observed in PLP-inoculated SJL mice would be paralleled by deficits in the motor and sensory transmission indices utilized in this study. In fact, both sensory and motor spinal cord transmission deficits were detected in EAE mice whether non-selective depolarizing agents such as potassium chloride and veratridine or a receptor selective agent such as AMPA was employed. While our data supports the hypothesized involvement of the glutamate system in the pathology of demyelinating diseases such as MS/EAE the fact that EAE also affected robust changes in the response to the sodium channel opener veratridine indicates that other system pathologies are involved. Based on both our preliminary qualitative analysis and the literature findings it appears that this methodology detects quantifiable changes in spinal cord transmission pathways during both the inflammatory period at the acute attack and the period of demyelination/axonal pathology occurring during chronic relapse/remission. Further studies will be needed to plot a quantitative time-course for PLP induced inflammation and axonal pathology and assess its impact on the expression of the neurological deficit and spinal cord transmission.

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Fig 1. Representative graph of the progression of the neurological symptoms (EAE) typically observed in SJL/J (H-2) mice inoculated with PLP 139-151 (Avg. +/- SEM: Vehicle EAE N=17; Vehicle Sham N=10). Typically the acute phase occurs at 2-3 weeks PI, followed by remission 1-week later.

Fig 2. Spinal cord depolarization elicited by motor and sensory application of veratridine (100 mM) in two behavioral subsets of EAE SJL/PLP Mice: chronically relapsing/remitting and remitting. Spinal cords were removed (PI 39-41) and assessed for motor and sensory transmission evoked by application of veratridine (10, 30, 100 mM). Subjects were divided into two behavioral subsets based on their neurological deficit profile as per established criterion (see Methods). Statistical analysis of the response to 100 mM veratridine using an unpaired t-test with Welch's correction revealed a significant difference between the behavioral subsets in terms of both motor (*$p<0.05$; $t=2.738$ $df=10$) and sensory (**$p<0.005$; $t=3.878$ $df=11$) transmission. [Panel A.: □ Motor Relapsing/Remitting (N=6); ■ Motor Remission (N=11); Panel B.: □ Sensory Relapsing/Remitting (N=4); ■ Sensory Remission (N=11).]

Fig. 3. Effect of the PI interval on the ability of AMPA to depolarize lumbar (panel A) and sacral (panel B) spinal cord tissue from SJL/J (H-2) PLP- EAE mice in a 2-compartment grease-gap bath. Score = Neurological Deficit (Avg. +/- SEM). Spinal cords were removed from both EAE and control (naïve, age-matched) mice at 2, 3, or 4

weeks PI. The DC potential between the two compartments was assessed in response to local application of AMPA (1.0, 10.0, 30.0 μM). All data are represented as the mean +/- SE of the group. Panel A: EAE mice displayed significant deficits in transmission mediated by lumbar application at both 2 weeks and 3 weeks PI (group effect: *$p<0.05$ ANOVA Naïve Control vs. EAE). . The transmission deficits were reversed by 4 weeks PI (group effect: †$p<0.05$ EAE 4 weeks PI vs. EAE 2 weeks PI and EAE 3 weeks PI). Panel B: Sacral application did not reveal transmission deficits. [Panel A: □ Control (score = 0); ▫ EAE 2 wks PI (score = 3.0 +/- 0.4); ▪ EAE 3 wks PI (score = 2.3 +/- 0.3); ■ EAE 4 wks PI (score = 1.2 +/- 0.2). Panel B.: □ Control (score = 0); ▫ EAE 2 wks PI (score = 2.9 +/- 0.4); ▪ EAE 3 wks PI (score = 2.4 +/- 0.3); ■ EAE 4 wks PI (score = 1.3 +/- 0.2).]

Fig. 4. Effect of the PI interval on the ability of KCl to depolarize lumbar (panel A) and sacral (panel B) spinal cord tissue from SJL/J (H-2) PLP-EAE mice in a grease-gap bath. Score = Neurological Deficit (Avg. +/- SEM). Spinal cords were removed from both EAE and control (naive, age-matched) mice at either 2 or 3 weeks PI. The DC potential between the two compartments was assessed in response to local application of KCl (0.30, 1.0, 3.0, 10.0 mM). All data are represented as the mean +/- SEM of the group data. Panel A.: EAE mice did not display a significant impairment in their response to

lumbar application of KCl. Panel B: EAE mice displayed significant deficits in KCl-mediated sacral depolarization at both 2 and 3 weeks PI (group effect: *p<0.05 ANOVA Naïve Control vs. EAE). [Panel A: □ Control (score = 0); ■ EAE 2 wks PI (score = 3.2 +/- 0.4); ■EAE 3 wks PI (score = 2.6 +/- 0.4: Panel B.: □ Control (score = 0); ■ EAE 2 wks PI (score = 3.3 +/- 0.4); ■ EAE 3 wks PI (score = 2.4 +/- 0.4).]

