



(22) Date de dépôt/Filing Date: 2002/04/24

(41) Mise à la disp. pub./Open to Public Insp.: 2002/11/21

(62) Demande originale/Original Application: 2 445 612

(30) Priorité/Priority: 2001/04/24 (US60/286,200)

(51) Cl.Int./Int.Cl. *A61K 31/74* (2006.01),
A61K 31/765 (2006.01), *A61K 31/785* (2006.01),
A61P 35/00 (2006.01)

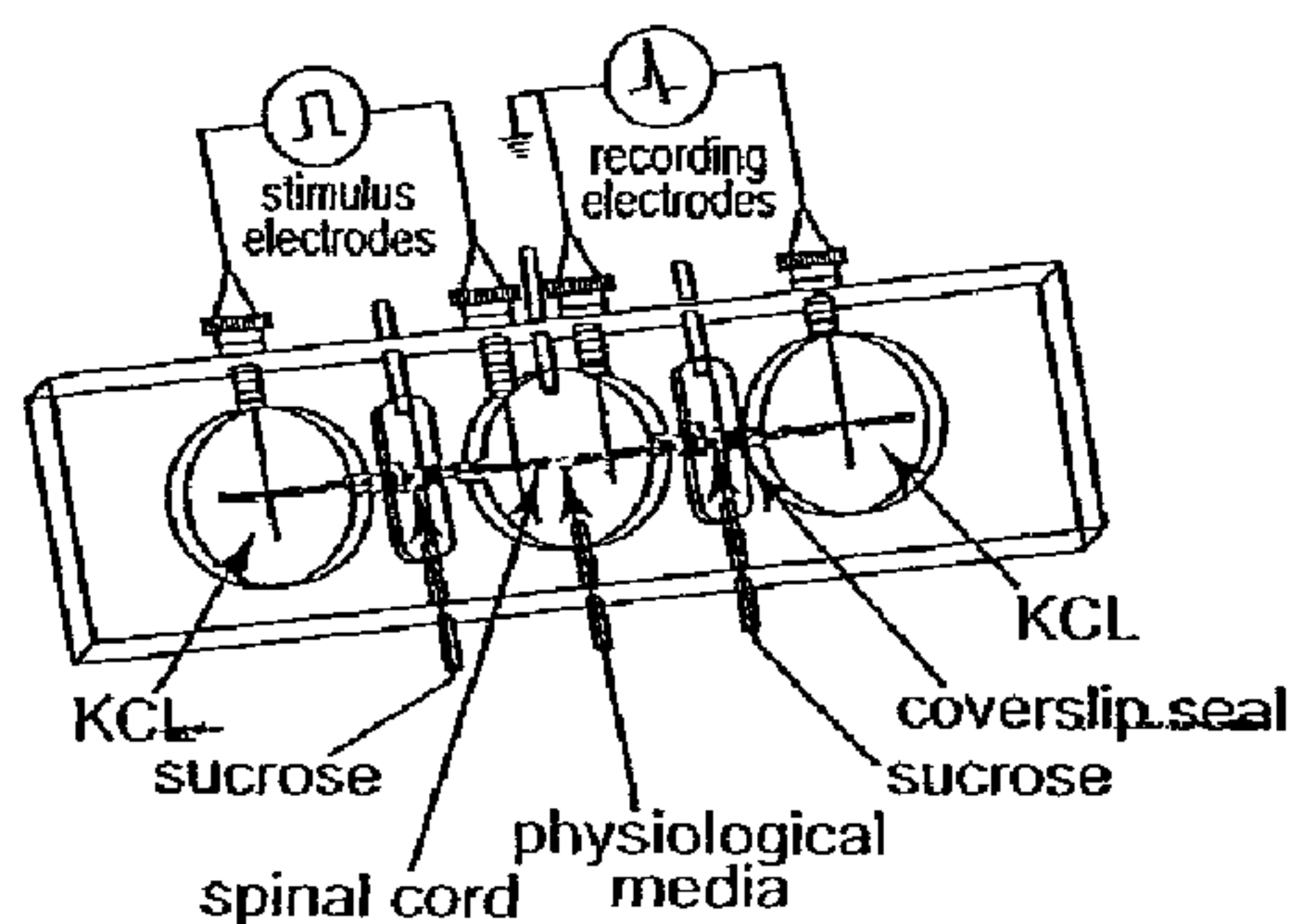
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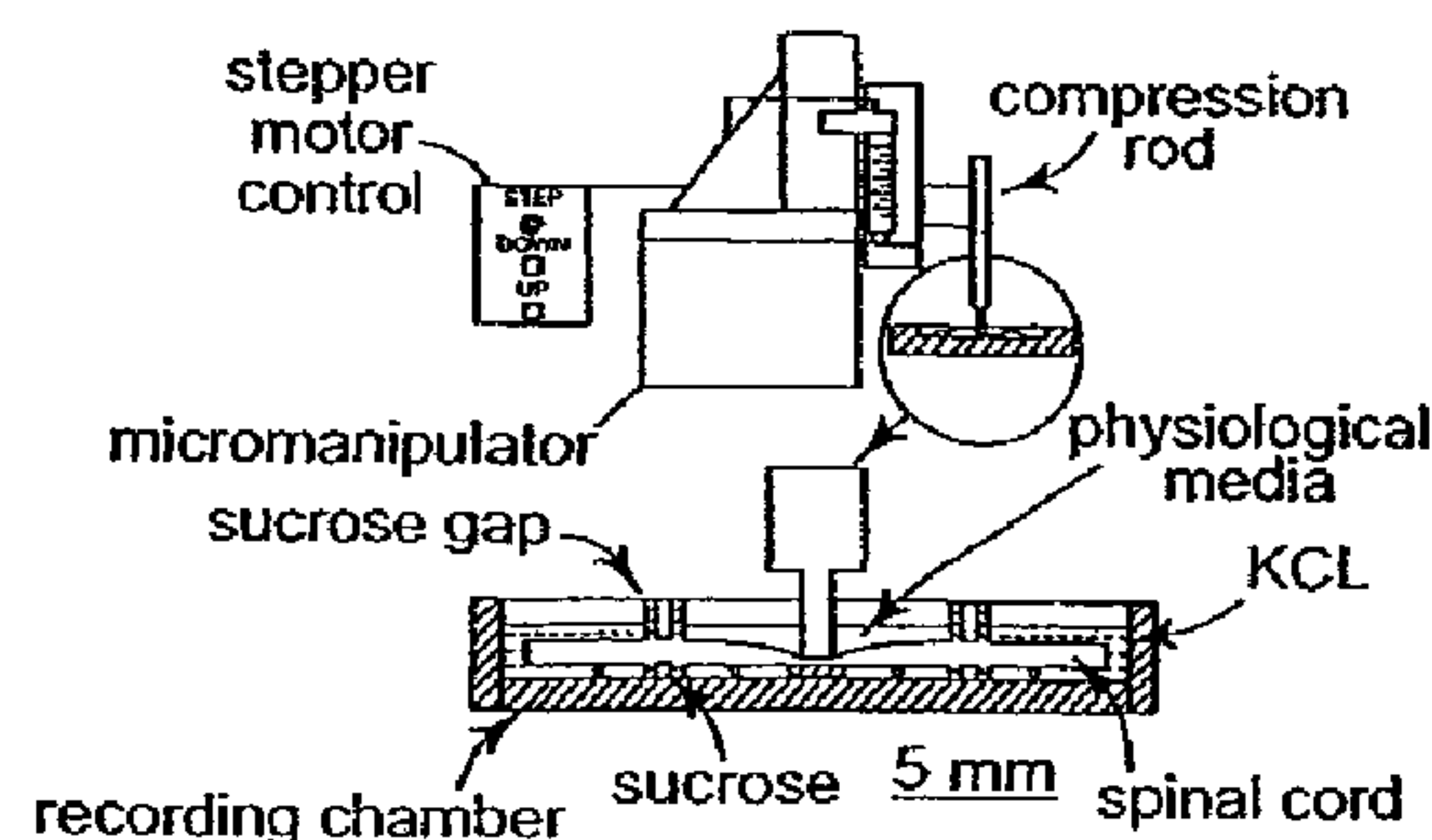
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(54) Titre : PROCÉDES ET COMPOSITIONS POUR TRAITER DES ENDOMMAGEMENTS DE TISSU NERVEUX
MAMMAIRE

(54) Title: METHODS AND COMPOSITIONS FOR TREATING MAMMALIAN NERVE TISSUE INJURIES



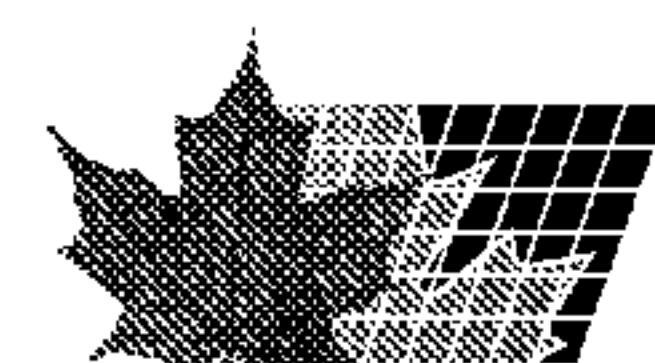
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(57) Abrégé/Abstract:

To achieve, an in vivo repair of injured mammalian nerve tissue, an effective amount of a biomembrane fusion agent is administered to the injured nerve tissue. The application of the biomembrane fusion agent may be performed by directly contacting



(57) **Abrégé(suite)/Abstract(continued):**

the agent with the nerve tissue at the site of the injury. Alternatively, the biomembrane fusion agent is delivered to the site of the injury through the blood supply after administration of the biomembrane fusion agent to the patient. The administration is preferably by parenteral administration including intravascular, intramuscular, subcutaneous, or intraperitoneal injection of an effective quantity of the biomembrane fusion agent so that an effective amount is delivered to the site of the nerve tissue injury.