Fig. 5. Effect of the PI interval on the ability of veratridine to depolarize lumbar (panel A) or sacral (panel B) spinal cord tissue from SJL/J (H-2) PLP-EAE mice in a grease-gap bath. Score = Neurological Deficit (Avg. +/- SE). Spinal cords were removed from both EAE and control (sham, age-matched) mice at 1, 2, 3, 4 or 5 weeks PI. The DC potential between the two compartments of the grease-gap bath was assessed in response to local application of veratridine (10.0, 30.0, 100.0 μM). All data are represented as the mean +/- SEM of the group data. Panel A: EAE mice displayed significant deficits in lumbar depolarization at both 2 and 3 weeks PI. Panel B: EAE mice displayed significant deficits in sacral depolarization at 2 through 5 weeks PI. Significant recovery was observed between 2 and 5 weeks PI. [*p<0.05, **p<0.01; ***p<0.001 ANOVA sham Control vs. EAE (group effect); †p<0.05 ANOVA EAE 2 wks PI vs. 5 wks PI (group effect, sacral)]. Deficits were not observed at 1 week PI. [Panel A.: □ Control (score = 0); ▒ pre-onset EAE 1 wk PI (score = 0); ▒ EAE 2 wks PI (score = 2.6 +/- 0.2); ■ EAE 3 wks PI (score = 2.2 +/- 0.4); ■ EAE 4 wks PI (score = 2.4 +/- 0.3); ■ EAE 5

wks PI (score = 1.1 +/- 0.3); Panel B.: □ Control (score = 0); ▒ pre-onset EAE 1 wk PI (score = 0); ▒ EAE 2 wks PI (score = 2.5 +/- 0.2); ■ EAE 3 wks PI (score = 2.3 +/- 0.5); ■ EAE 4 wks PI (score = 2.4 +/- 023); ■ EAE 5 wks PI (score = 1.2 +/- 0.3).]

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Table I. AMPA (30.0 μM) Latency to Peak in EAE and Naive Mice

Drug Application					EAE
Region	**Group**	**Weeks PI**	**Avg. Sec. (+/- SEM)**	**N**	**Deficit Score**
					Avg. Score (+/- SEM)
Lumbar	Naïve	age matched for 2-4 wks PI	306 (+/- 15)	35	0
Lumbar	EAE	2 wks PI	255 (+/- 10)	10	3.0 (+/- 0.4)
		3 wks PI	290 (+/- 20)	26	2.3 (+/- 0.3)
		4 wks PI	289 (+/- 21)	13	1.2 (+/- 0.2)
Sacral	Naïve	age matched for 2-4 wks PI	365 (+/- 24)	37	0
Sacral	EAE	2 wks PI	303 (+/- 35)	10	2.9 (+/- 0.4)
		3 wks PI	268 (+/- 13)*	26	2.4 (+/- 0.3)
		4 wks PI	295 (+/- 22)	12	1.3 (+/- 0.2)

Table I. Latency to AMPA-mediated (30.0 μM) depolarization in EAE and naive mice. Spinal cords were removed from SJL/PLP-inoculated EAE and naïve (age-matched) mice at either 2, 3, or 4 weeks PI and placed in a grease-gap bath. The DC potential between the two compartments was assessed in response to local application of AMPA (1.0, 10.0, 30.0 μM). In this table the data are represented as the mean +/- SEM

of the group for the latency to reach the maximum level of depolarization from the point of application of the 30 μM concentration of AMPA. The latency to maximal sensory depolarization was significantly decreased at 3 weeks PI (*$p<0.05$).

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Table II. KCl(10.0 mM) Latency to Peak in EAE and Naïve Mice

Drug Application		**Weeks PI**			**EAE**
Region	**Group**		**Avg. Sec. (+/- SEM)**	**N**	**Deficit Score**
					Avg. Score (+/- SEM)
Lumbar	Naïve	age-matched for 2-3 wks PI	289 (+/- 11)	16	0
Lumbar	EAE	2 wks PI	294 (+/- 13)	9	3.2 (+/- 0.4)
		3 wks PI	345 (+/- 61)	11	2.6 (+/- 0.4)
Sacral	Naïve	age-matched for 2-3 wks PI	264 (+/- 7)	26	0
Sacral	EAE	2 wks PI	276 (+/- 11)	10	3.3 (+/- 0.4)
		3 wks PI	259 (+/- 11)	10	2.4 (+/- 0.4)

Table II. Latency to KCl-mediated (10.0 mM) depolarization in EAE and naïve mice. Spinal cords were removed from SJL/PLP-inoculated EAE and naïve (age-matched) mice at either 2 or 3 weeks PI and placed in a grease-gap bath. The DC potential between the two compartments was assessed in response to local application of KCl (0.30, 1.0, 3.0, 10.0 mM). In this table the data are represented as the mean +/- SEM of the group for the latency to reach the maximum level of depolarization from the

point of application of the 10.0 mM concentration of KCl. Induction of EAE did not affect the latency to maximal depolarization.