ABSTRACT

To achieve, an *in vivo* repair of injured mammalian nerve tissue, an effective amount of a biomembrane fusion agent is administered to the injured nerve tissue. The application of the biomembrane fusion agent may be performed by directly contacting the agent with the nerve tissue at the site of the injury. Alternatively, the biomembrane fusion agent is delivered to the site of the injury through the blood supply after administration of the biomembrane fusion agent to the patient. The administration is preferably by parenteral administration including intravascular, intramuscular, subcutaneous, or intraperitoneal injection of an effective quantity of the biomembrane fusion agent so that an effective amount is delivered to the site of the nerve tissue injury.

METHODS AND COMPOSITIONS FOR TREATING MAMMALIAN NERVE TISSUE INJURIES

FIELD OF THE INVENTION

The present invention relates generally to methods for treating injured mammalian nerve tissue including but not limited to a spinal cord. Specifically, the invention relates to methods for treating injured nerve tissue through an in vivo application of a biomembrane fusion agent. Pharmaceutical compositions for treating an injured spinal cord are also described.

BACKGROUND OF THE INVENTION

Mechanical damage to the nervous system of mammals results in sometimes irreversible functional deficits. Most functional deficits associated with trauma to both the Peripheral Nervous System (PNS) or Central Nervous System (CNS) result from damage to the nerve fiber or axon, blocking the flow of nerve impulse traffic along the nerve fiber. This may be due to a physical discontinuity in the cable produced by axotomy. The blockage may also occur where the membrane no longer functions as an ionic fence, and/or becomes focally demyelinated [Honmou, O. and Young, W. (1995) Traumatic injury to the spinal axons (Waxman, S.G., Kocsis, J.D., Stys, P.K., Eds.): The Axon, New York: Oxford UP, pp 480-503; Maxwell, W.L. (1996): Histopathological changes at central nodes of ravier after stretch-injury, Microscopy Research and Technique, 34:522-535; Maxwell, W.L., Watt, C., Graham, D.I., Gennarelli, T.A. (1993): Ultrastructural evidence of axonal shearing as a result of lateral acceleration of the head in non-human primates, Acta Neuropathol, 86:136-144; Maxwell, W.L., Graham, D.I. (1997): Loss of axonal microtubules and neurofilaments after stretch-injury to guinea pig optic nerve fibers, J Neurotrauma, 14:603-614; Blight, A.R. (1993): Remyelination, Revascularization, and Recovery of Function in Experimental Spinal Cord Injury (Seil, F.J., Ed.): Advances in Neurobiology: Neural Injury and Regeneration, Vol. 59, New York, Raven Press, pp. 91-103]. In either case, functional deficits occur because of the break in nerve impulse conduction. Even the severe behavioral deficits associated with spinal cord injury is now understood to be largely due to the initial mechanical damage to white matter [Blight, A.R.: Morphometric analysis of a model of spinal cord injury in guinea pigs, with behavioral evidence of delayed secondary pathology, J. Neurolog. Sci., 103:156-171, 1991]. Delayed but progressive episodes of so-called "secondary injury" [Honmou and Young, W. (1995): Traumatic injury to the spinal axons (Waxman, S.G., Kocsis, J.D., Stys, P.K., Eds.): The Axon, New York: Oxford UP pp 480-503;

Young, W. (1993): Secondary injury mechanisms in acute spinal cord injury, J. Emerg. Med., 11:13-22.] subsequently enlarge the lesion leading to the typical clinical picture of a cavitated contused spinal cord, and intractable behavioral loss.

5 In the mammal, transection of the axon leads to the irreversible loss of the distal nerve process segment by Wallerian degeneration, while the proximal segment may survive. In the PNS, function may be restored by the endogenous regeneration of proximal segments down fasciculation pathways provided by both connective tissue and Schwann cell "tubes" which may persist for variable amounts of time post injury (Bisby, M.A. (1995): Regeneration of peripheral nervous system axons (Waxman, S.G., Kocsis, J.D., Stys, P.K., Eds.): The Axon Book, New York, The Oxford University Press, pp 553-578]. The level of the injury is critical to clinical fascicular repair however, as the rate of regeneration (about 1mm/day) may not be sufficient to avoid loss of target tissues dependent on its innervation (such as motor units in striated muscle). In the CNS, distal segments of nerve fibers do not regenerate, and their loss produces
10 nonfunctional "target" cells, which often require innervation to maintain their integrity. One ultimate strategy to enhance recovery from CNS injury is to induce or facilitate regeneration of white matter by various means.

 In the clinic, acute spinal cord transection is rare while compressive/contusive
20 mechanical damage is typical. In the PNS, transection, stretch injury as well as compression injury to nerve trunks are commonplace. However, severe, local, mechanical damage to any type of nerve fiber membrane may still initiate a process leading to axotomy and the irretrievable loss of distal segments. These events usually begin with a breakdown in the ability of the axolemma to separate and maintain critical differences in ions between the extracellular and intracellular
25 compartments -- in particular calcium.

 The devastating effects of injury to the mammalian spinal cord are not immediate. Severe mechanical injury initiates a delayed destruction of spinal cord tissue producing a loss in nerve impulse conduction associated with a progressive local dissolution of nerve fibers (axons)
30 [Honmou, O. and Young, W. (1995) The Axon (Waxman, S.G., et al., Eds.) pp. 480-529, Oxford University Press, New York; Griffin, J.W. et al. (1995) The Axon (Waxman, S.G., et al., Eds.) pp. 375-390, Oxford University Press, New York]. This loss of sensory and motor communication across the injury site can produce a permanent paralysis and loss of sensation in regions below the level of the spinal injury. Furthermore, it is clear the most damaging effects of
35 progressive "secondary injury" [Young, W. (1993) J. Emerg. Med. 11:13-22] of spinal cord

parenchyma relative to the loss of behavioral functioning is the effect it has on white matter. Localized mechanical, biochemical, and anoxic/ischemic injury to white matter may be sufficient to cause the failure of axolemmas to function as a barrier or fence to the unregulated exchange of ions [Honmou, O. and Young, W. (1995) *The Axon* (Waxman, S.G., et al., Eds.) pp. 480-529, Oxford University Press, New York]. This in turn compromises both the structural integrity of this region of the nerve fiber and its ability to conduct impulses along the cable. For example, elevated intracellular Ca^{2+} induces depolymerization of microtubules and microfilaments producing a focal destruction of the cytoskeleton [Griffin, J.W. et al. (1995) *The Axon* (Waxman, S.G., et al., Eds.) pp. 375-390, Oxford University Press, New York; Maxwell, W.L., et al. (1995) *J. Neurocytology* 24:925-942; Maxwell, W.L., et al. *J. Neurotrauma* 16:273-284].

The unrestricted movement of Ca^{++} down its electrochemical gradient into the cell leads to a destruction of membranes and the cytosol, and is an initial key event in all mechanical injury to nerve fibers as well as other ischemic injuries such as head injury and stroke [Borgens, R.B., Jaffe, L.F., Cohen, M.J. (1980): Large and persistent electrical currents enter the transected spinal cord of the lamprey eel, *Proc. Natl. Acad. Sci. U.S.A.*, 77:1209-1213; Borgens, R.B. (1988): Voltage gradients and ionic currents in injured and regenerating axons, *Advances in Neurology*, 47: 51-66; Maxwell, W.L. (1996): Histopathological changes at central nodes of ravier after stretch-injury, *Microscopy Research and Technique*, 34:522-535; Maxwell, W.L., Graham, D.I. (1997): Loss of axonal microtubules and neurofilaments after stretch-injury to guinea pig optic nerve fibers, *J. Neurotrauma*, 14:603-614; Maxwell, W.L., Watt, C., Graham, D.I., Gennarelli, T.A. (1993): Ultrastructural evidence of axonal shearing as a result of lateral acceleration of the head in non-human primates, *Acta Neuropathol*, 86:136-144; Honou and Young, 1995, Lee et al., 1999; Stys et. al., 1990]. Na^+ enters the localized region of the membrane insult as well, depolarizing the membrane and facilitating the release of intracellular Ca^{++} stores [Carafoli, E., Crompton, M. (1976): *Calcium ions and mitochondria* (Duncan, C.J., Ed.): Symposium of the Society for Experimental Biology: Calcium and Biological Systems, Vol. 30, New York, Cambridge University Press, pp. 89-115; Borgens, R.B., Jaffe, L.F., Cohen, M.J. (1980): Large and persistent electrical currents enter the transected spinal cord of the lamprey eel, *Proc. Natl. Acad. Sci. U.S.A.*, 77:1209-1213; 1988; Borgens, R.B. (1988): Voltage gradients and ionic currents in injured and regenerating axons, *Advances in Neurology*, 47: 51-66]. Potassium exodus also pushes the resting potential of the membrane towards the Nernst potential for K^+ contributing to the localized region of inexcitability and blockage of nerve impulse conduction down the cable in even intact membranes. Thus, when K^+ rushes down its

electrochemical gradient out of the cell, the resultant elevated extracellular concentration contributes to localized conduction block [Honmou, O. and Young, W. (1995) *The Axon* (Waxman, S.G., et al., Eds.) pp. 480-529, Oxford University Press, New York; Shi, R. et al., (1997) *Society for Neuroscience Abstracts*, 108:16]. However it is the progressive chain
 5 reaction of events set in motion by Ca^{++} entry into the cell that initially leads to progressive dissolution of the axon – aided in later stages of the acute event by additional complex molecular processes such as the initiation of lipid peroxidation pathways and formation of “free radical” oxygen metabolites.

10 There are several classes of molecules that have already been shown to be able to seal cell membranes or to actually fuse membranes together [Nakajima, N., Ikada, Y. (1994): Fusogenic activity of various water-soluble polymers, *J. Biomaterials Sci., Polymer Ed.*, 6:751-9]. These biocompatible polymers can also resolve discontinuities in the plane of the membrane into an unbroken plasmalemma, and/or become inserted into the membrane defect, sealing it and
 15 reversing permeabilization.