Table IIIA. Veratridine (100.0 μM) Latency to Peak in EAE and Sham Mice

Drug Application					EAE
Region	**Group**	**Weeks PI**	**Avg.Sec. (+/- SEM)**	**N**	**Deficit Score**
					Avg. Score (+/- SEM)
Lumbar	Sham	2 wks PI	501 (+/- 31)	20	0
		3 wks PI	519 (+/- 23)	19	0
		4 wks PI	464(+/- 35)	18	0
		5 wks PI	436 (+/- 25)	19	0
Lumbar	EAE	2 wks PI	411 (+/- 30)	21	2.6 (+/- 0.2)
		3 wks PI	570 (+/- 53)	8	2.2 +/- 0.4)
		4 wks PI	532 (+/- 52)	16	2.4 (+/- 0.3)
		5 wks PI	526 (+/- 42)	11	1.1 (+/- 0.3)

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Table III B. Veratridine (100.0 μM) Latency to Peak in EAE and Sham Mice

Drug Application		Weeks PI	Avg.Sec.(+/- SEM)	N	EAE Deficit Score
Region	Group				Avg. Score (+/- SEM)
Sacral	Sham	2 wks PI	449 (+/- 29)	20	0
		3 wks PI	453 (+/- 18)	19	0
		4 wks PI	478 (+/- 28)	20	0
		5 wks PI	451 (+/- 16)	19	0
Sacral	EAE	2 wks PI	458 (+/- 32)	16	2.5 (+/- 0.2)
		3 wks PI	507 (+/- 26)	7	2.3 (+/- 0.5)
		4 wks PI	428 (+/- 17)	17	2.4 (+/- 0.2)
		5 wks PI	532 (+/- 41)	13	1.2 (+/- 0.3)

Table III. Latency to veratridine-mediated (100.0 μM) depolarization in EAE and naïve mice. Spinal cords were removed from SJL/PLP-inoculated EAE and sham (age-matched) mice at between 2 and 5 weeks PI and placed in a grease-gap bath. The DC potential between the two compartments was assessed in response to local application of veratridine (10.0, 30.0, 100.0 μM). In this table the data are represented as the mean +/- SEM of the group for the latency to reach the maximum level of depolarization from the point of application of the 100.0 μM concentration of veratridine. Induction of EAE did not affect the latency to maximal depolarization.