For over thirty years polyethylene glycol (PEG) has been known to fuse many cells together to form one giant cell. Application of this hydrophilic macromolecule has been exploited to form multicellular conjugates for the purpose of exchanging genetic material,
 20 hybridoma formation, or as a model for endogeneous vesicle fusion [Davidson, R.L., O'Malley, K.A., Wheeler, T.B. (1976): Induction of mammalian somatic cell hybridization by polyethylene glycol, *Somat. Cell Genet.*, 2:271-280; Lee, J., Lentz, B.R. (1997): Evolution of lipid structures during model membrane fusion and the relation of this process to cell membrane fusion, *Biochemistry*, 36:6251-6259; Lentz, B.R. (1994): Induced membrane fusion; Potential
 25 mechanism and relation to cell fusion events, *Chem. and Phys. of Lipids*, 73: 91-106]. PEG has also been used to fuse many pheochromocytoma cells (PC-12; neuron like cells) together to produce large single units facilitating neurophysiological measurements in vitro as well as fusing the severed ends of single invertebrate giant axons in vitro [O'Lague, P.H., Huttner, S.L. (1980): Physiological and morphological studies of rat pheochromocytoma cells (PC12) chemically fused
 30 and grown in culture, *Proc. Nat. Acad. Sci. USA*, 77:1701-1705; Krause, T.L., Bittner, G.D. (1990, 1991): Rapid morphological fusion of severed myelinated axons by polyethylene glycol, *PNAS*, 87: 1471-1475].

Methods and compositions for treating mammalian spinal cord injuries are needed. The
 35 present invention addresses these needs.

SUMMARY OF THE INVENTION

The present invention is directed to methods and compositions for the in vivo repair of injured mammalian nerve tissue. The invention is more particularly directed to a composition containing an effective amount of a biomembrane fusion agent (see Definitions section below) to be delivered to the site of an injury (see Definitions section below) to nerve tissue, particularly nerve tissue of the spinal cord or the peripheral nervous system. The biomembrane fusion agent may be directly contacted with the nerve tissue at the site of the injury or may be administered to the patient parenterally. Preferably, the biomembrane fusion agent is of such an amount that its delivery to the site of the injury through the blood supply after injection of the biomembrane fusion agent into the patient is effective to repair injured nerve fibers. The injection may be an intravascular, intramuscular, subcutaneous, or intraperitoneal injection of an effective quantity of the biomembrane fusion agent so that an effective amount is delivered to the site of the nerve tissue injury.

Preferably, the biomembrane fusion agent takes the form of a hydrophilic polymer in the form of a polyalkylene glycol or oxide such as a polyethylene glycol, a polyethylene glycol/polypropylene glycol block copolymer such as ethylene oxide-propylene oxide-ethylene oxide (EPAN), or another hydrophilic biocompatible surfactant such as dextrans. The surfactant is preferably nonionic and may take the form of an amphipathic polymer such as a poloxamine. Most preferably, the biomembrane fusion agent is polyethylene glycol (PEG) ($\text{H}(\text{OCH}_2\text{CH}_2)_n\text{OH}$), where n preferably ranges from 4 to about 570 or more, more preferably about 30 to about 100. PEG is used as a solvent for many compounds used in medicine. For example, PEG is used as a carrier for contrast media used in radiology, and a solvent for hemopoietic factors infused into hemophilic patients. A suitable alternative is a poloxamer (see Definitions section below). Some of these triblock polymers consist of PEG polymers with a propylene glycol core. The sizes of the individual polymeric chains are not critical to the action of the poloxamer, and the poloxamer can also be injected into the blood stream or applied topically in the same manner as PEG. (Poloxamers are also amphipathic polymers to a greater or lesser extent depending on the relative numbers of ethylene glycol and propylene glycol groups.)

In the development of the present invention, the distribution of a biomembrane fusion agent, more particularly, PEG, in animals with spinal cord injuries was traced and it was found that PEG specifically targets the hemorrhagic injury in spinal cord following any means of

introducing it to the blood supply (for example, parenterally such as intravenous, subcutaneous, or intraperitoneal injection, transdermally, orally, through buccal administration or via another route of administration). Furthermore, PEG appears to more uniformly bathe the injury site when delivered by the blood supply than when it is applied to the injury directly. In testing the application or administration of PEG to spinal cord injured guinea pigs, it has been observed that the recovery of functions (both in nerve impulse conduction through the spinal cord injury and behavioral recovery) has been identical to that previously determined in response to topical (direct) application of PEG to the site of nerve tissue injury.

This is a dramatic and unexpected finding. A single dose of a biomembrane fusion agent such as PEG in aqueous solution administered beneath the back skin (subcutaneous injection) will reverse many functional deficits in severe or traumatic spinal cord injuries in guinea pigs when the dose is administered up to six (6) to eight (8) hours post injury. The PEG migrates to and selectively attaches to the site of a mammalian nerve tissue injury and functions there as a biomembrane fusion agent.

Tests show that the application or administration of a biomembrane fusion agent such as PEG to severe spinal cord crush/contusion injuries in situ produces functional recovery of an identified spinal cord mediated behavior in test mammals as well as a rapid recovery of recorded nerve impulses ascending the spinal cord through the original lesion. These physiological and behavioral recoveries following severe spinal cord injury in the test mammals are not temporary but rather stable, even improving with the passage of time. Moreover, the application of a biomembrane fusion agent such as PEG can be delayed for at least 8 hours after spinal cord injury without a loss in its effectiveness.

Accordingly, the present invention contemplates a method and a composition for treating injured mammalian, preferably human, nerve tissue wherein an effective amount of a biomembrane fusion agent exemplarily including a hydrophilic polymer such as a polyalkylene glycol (or oxide), or block copolymers and mixtures thereof, or a biocompatible surfactant such as a nonionic amphipathic polymer (e.g., a poloxamer or a poloxamine), or mixtures thereof, is administered to a patient for delivery to the nerve injury site via the patient's vascular system. Preferably, the treatment includes an injection of the biomembrane fusion agent into a patient parenterally, including intravascularly, intramuscularly, subcutaneously, intraperitoneally, or through any other path which results in a delivery of the biomembrane fusion agent to the site of the injury via the vascular system.

Where the biomembrane fusion agent is a polyalkylene glycol, it can preferably and particularly take the form of C₁ to C₁₀ polyalkylene glycol such as polymethylene glycol, polyethylene glycol, polypropylene glycol, polybutylene glycol, polypentylene glycol, polyhexylene glycol, polyheptylene glycol, polyoctylene glycol, polynonylene glycol, and polydecylene glycol, including branched and structural isomers thereof. The biomembrane fusion agent may more generally take the form of any mixture of acceptable individual agents, such as mixtures of two or more polyalkylene glycols, including branched and structural isomers thereof, mixtures of polyalkylene glycols with block copolymers of polyalkylene glycols, and mixtures of block copolymers of polyalkylene glycols. The use of polyethylene glycol, polypropylene glycol and polyethylene glycol polypropylene glycol block copolymers (e.g., poloxamer 188) are particularly preferred for use in the present invention, with polyethylene glycol being most preferred. In some applications, administration is facilitated by using a biomembrane fusion agent having a reduced viscosity, e.g., reduced relative to room-temperature viscosity by heating. Polyethylene glycol polypropylene glycol block copolymers (e.g., poloxamer) appear to have an acceptably low viscosity. However, it is clear that a suitably low viscosity may be attained by selecting a low-molecular-weight molecule as the biomembrane fusion agent and injecting the agent after heating the agent to a permissibly elevated temperature.

In one form of the invention, a method and a composition for treating an injured mammalian spinal cord also involves directly or indirectly (by any route of administration including through the vascular system) administering an effective amount of a potassium channel blocker to the site of nerve tissue damage, together with an effective amount of a selected biomembrane fusion agent. The potassium channel blocker can be, for example, an amino-substituted pyridine, such as 4-aminopyridine.

Yet other aspects of the invention provide compositions for treating an injured mammalian nervous system, such as an injured mammalian spinal cord, that include effective amounts of a biomembrane fusion agent and optionally a potassium channel blocker as described above. It has been unexpectedly found that such compositions synergistically treat a damaged spinal cord.

Where the biomembrane fusion agent takes the form of polyethylene glycol, it is administered in an effective amount and preferably within the dosage range of about 15 to 50 mg of PEG per body weight of the patient in kilograms where the PEG has a weight of about 1500

to 4000 Daltons. The fusion agent is preferably administered in combination with a pharmaceutically acceptable carrier, additive or excipient, more preferably in a sterile injectable saline such as lactated Ringer's solution or any other IV "fluids" commonly administered after trauma as a treatment for shock and/or blood loss. Any polyalkylene copolymer having a safe clinical use as an injectable treatment in other contexts is suitable for use in a method for treating injured nerve tissue in accordance with the present invention.

Where the fusion agent is poloxamer, a polyethylene – polypropylene – polyethylene block copolymer, or a poloxamine, it is administered preferably in an isotonic sterile saline such as a lactated Ringer's solution, USP sterile isotonic saline solution, Krebs's solutions, or other IV "fluids" solution at fusion agent dosages of 50 – 150 mg / kg of the patient's body weight, for instance, about 100 mg/kg of body weight. The aqueous solution is prepared in such a way that the injection is approximately 1 cc. Poloxamers are preferably accompanied by a potent antioxidant. For instance, 0.4 g of a natural antioxidant, Vitamin C, may be added to the stock solution of 350 mg/Kg P188. Any nonionic surfactant or amphipathic polymer having a safe clinical use as an injectable treatment in other contexts is suitable for use in a method for treating injured nerve tissue in accordance with the present invention.

The methodology of the present invention will permit a physician or medical practitioner (e.g., neurosurgeon) to physically and functionally reconnect transected nerve cell processes (axons), as well as immediately rescue crushed nerve processes that would otherwise progress on to axotomy and the irreversible loss of the distal axonal segment. This result is surprising. The methodology of the present invention is unexpected and dramatic for at least four more significant reasons:

1) A biomembrane fusion agent as disclosed herein can be delivered by tuberculin syringe and a fine (26 gauge) needle inserted just under the sheath of peripheral nerves near the site of crush or stretch and/or by IV injection. This operation has been performed with PEG and poloxamer in adult guinea pigs with focal crush injuries to the sciatic nerve of the leg. Observations revealed very rapid recoveries (minutes to 1 hour) of nerve impulse conduction through the injury and recoveries of muscle function in the lower leg (originally extinguished by the crush of the relevant nerve).

2) Administration of a biomembrane fusion agent through the blood supply of a patient with injured nerve tissue relieves the attending neurosurgeon of the absolute requirement to

surgically expose the site of the nerve tissue injury, for instance, to remove the tough covering of the spinal cord (the dura), before a topical application of the fusion agent is made.

3) Introduction of biomembrane fusion agents through the blood supply enormously facilitates the time in which these agents could be delivered clinically. The fusion agents can be delivered as a component of IV fluids that are standardly begun even at the accident site minutes to hours after injury.

4) Introduction of a biomembrane fusion agent such as PEG and/or poloxamer through the vasculature (blood supply) also enables the use of this therapy in cases of severe head injury, as well as cerebral hemorrhage (stroke). These traumas would not have been accessible to the topical application and removal of fusion agent solutions, but are perfectly accessible to the treatment by IV injection through the normal IV fluids continuously delivered to trauma patients. Head injury and stroke are hemorrhagic events identical to spinal cord injury in that cells in these regions of the brain begin to undergo dissolution and death after they become permeabilized by even a temporary restriction of blood supply. The breaches in the membranes of the nerve cells can be molecularly sealed and the cells rescued by fusion agent application just as in spinal cord trauma.

An injection of a biomembrane fusion agent pursuant to the present invention should be made as soon as possible after a severe injury to the central nervous system. Since the biomembrane fusion agent is delivered via the blood stream, this methodology can be used to treat any form of traumatic damage to the peripheral nervous system (crush or injury where nerve fibers are not completely severed), any form of damage to the spinal cord where the cord itself is not severed into two pieces, any type of traumatic damage to the brain such as blunt force trauma or concussion, and stroke or cerebral aneurysms.

It is therefore an object of the invention to provide methods and compositions for treating a mammalian nerve tissue damage to at least partially restore nerve function.

These and other objects and advantages of the present invention will be apparent from the descriptions herein.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1A-1 B depict experimental apparatuses used in studies described herein. FIG. 1A depicts a top view of the double sucrose recording chamber. In FIG. 1A, from left to right, the first large compartment contains 120 mM KCl, the central large compartment contains the physiological test solutions, such as oxygenated Krebs' solution, and the third compartment also contains 120 mM KCl. The small chambers on either side of the central compartment contain 230, mM sucrose. Seals fashioned from coverslips are secured in place with high vacuum silicone grease at the locations shown to inhibit the exchange of the various media from one compartment to the next. AgAgCl electrodes for recording and stimulation are in series with socket connectors at the locations shown. In the top portion of FIG. 1B, a side view of the apparatus used to produce a standardized crush to the isolated spinal cord at its midpoint within the central compartment is shown. The position of the spinal cord injury within the central chamber is shown in the lower portion of FIG. 1B. The apparatuses are further described below.

FIGS. 2A-2B depict electrophysiological recordings showing compound action potentials (CAPs) of control, and PEG/4-AP treated spinal cords. In FIG. 2A, untreated spinal cord strips were treated with 100 μ M 4-AP at 1 hour post-injury. In FIG. 2B, 100 μ M 4-AP was administered 1 hour post-PEG application. FIG. 2C is a bar graph of group data showing percent amplitude increase for 5 control and 5 PEG-treated spinal cords.

FIG. 3 depicts a proposed mechanism of the synergistic effect of PEG and 4-AP as more fully described in Example 1. The membrane lesion obtained by mechanical compression is depicted by holes. Small arrowheads represent potassium channels.

FIG. 4 depicts an experimental setup used in the examples. Nerve impulse pathways were interrupted by crushing the spinal cord in the midthoracic region (red circuit). A control procedure demonstrated that a failure to detect SSEPs was due to a failure of ascending nerve impulse conduction through the lesion by stimulation of a neural circuit unaffected by the injury.

FIG. 5 depicts a surgical exposure performed on the sciatic nerve of a test mammal and shows the branches (which are cut – see methods) of the sciatic nerve and the gastrocnemius muscle. Note the position of the two transducers, one measuring the force of muscle contraction, the other the displacement of the hind paw. The relative position of the hook electrodes

stimulating the sciatic nerve proximal to its insertion on the gastrocnemius is shown as is the placement of bipolar disc electrodes on the muscle to record the spread of APs in response to stimulation. All records are acquired simultaneously on three channels of recording equipment, a fourth channel being used to display an event marker triggered by the stimulation pulse. For
5 illustration purposes only, the drawing is not made to scale.

FIGS. 6A-6D are four photographic representations showing polyethylene glycol labeling in crushed guinea pig spinal cord. In FIGS. 6A-6D, the distribution of FI-PEG in crushed spinal cord is shown using three types of application. The application of PEG was made within 1/2
10 hour of the constant displacement crush injury, and evaluated by fluorescent microscopy of 50µm thick frozen cross sections about 24 hours later. In FIG. 6A, a typical control section is shown in darkfield - the image digitally enhanced to reveal the very faintly labeled spinal cord. Such uninjured control sections were obtained by harvesting a segment of the spinal cord at least 3-4 vertebral segments from the injury site. Note the characteristic labeling of PEG in uninjured
15 spinal cord at the level of detection. The arrows point to weakly labeled regions of vasculature in the gray matter and at the pial surface. FIG. 6B shows strong labeling of PEG at the epicenter of the crush produced by a 2-minute topical application of PEG to the lesion as in previous reports. Arrows point to relatively unlabeled central regions of this injury. In FIGS. 6C and 6D, heavy FI-PEG labeling is shown associated with subcutaneous and intravenous injection
20 respectively. In FIG. 6C, the arrow points to a cyst forming around the swollen central canal. Note the extensive labeling of only the injury site by all methods. The scale bar = 500µm

FIGS. 7A and 7B are graphs of electrical records showing loss and recovery of conduction in crushed guinea-pig sciatic nerves after administration of PEG. The first electrical
25 record at the top of both FIGS. 7A and 7B shows a typical SSEP recording in response to tibial nerve stimulation. Note the early and late arriving evoked potentials (P1 and P2) in the intact spinal cord, and their immediate elimination by the spinal cord injury. Though not shown for every record, the median nerve control procedure was performed any time an SSEP was not recorded, demonstrating the failure to record CAPs was due to the injury. In FIG. 7A, a typical
30 set of records is shown for one control animal to the 1 month time point when the study was concluded. Note the complete lack of SSEP conduction and the robust Median nerve induced SSEP. In FIG. 7B, a typical set of electrical records for a PEG-treated animal is shown. Note the elimination of the tibial nerve derived SSEP by the spinal cord injury, and the positive median nerve control procedure performed at the same recording time. Before the end of the
35 first day post-injury, SSEP conduction was restored by this subcutaneous PEG injection made 6

hours after the injury. Recovered evoked potentials continued to improve in amplitude and latency during the next month of observation, and in no case were recovered SSEPs lost after their recovery. The insert displays the amplitude and time base for all records except median nerve stimulations, which were recorded at $\frac{1}{2}$ this sensitivity, but using the same time base.

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FIGS. 8A – 8C are tracings of captured and superimposed video images of a guinea pig during a period of CTM stimulation with a monofilament probe, showing behavioral recovery following subcutaneous PEG administration. These tracings are derived from stop motion videotape analysis of cutaneous trunci muscle (CTM) stimulation regimens in which the entire CTM receptive field is first determined in the uninjured guinea pig (circumscribed). Probing inside this region of back skin with a monofilament probe produces back skin contractions, while probing outside the region does not. This line is drawn on the shaved back of the sedate animal with a marker while the investigator probes the region. The entire procedure is videotaped from above, and the various regions of both intact receptive fields and areflexia are reconstructed from these video images. Note that in all animals, the midthoracic spinal cord injury eliminates CTM responsiveness below the level of the injury on both sides (circumscribed). In control animals (FIG. 8A), this region of areflexia remained unchanged for the duration of the experiment. In PEG-treated animals (FIG. 8B), a variable region of the lost receptive CTM fields recovered within a short time of treatment. That region shows a region of CTM recovery for this one animal comprising about 55% of the original area of CTM loss. The inset (FIG. 8C) shows the 4-week video image which was used to reconstruct the regions of intact and nonfunctional receptive fields. The dot matrix allows precise alignment and superimposing of receptive fields, as well as a deeper analysis of the vector of skin movement, the velocity of skin contraction and latency when required (data not shown).