Fig. 1

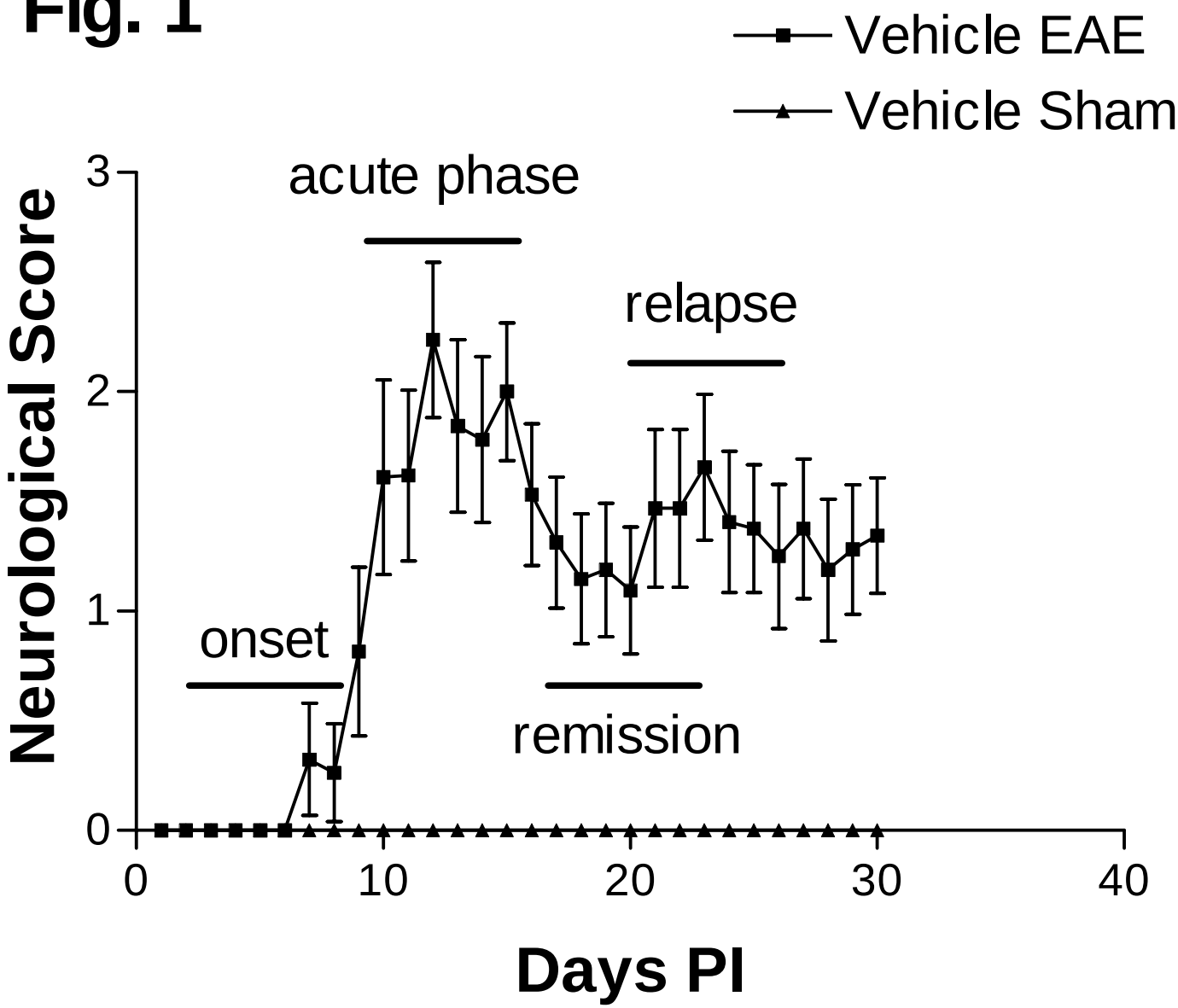
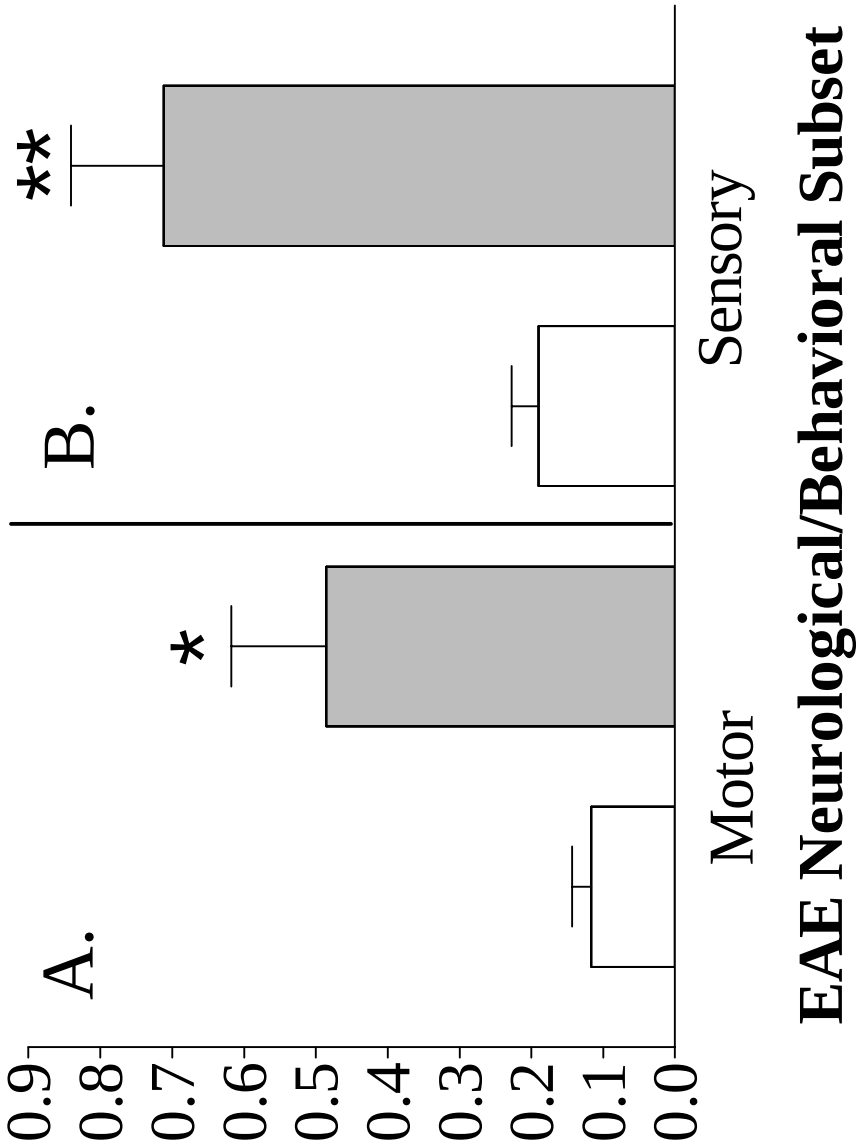