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FIGS. 9A-9D depict a portion of a neurological examination for outcome measures and recovery from paraplegia. A dog is placed on its side while a neurologist tests for the presence of superficial pain (A), deep pain (B), and conscious proprioception (C and D). Skin of the flank and limbs was pinched sharply with hemostats probing for a reaction from the subject during tests of superficial pain response. Deep pain response was similarly determined, but by a sustained and sharp squeeze of the joints of the digits. Positive responses were provided for comparison by testing the fore limbs. The responses were quantified by a 1–5 score: 1 = no detectable response; 2 = a response at the limits of detection, indicated by an increased state of arousal, increased respiration or pulse; 3 = consistent attention to the painful stimulus but without any overt defensive behavior; 4 = mildly defensive behavior such as abrupt turning of

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the head towards the stimulus, and whining; 5 = completely normal response to painful stimuli including yelping, biting, and aggressive behavior. These scores were obtained for both sides of the body and averaged. Conscious proprioceptive placing (CP) and weight support was tested in dogs by providing lateral support of the hind limbs, and turning one hind paw “under” so that the dorsal surface of the paw (and the animal’s weight) rested on the table (inset C). A normal animal briskly replaces the paw to a normal stance instantly after the examiner releases the paw. Paraplegic animals rest in this “knuckled under” stance for extended periods of time. Testing the fore leg provided a positive control. The test was performed on each side of the body, and scored on each side: 1 point = complete absence of CP, and 2.5 points for a positive CP response. These scores were then summed for each animal. Voluntary locomotion (not shown) was evaluated with a similar 1-5 point score: 1 = complete inability to step or voluntary ambulate; 2 = stepping and load bearing at the limit of detection, at best a few steps before falling (paresis); 3 = longer sequences of stepping, poorly coordinated before falling (paresis), and unable to climb stairs; 4 = more robust and effective walking but with clear deficits in coordination, effective weight support, but able to climb stairs; 5 = completely normal voluntary walking, indistinguishable from a normal animal. All neurological exams were videotaped for reference and half points were permitted at the examiner’s discretion. A total neurological score (TNS) was determined for each animal at each testing period by summing the scores of these 4 independent tests. Thus the range of a possible score for any one animal was 4 (a totally paraplegic animal) to 20 (a totally normal animal, indistinguishable from an uninjured one).

FIG. 9E shows a comparison of control and PEG-treated animals (FIG. 9A-9D) for each of the four outcome measures at approximately 3 days post injury (about 48 hours after the last PEG injection), 1 week, and 6–8 weeks post injury. The y -axis for each bar graph is the percentage of the population (i.e., 25, 50, 75%). DP = deep pain, SP = superficial pain, P = proprioceptive placing, and L = voluntary locomotion. Asterisks note when a test for proportions (Fisher’s exact test, two tailed) or a comparison of the means (Students T, or the Welch variation) revealed statistical significance. Note the clear recovery of outcome measures within 48 hours of the last PEG injection in that group, and the striking improvement in TNSs in PEG-treated dogs at every period of evaluation.

FIG. 10A shows a sedated dog and electrode placement in electrophysiological tests for conduction through a spinal cord injury to determine a Somatosensory Evoked Potential (SSEP). At each evaluation, four to seven sets of evoked potentials (SSEPs) were stimulated, recorded, averaged, and stored using a Nihon KohdenTM ME#B – 5304K 4 Neuropak recorder. More

particularly, FIG. 10A shows the sedated dog and the placement of bipolar stimulating pin electrodes, inserted subcutaneously, in the hind limb at the distal popliteal area approximately 0.5 – 1 cm apart. These electrodes stimulated the tibial nerve of the hind limb (red wires). A similar procedure was used to stimulate the median nerve of the forelimb (wires). Trains of square wave stimulations (0.5 – 3.0 mA amplitude, 200/min) were applied to evoke compound nerve impulses from these nerves. To record evoked potentials, scalp needle electrodes were inserted subcutaneously over the somatosensory cortex contralateral to the side stimulated, while reference electrodes were inserted on the opposite side between the mastoid and the pinna of the ear. The placement of recording electrodes was facilitated by stimulation of the median nerve at the outset, a neural circuit above, and unaffected by, the spinal cord injury (inset, circuit 2). This procedure also provided a positive control recording to validate the frequent inability to record evoked potentials stimulated at the hind limb - but whose ascending potentials are blocked by the spinal cord lesion (inset, circuit 1).

FIG. 10B is a graph of a complete set of SSEP recordings from the procedure of FIG. 10A. A lower group of waveforms in this pair are the three individual trains of 200 stimulations as discussed, and an upper waveform is the averaged evoked SSEP (only such averaged SSEPs are provided in subsequent records, FIGS. 11A and 11B). This record is of a control procedure. Note the clear evoked potential, recorded approximately 10 ms after stimulation of the median nerve.

FIG. 10C is a graph showing a portion of an electrical recording, displaying three trains of stimulation, as well as the averaged SSEP as in FIG. 10B. This record was in response to stimulation of the tibial nerve in the same paraplegic dog providing the record in FIG. 10B, approximately 4 days post-injury. The complete elimination of SSEP conduction through the lesion is characteristic of all neurologically complete paraplegic animals meeting the criteria described in the text, both in this and all previous reports using identical procedures (R.B. Borgens *et al.*, *J. Restorative Neurology and Neurosci.* 5, 305 (1993); R.B. Borgens *et al.*, *J. Neurotrauma* 16, 639 (1999)). SA = stimulus artifact; time base = 50 msec full screen, 5 msec/div; sensitivity = 1.25 μ V/ div.

FIGS. 11A and 11B relate to PEG induced recovery of nerve impulse conduction through the site of spinal injury. In FIG. 11A, a 6-week progression of recovery of conduction through the lesion is shown for a PEG-treated dog. Each trace is the averaged waveforms of 3–4 trains of 200 stimulations as described in FIGS. 9A-9E. There is complete absence of an SSEP in this

paraplegic animal prior to surgery, and approximately 4 days later. The third trace is a median nerve control procedure. There is no evidence of recovered conduction at 1 week post injury. By 6 weeks post-surgery, two distinct evoked cortical potentials had returned, a typical early arriving peak of approximately 26 msec latency (P 1), and a later arriving peak (P 2), of approximately 45 msec latency.

In FIG. 11B, a low amplitude, long duration, but reproducible evoked potential recovered within 15 min of a slow injection of PEG is shown. This atypical SSEP appeared to segregate into an early arriving peak of about 15-20 msec latency, and a more condensed and later arriving peak (P.2) of about 32-35 msec latency. SA = stimulus artifact. The time base and sensitivity scale is for both FIG. 11A and FIG. 11B.

DEFINITIONS

The term "nerve tissue" as used herein refers to any vertebrate nerve tissue, particularly including cells of the central nervous system (CNS) and peripheral nervous system. More particularly, nerve tissue includes spinal cord neuronal structures, peripheral nervous system nerves, and nerve cells of the brain.

The word "injury" is used herein to generally denote a breakdown of the membrane of a nerve cell, such that there is a collapse in the ability of the nerve membrane to separate the salty gel on their insides (cytoplasm) from the salty fluid bathing them (extracellular fluid). The types of salts in these two fluid compartments is very different and the exchange of ions and water caused by injury leads to the inability of the nerve to produce and propagate nerve impulses – and further to the death of the cell. The injury is generally a structural, physical or mechanical impairment and may be caused by physical impact, as in the case of a crushing, compression, or stretching of nerve fibers. Alternatively, the cell membrane may be destroyed by or degraded by a chemical imbalance or physiological malfunction such as anoxia (e.g., stroke), aneurysm or reperfusion. In any event, an "injury" as that term is used herein more specifically contemplates a nerve membrane defect, interruption, breach, or rupture (in the phospholipid bilayer) which can be treated and sealed by the administration of a biomembrane fusion agent as described herein.

The term "biomembrane fusion agent" is used herein to designate any and all molecules which are not only compatible with vertebrate, and more specifically mammalian, nerve cells but also have an affinity for nerve cell membranes so as to attach to injured nerve cells at the site of

an injury. A biomembrane fusion agent thus serves in part as a kind of biological cement or filling material which bridges over ruptures in neuronal structures. This sealing is extremely rapid (minutes) and facilitates the repair of the damaged neuronal structures by natural physiological processes which are complete at much later times (1-7 hours). The sealing of neuronal membranes as described herein naturally arrests or inhibits the progressive destruction of nervous tissue after an injury to the nerve cell. Exemplary biomembrane fusion agents include hydrophilic polymers such as polyalkylene glycols (polyalkylene oxides) and polyalkylene glycol block copolymers such as polyethylene glycol/polypropylene glycol block copolymers (e.g., poloxamer 188) and ethylene oxide-propylene oxide-ethylene oxide (EPAN), and further include biocompatible surfactants, particularly nonionic surfactants and more particularly amphipathic polymers such as poloxamines. Poloxamers may also be considered to be amphipathic polymers. Poloxamers are hydrophilic to the extent that there is a greater number or greater weight percentage of ethylene glycol groups as opposed to propylene glycol groups. A biomembrane fusion agent at that term is used herein may comprise a collection, mixture, or combination of individual biomembrane fusion agents each of which is effective in its own right to seal ruptures in nerve membranes.

The term "effective amount" when used herein with reference to a biomembrane fusion agent denotes a quantity of the agent which, when administered to a patient or subject, is sufficient to result in a measurable improvement in electrical and/or behavioral function of a nerve which has been so damaged or injured that normal functioning is not possible. As discussed below, the efficacy of the treatment may be determined in a variety of ways, including methods which detect restoration of nerve function. With respect to the use of the term "effective amount" with other agents, for example, potassium channel blockers, that term is used to describe an amount of an agent effective within the context of that agent's use in the present invention.

The term "hydrophilic polymer" means any macromolecule (molecular weights of 200 daltons and greater) which exhibits an affinity for or attraction to water molecules and which comprises multiple instances of an identical subunit ("monomer") connected to each other in chained and/or branched structures.

A "surfactant" is a molecule exhibiting both an affinity for or attraction to polar molecules such as water and an affinity for or attraction to non-polar molecules such as lipids, fats, oils, and greases. A "nonionic surfactant" is electrically neutral, i.e., carries no positive or

negative charge. However, a nonionic surfactant may have localized quantum variations in charge leading, for example, to a polar substructure evidencing an affinity for other polar molecular structures such as water molecules. In the context of the present disclosure, surfactants include amphipathic polymers.

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An “amphipathic polymer” as that term is used herein relates to polymers which have localized quantum variations in charge giving rise to polar substructures and non-polar substructures. The polar substructures evidence an affinity for or attraction to other polar molecular structures such as water molecules (hydrophilic), while the nonpolar substructures exhibit an affinity or attraction for nonpolar molecules such as lipids, oils, greases, fats, etc. (lipophilic).