Fig. 2



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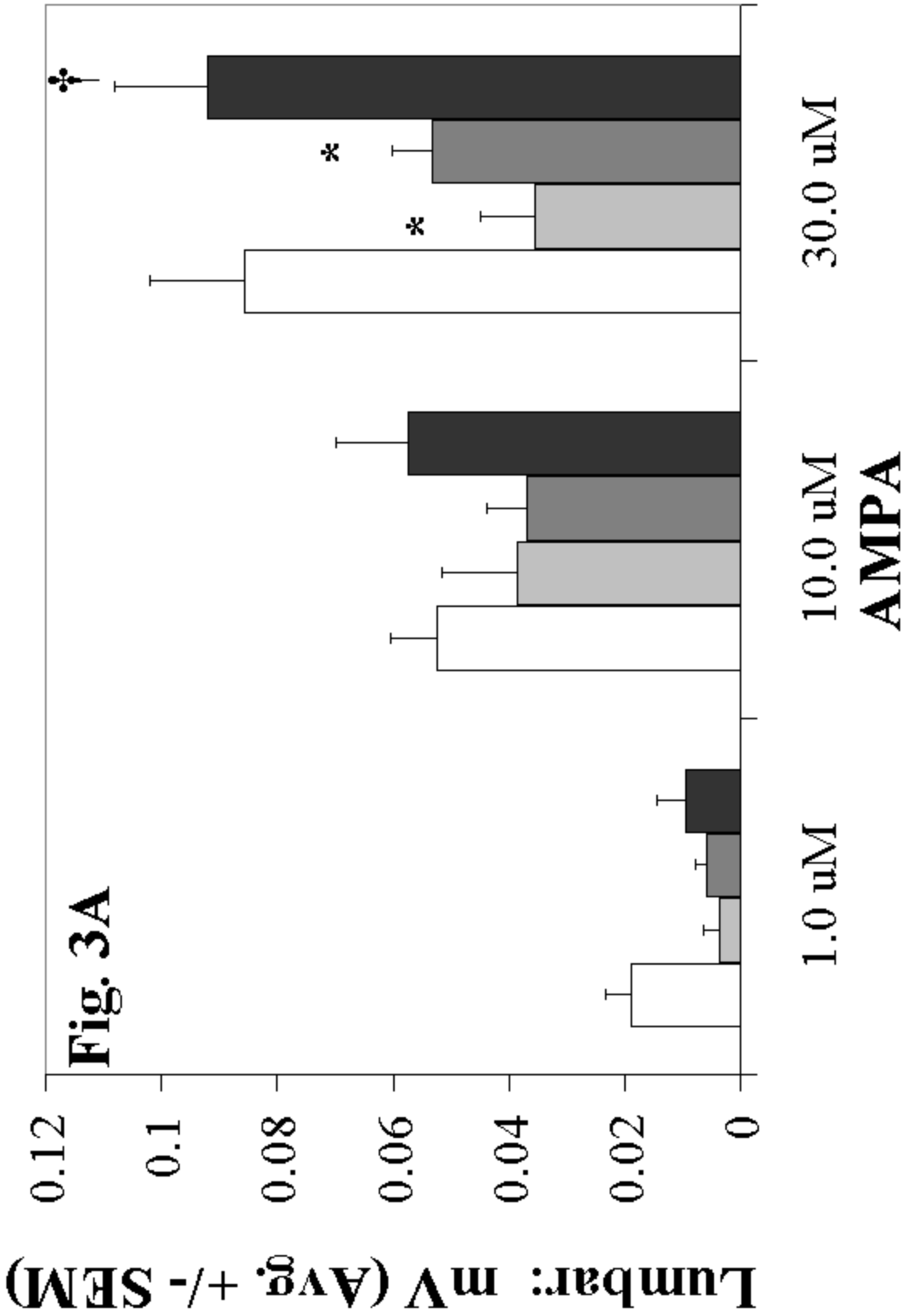