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Poloxamers, also called non-ionic detergents, and/or triblock polymers, comprise a polyethylene glycol chain(s) (block 1), then a polypropylene glycol chain (block 2), followed by a polyethylene glycol chain(s) (block 3). These compounds can be synthesized in numerous conformations and molecular weights. The weights of the various “blocks” can even vary between themselves – leading to a complicated nomenclature. What all of the poloxamers have in common is a hydrophobic head group (block 2), surrounded by hydrophilic (PEG) chains. The hydrophobic “head” is believed to insert itself into the “hole” in a membrane (where the hydrophobic interior of the bilamminer membrane is exposed) while the hydrophilic PEG arms interdigitate and link with or attach to the nearby, more normal, membrane.

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The term “poloxamine” denotes polyalkoxylated symmetrical block polymers of ethylene diamine conforming to the general type $[(\text{PEG})_x-(\text{PPG})_y]_2-\text{NCH}_2\text{CH}_2\text{N}-[(\text{PPG})_y-(\text{PEG})_x]$

The word “biocompatible” means that a substance can be placed into intimate contact with biological structures, including cells and cellular membranes, without detriment to the continued physiological functioning of the contacted cells and membranes.

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The term “polyalkylene glycol” refers to a molecule having the chemical formula $\text{H}(\text{O}[\text{CH}_2]_m)_n\text{OH}$ where m and n are nonzero integers. The integer m has the following values for exemplary polyalkylene glycols: polymethylene glycol (m=1), polyethylene glycol (m=2), polypropylene glycol (m=3), polybutylene glycol (m=4), polypentylene glycol (m=5), polyhexylene glycol (m=6), polyheptylene glycol (m=7), polyoctylene glycol (m=8), polynonylene glycol (m=9), and polydecylene glycol (m=10), including branched and structural

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isomers thereof. Pursuant to the present disclosure, polyalkylene glycols have a molecular weight between about 200 and about 25,000 daltons, and preferably between about 400 daltons and about 3500 daltons.

5 The word "carrier" is used herein to denote a liquid matrix, medium or solvent in which molecules of a biomembrane fusion agent are dispersed or distributed. A pharmaceutically acceptable carrier is one which is biocompatible to vertebrate and more particularly mammalian tissues. Generally acceptable carriers include water, saline solutions, among numerous others.

10 By definition a "potassium channel blocker" or " K^+ channel blocker" is any agent that specifically and sterically inserts itself into (or otherwise deactivates) any of the several and growing classes of K^+ channels. This includes both fast and slowly activating channels and both "voltage gated or non-gated" channels. Almost all channels for K^+ are "gated" by the voltage across the cell membrane. When these channels are open, K^+ tends to move from the cytoplasm
15 into the extracellular fluid because it is about 100 times more concentrated inside than outside the cell. This K^+ exodus (which among other things helps extinguish the nerve impulse, bringing the membrane potential back to a resting state) can thus be "blocked". In regions of demyelination or membrane potential polarization, K^+ channel blockade can both increase excitability, as well as extend the distance along a nerve fiber in which a nerve impulse can travel
20 before it is extinguished. In spinal cord injury, this may only be a few millimeters of nerve fiber damage, with absolutely normal membrane on either side. There are many known K^+ channel blockers including reversible blockers (TEA) and some proteins (synthesized from snake venoms) that irreversibly block these channels. Potassium channel blockers include substituted pyridines and, more particularly, amino-substituted pyridines. The application of K^+ channel
25 blockers to spinal cord repair as described herein involves the fast potassium channel, type I, blocker 4-AP (4-aminopyridine) and its analog 3, 4 di-aminopyridine. Too high a dosage, or the use of the other blockers (more non specific and poorly reversible) may lead to convulsions and even death.

30 The delivery of a biomembrane fusion agent via a vascular system of a patient entails the administration of a biomembrane fusion agent via a pathway including one or more veins and/or arteries of the patient. Instead of direct application in which the agent is injected into the patient at the site of exposed nerve tissue, the vascular-system-mediated delivery of a biomembrane fusion agent contemplates an administration and subsequent conveyance of the agent to the site
35 of an injured nerve via the vascular system of the patient. The administration of the

biomembrane fusion agent is preferably by injection, for example, via a hypodermic needle or catheterization, either directly into a vein or artery or indirectly by subcutaneous injection into muscle tissue or intraperitoneally. Other methods may also be effective, for example, by ingestion, transmembrane delivery (including transdermal delivery), by suppository, through
5 inhalants, buccally, or by implantation.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

For the purposes of promoting an understanding of the principles of the invention,
10 reference will now be made to preferred embodiments and specific language will be used to describe the same. It will nevertheless be understood that no limitation of the scope of the invention is thereby intended, such alterations and further modifications of the invention, and such further applications of the principles of the invention as illustrated herein, being contemplated as would normally occur to one skilled in the art to which the invention relates.

15 The present invention provides methods and compositions for treating injured nerve tissue of a vertebrate. The methods and compositions are designed to at least partially restore nerve function in the vertebrate. In one aspect of the invention, methods are provided for treating an injured or damaged vertebrate spinal cord that include contacting the spinal cord with an effective amount of a biomembrane fusion agent. The compositions include a biomembrane
20 fusion agent, preferably a polyalkylene glycol such as polyethylene glycol (chemical formula: $H(OCH_2CH_2)_nOH$) and/or a nonionic surfactant such as an amphipathic polymer (e.g., a poloxamer or a poloxamine), and/or mixtures or copolymers thereof. In alternative embodiments, the method may include treating the nervous system with a potassium channel blocker, preferably a substituted pyridine, such as an amino-substituted pyridine, either before, during or
25 after contacting the spinal cord with the biomembrane fusion agent. Other aspects of the invention provide compositions for treating an injured nervous system of a vertebrate. The preferred compositions include a biomembrane fusion agent and a potassium channel blocker.

The preferred biomembrane fusion agent is a polyalkylene glycol. A wide variety of polyalkylene glycols may be used, including those, for example, where the alkylene group is
30 methylene, ethylene, propylene, butylene, pentylene, hexylene, heptylene, octylene, nonylene, and decylene, including branched and structural isomers thereof. Preferably, the polyalkylene glycol will be water-soluble and is selected from the group consisting of polyethylene glycol, polypropylene glycol and block copolymers of polyethylene glycol and polypropylene glycol. A more preferred polyalkylene glycol is polyethylene glycol. Although a wide range of molecular
35 weight polyalkylene glycols may be used (between about 200 daltons and about 25,000 daltons)

depending on the ability of the polyalkylene glycol to pass through various biological barriers such as the digestive tract, polyalkylene glycols and polyalkylene glycol block copolymers of molecular weight of about 400 to about 3500 daltons are preferred. Such biomembrane fusion agents may be synthesized by methods known to the art or may be purchased commercially.

5 The biomembrane fusion agent may also be a polyalkylene glycol/protein conjugate as known in the art, wherein the protein preferably aids in scavenging free radicals. For example, the biomembrane fusion agent, such as polyethylene glycol or other alkylene oxide, may be conjugated to catalase to form PEG-catalase, or to superoxide dismutase to form PEG-SOD. Such conjugates are available commercially from Sigma, St. Louis, Mo. The biomembrane
10 fusion agent, may also be conjugated to a biodegradable surgical glue, such as a commercial fibrin glue, to facilitate and stabilize reattachment and fusion of severed nervous tissue.

Alternatively, the biomembrane fusion agent may be a biocompatible surfactant, preferably a nonionic surfactant and more preferably an amphipathic polymer such as a poloxamer or a poloxamine.

15 The biomembrane fusion agent may be provided in a pharmaceutically acceptable carrier. Such carriers include, for example, water, preferably sterile and including distilled water, and any other pharmaceutically acceptable carrier known to the art that will not have an adverse effect on the treatment. Sterile distilled water is a preferred carrier in work to date.

20 The biomembrane fusion agent is administered to the patient as soon after injury as possible and prior to irreversible dissolution of axonal membranes and the myelin sheath. Although this time period may vary depending on the nature and extent of the injury, the fusion agent is typically administered immediately after the injury occurs, and preferably not later than about 24 hours post-injury, but is typically administered between about 1 hour to about 8 hours post-injury. Though early treatment is preferred, administration of the biomembrane fusion
25 agent may still be beneficial for up to 2 weeks after the initial nerve injury (called the "primary injury"). This is because nerve injury is a continuous, slow, progressive event, especially in spinal cord where it is called "secondary injury" (Tator and Fehlings 1991, J. Neurosurgery 75:15-26).

30 The biomembrane fusion agent may be delivered to the site of injury by any suitable method. Preferably, the biomembrane fusion agent is administered through the vascular system of the subject or patient. The fusion agent may be injected directly into the vascular system or indirectly by injection intramuscularly, subcutaneously or intraperitoneally. It has been discovered that an indirect administration of a biomembrane fusion agent such as polyethylene glycol via the vascular system of the patient unexpectedly results in a selective adherence of the
35 fusion agent (e.g., PEG, poloxamer or other agent) to the injured nerve tissue. There is little or

no adherence to undamaged nerve tissue. Without being limited by way of theory, it is believed that by adhering to damaged nerve tissue, the biomembrane fusion agent promotes the natural healing processes of the damaged nerve cells.

Where the biomembrane fusion agent is a polyalkylene glycol such as PEG, the fusion solution comprises fusion agent in an amount of typically about 15 to about 50% by weight and preferably is administered in doses of about 15 – 50 mg PEG per body weight in kilograms of the patient where the PEG has a weight of 1500 – 4000 Daltons. Where the biomembrane fusion agent is an amphipathic polymer such as a poloxamer or a poloxamine, the fusion solution typically contains fusion agent in an amount of about 15 to about 50% by weight and is administered in dosages of about 15 – 150 mg poloxamer or poloxamine per body weight in kilograms of the patient.

Where the agent is applied directly to damaged nerve tissue which has been exposed, for example, via surgical procedures, the agent may be applied with any suitable liquid dispensing device. Although the percentage by weight of the fusion agent in the direct-application composition may vary, the composition typically includes fusion agent in an amount of at least about 40% by weight, more preferably about 40% to about 50% by weight, and most preferably about 50% to about 55% by weight.

In the case of a direct-contact application, the injured site is exposed to the fusion agent for a time period effective for treating the injury. This time may vary depending on the size of the lesion, the extent and nature of the injury, the biomembrane fusion agent used, and the concentration of the biomembrane fusion agent. The lesion is typically exposed to the agent for at least about one minute and more preferably at least about 2 minutes. In preferred embodiments, the fusion agent is removed from the injured tissue being treated prior to the occurrence of deleterious tissue changes. In a further preferred embodiment, the injured tissue is exposed to the fusion agent for no more than about 5 minutes. After the injured region of the nervous system is treated with the fusion agent, it may be removed by aspiration and the treated site washed with a biowashing solution, such as isotonic Krebs's solution as described in the examples. Excess fusion agent and/or Krebs's solution can then be removed by aspiration.

In another form of the invention, the method may further include administering to the patient or subject an effective amount of a potassium channel blocker. In the case of a direct-contact application of a biomembrane fusion agent, the injured site is contacted with an effective amount of a potassium channel blocker in addition to the biomembrane fusion agent. A variety of potassium channel blockers may be used, including substituted pyridines. Preferred potassium channel blockers include those that improve action potential conduction in injured tissue, including 3,4-diaminopyridine, 4-methylaminopyridine and ampidine. In a preferred form of the

invention, the pyridine is substituted with an amino group, more preferably at the 4-position of the ring. Moreover, it has unexpectedly been discovered that treatment of an injured mammalian spinal cord with a potassium channel blocker, such as 4-aminopyridine, after treatment with a fusion agent, such as polyethylene glycol, can result in synergistic repair of the spinal cord. For example, compound action potentials (CAPs) increase in conduction when both agents are used by a percentage greater than the sum of the percent increase in conduction of the CAPs when injured spinal cords are treated alone with either the fusion agent or the potassium channel blocker.

Although the injured nervous system may be contacted with the potassium channel blocker prior to or at the same time as treating with the fusion agent, the system is preferably contacted with the blocker after the treatment with the fusion agent. The potassium channel blocker may be applied in a fashion similar to the fusion agent. The amount of the potassium channel blocker effective in treating or repairing the injured nervous system, such as injured mammalian spinal cord, will also similarly depend on the factors mentioned above. When the potassium channel blocker is 4-aminopyridine, it is typically applied at a concentration of about 10-100 ng/ml cerebrospinal fluid and further preferably about 50-100 ng/ml cerebrospinal fluid. After treatment with 4-aminopyridine, it can similarly be removed by aspiration and the lesion site washed with the biowashing agent.

In yet other forms of the invention, the method may include treating the injury with a polyalkylene glycol, as well as with other conventional management compounds and/or compositions. For example, in addition to treatment with a polyalkylene glycol, the injury may be treated with a steroid, such as methylprednisolone.

A wide variety of injuries may be treated in the present invention. In various forms of the invention, the injury may arise from a compression or other contusion of the spinal cord, crushing of the spinal cord or severing of the spinal cord, or anoxia (e.g., stroke), aneurysm or reperfusion.

The efficacy of the treatment may be determined in a variety of ways, including methods which detect restoration of nerve function. For example, restoration or increase in conduction of action potentials, such as CAPs, through the injured site may be used as an indicator that nerve function has at least partially been restored as described in the examples. Nerve function is considered to have been at least partially restored if there is an increase in the conduction of action potentials after treatment. Preferably, the treatment will be conducted sufficiently to achieve at least about 10% increase in conduction of CAPs. Moreover, restoration of anatomical continuity may also be observed by examination with high-resolution light microscopy and/or by diffusion of intracellular fluorescent dyes through the repaired nervous tissue, such as repaired

axons, or by direct observation of repaired axonal membranes. Additionally, in human applications, the efficacy of preferred treatments may be observed by the restoration of more than one spinal root level as determined by the American Spinal Injury Association (ASIA) motor score and/or the National Animal Spinal Cord Injury Study (NASCIS) score as known in the art and as described in Wagih et al., (1996) Spine 21:614-619. Furthermore, in veterinary applications, behavioral analysis of the cutaneous trunci muscle (CTM) reflex, as more fully discussed in the examples, may also be used to determine the efficacy of the treatment, and whether nerve function has at least partially been restored. Using this analysis, nerve function is considered to have been at least partially restored if there is an increased reflex behavior after treatment, but treatments are desirably preferred so as to achieve at least about a 10% increase in the area of CTM behavioral recovery.

In yet other aspects of the invention, compositions for treating an injured nervous system of a vertebrate are provided. The compositions are designed to at least partially restore nerve function as described below. In one form, a composition includes a biomembrane fusion agent and a potassium channel blocker. Although a wide variety of biomembrane fusion agents and potassium channel blockers that are mentioned above may be included in the composition, a preferred biomembrane fusion agent is a polyalkylene glycol and a preferred potassium channel blocker is a substituted pyridine. In more preferred forms of the invention, the polyalkylene glycol is polyethylene glycol and the potassium channel blocker is an amino-substituted pyridine, such as 4-aminopyridine. The composition may be in a pharmaceutically acceptable carrier as described above.

Although the methods and compositions of the invention are useful in treating a wide variety of vertebrates, they may be advantageously used to treat mammals and preferably humans. Moreover, although the methods and compositions are advantageously and surprisingly useful in treating the spinal cord, they may also be used in treating the peripheral nervous system and/or central nervous system, or other areas in which damaged axons are present.

Reference will now be made to specific examples illustrating the compositions and methods described above. It is to be understood that the examples are provided to illustrate preferred embodiments and that no limitation to the scope of the invention is intended thereby.

EXAMPLE 1

Potassium Channel Blockade as an Adjunct to PEG-Mediated Recovery of Conduction

This example shows that treatment of injured spinal cords in vitro with both a potassium channel blocker and a biomembrane fusion agent allows synergistic recovery of compound action potentials (CAPs).

It is a common feature of injured cells to lose intracellular potassium to the extracellular milieu through compromised membrane. In axons, this may be sufficient to suppress action potential conduction. Thus, it was attempted to determine if blockage of fast potassium channels with 4-AP would affect the properties of conduction immediately following PEG repair.

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Analysis was also performed in the double sucrose recording chamber.

In Vitro Isolation of the Spinal Cord

Adult female guinea pigs of 350-500 gram body weight were used for these studies. The spinal cord was isolated from deeply anesthetized animals [(60 mg/kg ketamine hydrochloride, 0.6 mg/kg acepromazine maleate, and 10 mg/kg xylazine, intramuscularly (i.m.)). Following anesthesia, the animal was perfused transcardially with cold (15°C) Krebs' solution (NaCl, 124 mM; KCl, 2 mM; KH₂PO₄, 1.2 mM; MgSO₄, 1.3 mM; CaCl₂, 11.2 mM; dextrose, 10 mM; NaHCO₃, 26 mM; sodium ascorbate, 10 mM; equilibrated with 95% O₂, and 5% CO₂). The vertebral column was rapidly removed using bone forceps and scissors by previously described techniques [Shi, R. and Blight, A.R. (1996) *J. of Neurophysiology*, 76(3):1572-1579; Shi, R. and Blight, A.R. (1997) *Neuroscience* 77(2):553-562]. The spinal cord was divided into four longitudinal strips, first by midline sagittal division, then by separating the dorsal and ventral halves with a scalpel blade against a plastic block. Only the ventral white matter was used for this study. These 35-38 mm long strips of spinal cord white matter will usually be referred to below as "cords" or "spinal cords" for ease of description. Spinal cords were maintained in continuously oxygenated Krebs' solution for an hour before mounting them within the recording chamber. This was to ensure their recovery from dissection before experiments were begun.

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Double Sucrose Gap Recording Technique

The double sucrose gap recording chamber is shown in FIGS. 1A and 1B and has already been described in previous publications [Shi, R. and Blight, A.R. (1996) *J. of Neurophysiology*, 76(3):1572-1579; Shi, R. and Blight, A.R. (1997) *Neuroscience* 77(2):553-562]. Briefly, the strip of isolated spinal cord white matter was supported in the three-compartment chamber. The central compartment was continuously superfused with oxygenated Krebs' solution (about 2 ml/min) with a peristaltic pump. The compartments at both ends were filled with isotonic (1120 mM) potassium chloride, and the gap channels with 230 mM sucrose. The white matter strip was sealed on either side of the sucrose gap channels with shaped fragments of glass coverslips that

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also blocked the flow of fluid in the narrow gap between the coverslip and the tissue surface.

Note that the central chamber is at ground potential for recording. The sucrose solution was run continuously through the gap at a rate of 1 ml/min. Axons within the spinal cord strip were stimulated and compound action potentials (CAPs) were recorded at the opposite end of the

5 white matter strip by silver-silver chloride electrodes positioned within the side chambers and the central bath as shown in FIG. 1B. Specifically, action potentials were stimulated at the left side of the spinal cord strip as shown in the figure, conducted through the spinal cord in the central compartment (also including the injury site), and recorded at the right side of the spinal cord strip as shown. Stimuli were delivered through stimulus isolation units in the form of 0.1 msec
10 constant current unipolar pulses. A conventional bridge amplifier with capacity compensation (Neurodata Instruments) was used to amplify the signal. This data was digitized and stored on video tape with a Neurodata Instruments Neurocorder for subsequent analysis. During the experiment, the oxygenated Krebs' solution continuously perfused the isolated spinal cord tract, while temperature was maintained at 37°C.