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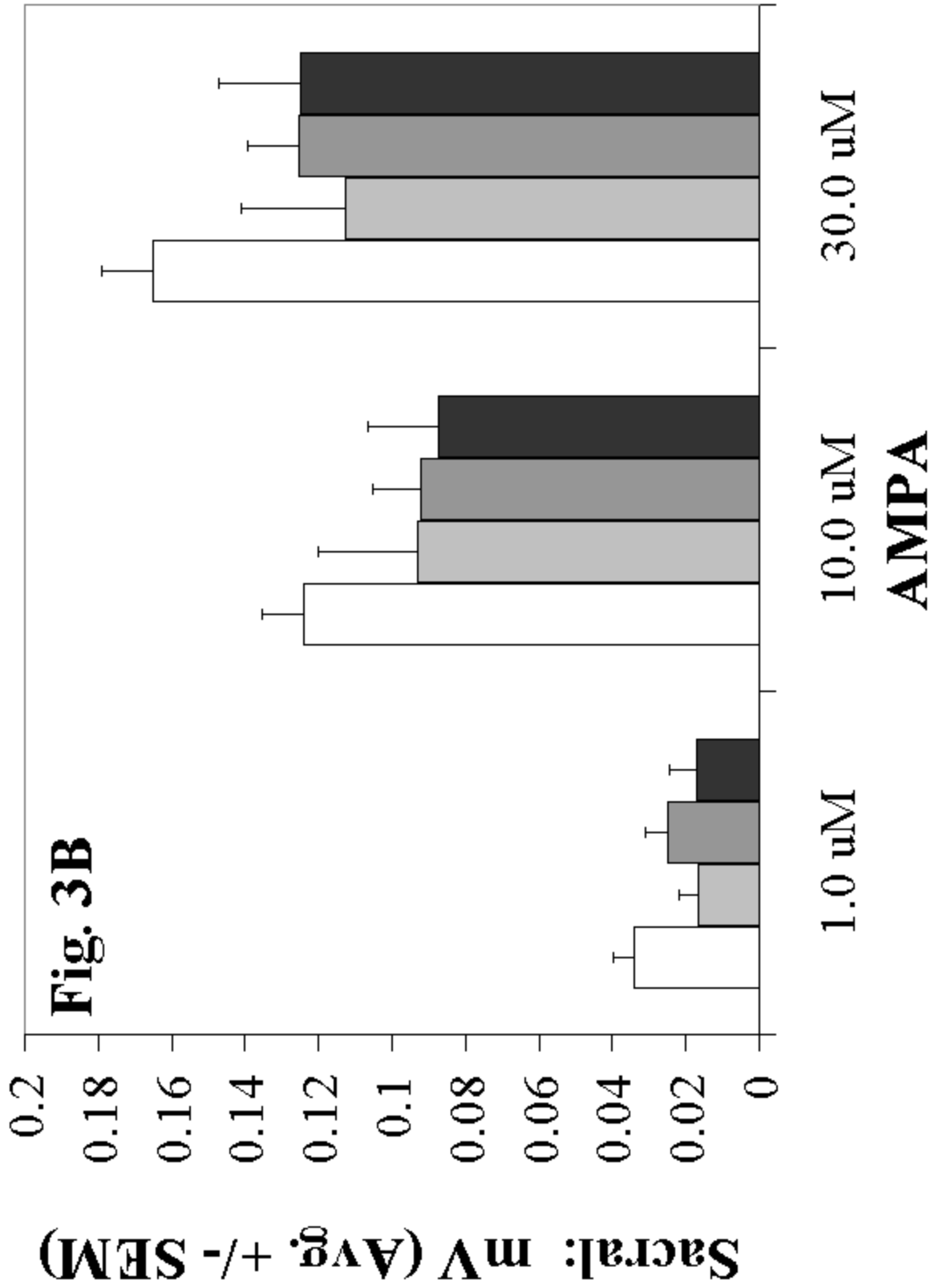

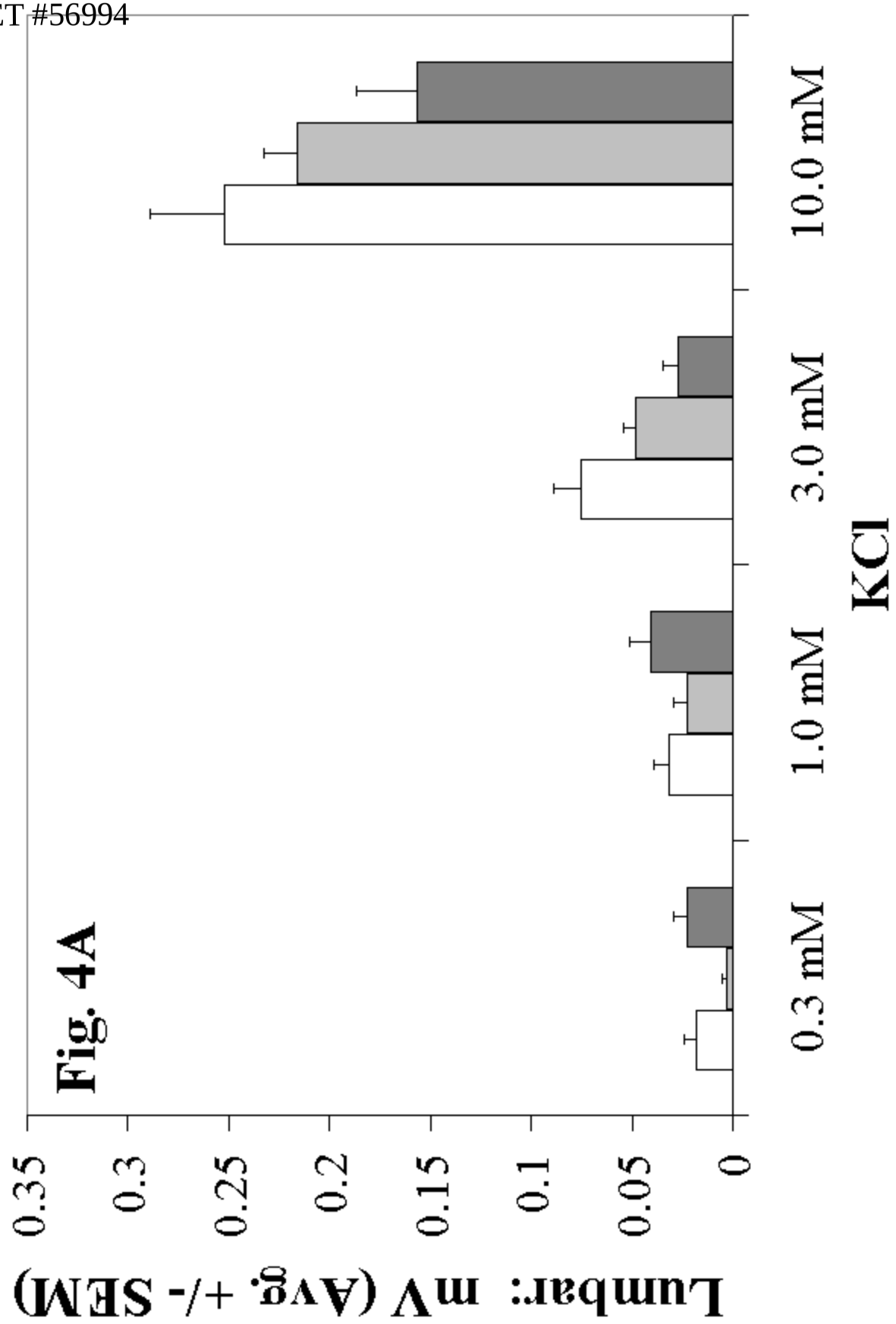

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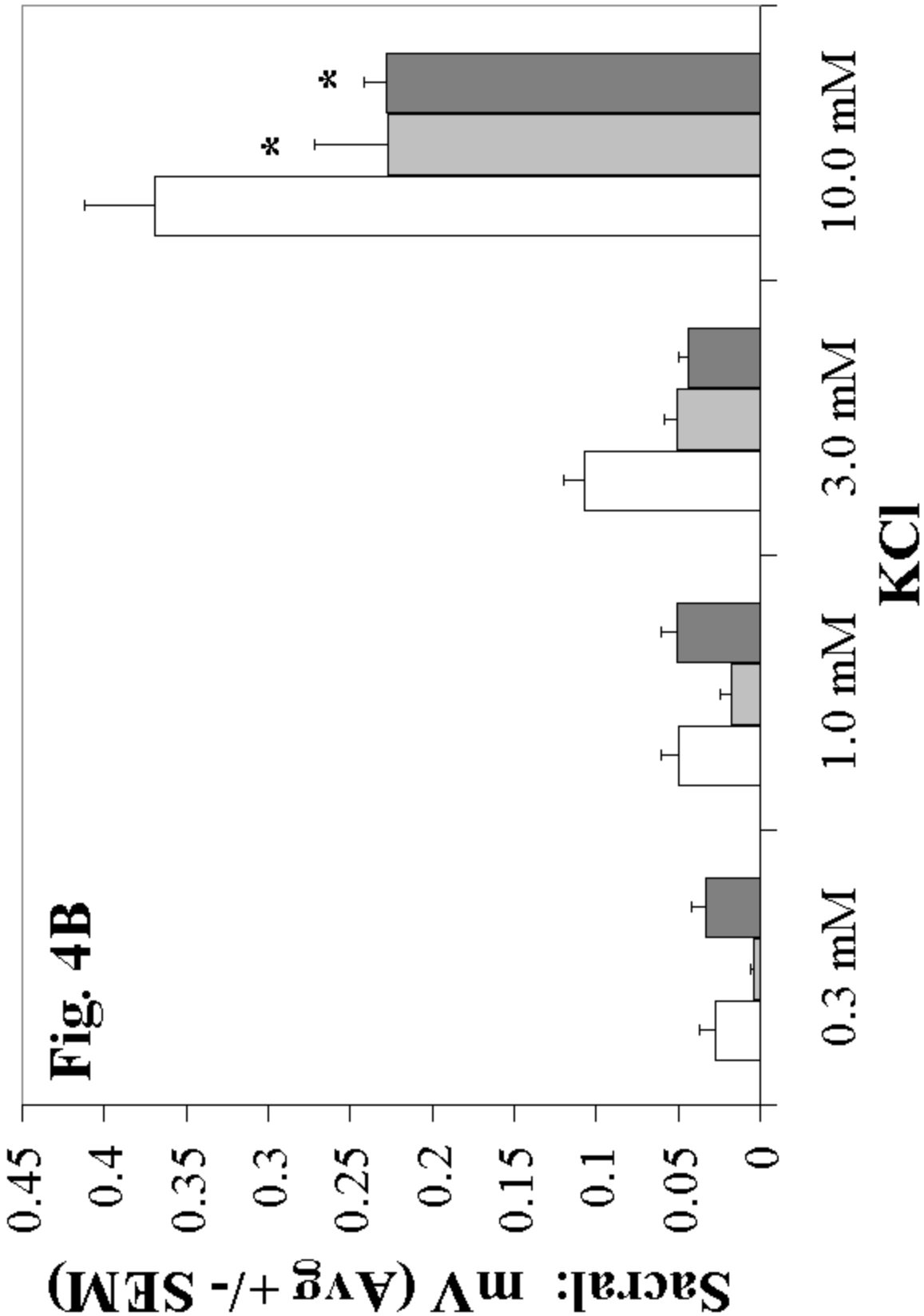

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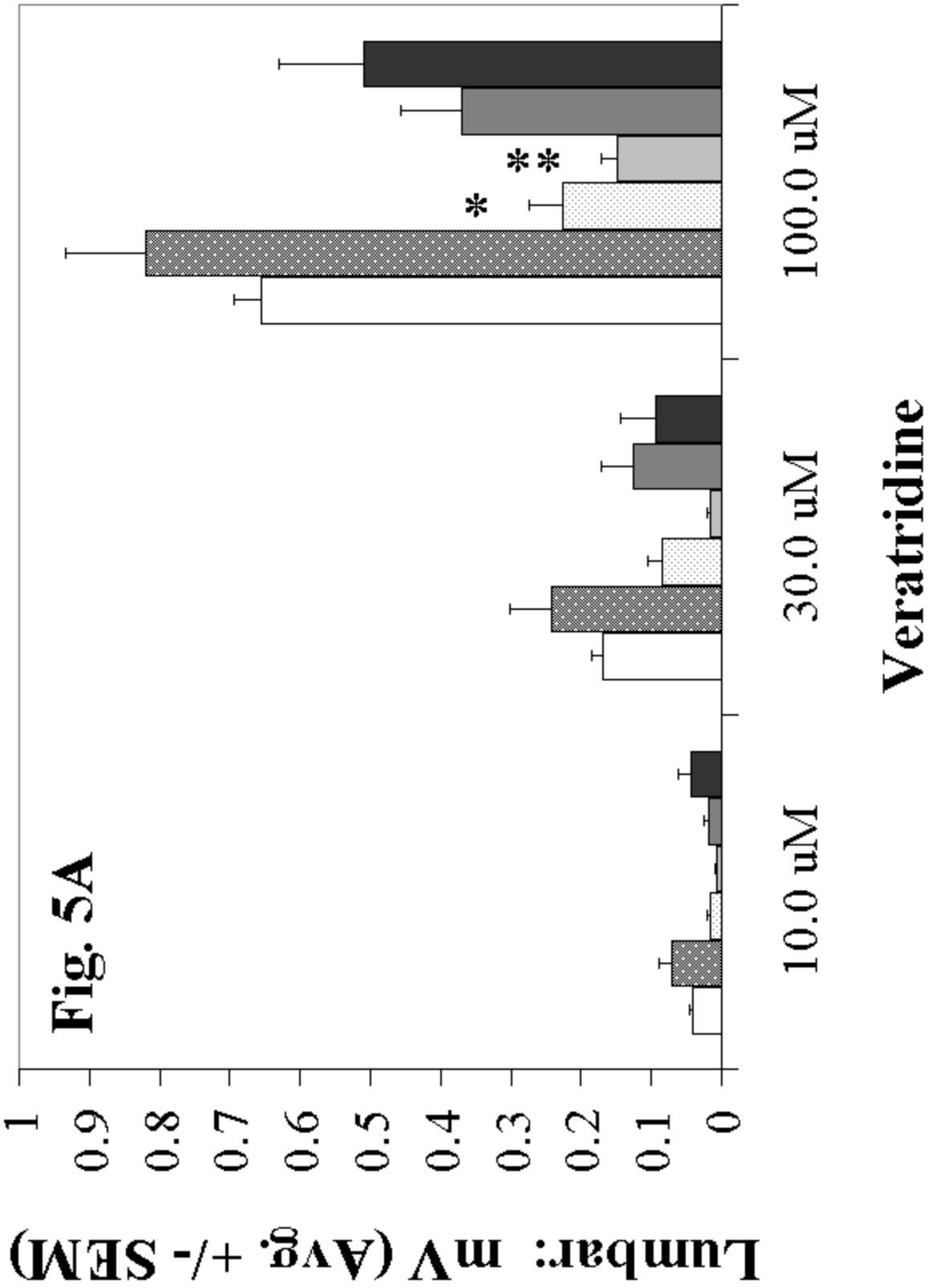

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