15 Every electrophysiological test was digitized in real time and captured to the computer for subsequent quantitative evaluation. All records were also recorded on VHS magnetic tape as a means of back up documentation. All solutions used in the PEG repair process were made on the day of their use.

20 The Compression Injury

A standardized compression injury was produced with a stepper-motor controlled rod which compressed the spinal cord while suspended inside the recording chamber (FIG. 1 B). Briefly, the isolated white matter strip was compressed against a flat, raised plastic, plexiglass stage at the center of the recording chamber with the flattened tip of a plexiglass rod. The tip was
25 advanced downward to contact the tissue at a standardized rate of about 25 pm/s. The downward movement of the rod was controlled with a stepper motor to produce a finely graded crush just sufficient to eliminate all CAP propagation (which was monitored continuously during the procedure). The end of the rod with the flattened tip provided a compression surface of 2.5 mm along the length of the tissue, and a transverse width of 7 mm, such that it was always wider than
30 the spinal cord strip, even under full compression. Positioning of the compression rod was accomplished with a micromanipulator. CAPs were simultaneously recorded during the injury process. Compression was stopped when CAPs were completely eliminated. The state of complete CAP failure was maintained for an additional 15 seconds before the rod was rapidly withdrawn from the cord's surface to relieve pressure. The recovery of the CAP was then
35 documented. The basic recovery profile following such standardized compression in normal

Krebs' solution has been previously characterized and published [Shi, R. and Blight, A.R. (1996) J. of Neurophysiology, 76(3):1572-1579].

PEG Repair Procedure

5 The PEG repair procedure included the following steps:

1) Typical physiological functioning of the isolated white matter strip removed to the recording chamber required about 1/2 to 1 hour of incubation time while immersed in oxygenated Krebs' solution to stabilize. In initial experiments, once the CAP propagation had stabilized, the Krebs' solution was replaced with Ca^{2+} -free Krebs' (Ca^{2+} replaced with an
10 equimolar amount of Mg^{2+}).

2) The spinal cord strip was then crushed by the techniques described above, while simultaneous stimulation and recording continued.

3) A solution of PEG in distilled water (50 % by weight) was applied by a pressure injection through a micropipette. A vital dye was added to the PEG solution to monitor its
15 continuous application to the lesion site in a stream about 0.5 mm wide for about 1-2 minutes. The PEG was applied to one side of the lesion, washed over it, and immediately removed by constant aspiration on the other side using a second pipette.

4) Immediately following the PEG application, the bathing media in the central chamber was replaced with a continuous stream of oxygenated normal Krebs' solution. The physiological
20 properties of the PEG-treated spinal cord were monitored continuously for 1 hour. Usually, a weak recovering CAP was evident within 6-15 minutes of the PEG application.

Tests were made of the response of "recovering" axons to the additional application of the fast potassium channel blocker, 4 aminopyridine (4-AP). In this trial, 5 separate cords were treated with an application of PEG as described above and compared to 5 control cords. One
25 hour after compression, 100 pM 4-AP (in Krebs' solution) was applied for 15 minutes and then washed free with normal Krebs' solution as described above.

FIG. 2A shows the enhancement of the CAP in crushed (but untreated with PEG) spinal cord by 4-AP. In this individual record, the initial recovered CAP at 1 hour post injury is shown,
30 and the enhanced CAP following 100 pM 4-AP treatment is superimposed upon it. Following documentation of the 4-AP enhanced CAP, the blocker was washed out, and the media in the central compartment was replaced with normal Krebs' solution. The CAP fell to pretreatment levels by 15 minutes and was indistinguishable from the original record. This final waveform is superimposed on the other two CAPs in FIG. 2A but cannot be discriminated from the

pretreatment electrical record. In this single test, 4AP reversibly enhanced the recovered CAP by about 40%.

FIG. 2B shows an identical test performed on a PEG-treated spinal cord, in which 4-AP was administered at 1 hour post PEG application. In this individual test, the second CAP was
5 reversibly enhanced by about 70%.

Following the near doubling of the CAP, 4-AP was washed out as described, and the CAP fell to pretreatment levels as in controls (FIG. 2A).

FIG. 2C shows the group data, including 5 spinal cords in each group. The percent enhancement of the PEG-mediated recovery for the group data mirrors that discussed above for
10 the individual experiments (about 70% enhancement in the experimental group; about 40% in the control group). This experimental enhancement was statistically significantly greater than that observed in the controls. ($p < 0.05$, unpaired Student's t test)

Although not being limited by theory, FIG. 3 depicts a proposed mechanism of the synergistic effect of PEG and 4-AP. A severe mechanical compression of a myelinated axon is
15 diagrammed at the top. Note that the myelin sheath envelops high densities of fast W channels clustered at the paranodal region. Severe crush leads to an exposure of the potassium channels of the paranodal region by a withdrawal or collapse of the myelin lamella at this site [Shi, R. and Blight, A.R. (1996): Neuroscience, 77:553-562]. Exposure of the voltage gated potassium channels after injury would elevate K^+ conductance further impeding conduction across this
20 damaged portion of the membrane (gray region showing "holes" in the compromised membrane). In control preparations, partial to complete conduction block results from this localized disturbance of the axolemma, which may progress to complete separation of the axon and loss of the distal axonal segment by Wallerian degeneration (left side of FIG. 3). In PEG-treated axons (right side of FIG. 3), the membrane repair leads to preservation of injured
25 axons as well as improvements in their conduction capabilities (gray regions; membrane holes now sealed). However, elevated K^+ conductance through K^+ channels that are still exposed at the site of repair in PEG-treated nerve fibers might still suppress conduction to some extent. Blockade of these channels with 4 AP (FIG. 3, small arrow heads; lower right) would be expected to reduce any outward K^+ conductance and thus enhance conduction.

30 Spinal cords were crushed, isolated and treated with PEG as described.

SUMMARY OF RESULTS

Within a few minutes after the application of the water-soluble polymer PEG, an immediate recovery of CAP propagation through the lesion occurred. The recovered CAP amplitude slowly increased with time to a peak of about 20% of the initial CAP amplitude.
35 Moreover, this level of recovery a) was always statistically significantly higher than control

amplitudes, b) was observed at every time point tested, and c) occurred in 100% of the experimentally treated spinal cords. It is clear that a topical application of PEG can immediately repair severe compression injury to the mammalian spinal cord leading to significant increases in functional recovery as defined by the enhanced capacity to propagate nerve impulses through the lesion. This report is the first to demonstrate PEG-mediated repair of crushed mammalian nervous tissue.

We have shown that a physiological, balanced media and the aforementioned PEG solution, is all that is required to produce functionally significant repair in mammalian spinal cords (see below). Moreover, in other experiments, where completely transected guinea pig spinal cords were fused with PEG, it has been revealed there was no specific PEG molecular weight critical to the process, having tested PEG solutions using 400, 1400, 1800, 2000, and 3700 daltons.

In this physiological study, similarities and differences between the natural mechanisms of axonal repair and those mediated by PEG have been determined. First, a least squares linear regression analysis of preand postinjury CAP amplitudes suggests that PEG-mediated repair can occur across all levels of stimulus thresholds, reflecting axon diameters, as does the natural recovery process in untreated spinal cord strips. In other words, all spinal axons regardless of their caliber are equally susceptible to PEG mediated repair [see Shi, R. and Blight, A.R. (1996) Neuroscience 77:553-562 for a similar analysis of axonal recovery from compression injury]. The differences between natural repair and that produced by PEG application are more striking. First, this injury is very severe; 30% of control spinal cords never recovered any capacity to conduct CAPs during the 1 hour period of evaluation following injury. On the other hand, there was no instance where PEG did not initiate a measurable physiological recovery. On a more subtle level, there appears to be a slightly reduced CAP amplitude during the period of relative refractory in only PEG-mediated CAPs relative to control cords. One explanation for this observation may be that in control cords a severely compromised and dysfunctional population of axons may become completely nonfunctional, revealing more normal conduction properties in that population that survive the injury. PEG may rescue a portion of such severely compromised axons, recruiting them into the CAP, and perhaps accounting for its slightly different conduction properties.

The above-described in vitro evaluation of the anatomy of axonal repair following mechanical compression has revealed that a 2 minute application of PEG produced sealing of membrane lesions at the site of a standardized compression. Sealing was indicated by the exclusion of horseradish peroxidase uptake by injured fibers in the PEG-treated group compared to sham-treated spinal cords. Such immediate repair of

membrane breaches sufficient to inhibit the uptake of large molecular weight dyes should also arrest or reduce permeabilization, allowing the nonspecific flux of ions across it. Although not being limited by theory, it is believed that this "sealing" behavior of PEG both restores excitability and reverses anatomical dissolution of the nerve fiber.

- 5 This procedure may advantageously applied to treat severe, acute neurotrauma. In addition to immediate improvements in conduction, repair of crushed axons in peripheral nerves leading to a rescue of their distal segments would provide the added benefit of reducing atrophy or degeneration of target cells or so called "end organs." Moreover, PEG-mediated fusion of even transected axons could become a component of microsurgical grafting techniques since the
- 10 conventional resection of peripheral nerve trunks prior to fascicular grafting exposes the severed tips of proximal and distal axonal segments, making them available for fusion.

EXAMPLE 2

Rapid Recovery from Spinal Cord Injury Following

15 Subcutaneously Administered Polyethylene Glycol

This example demonstrates that a biomembrane fusion agent, specifically the hydrophilic polymer PEG, can be safely introduced into the bloodstream by several routes of administration, and that the administered PEG specifically targets a hemorrhagic contusion of an adult guinea pig spinal cord. A single subcutaneous injection (30% weight by weight in sterile saline) made 6

20 hours after spinal injury was sufficient to produce a rapid recovery of CAP propagation through the lesion, accompanied by a significant level of behavioral recovery in only PEG-treated animals.

The results of these tests demonstrate (1) that PEG specifically targets the spinal cord contusion independent of whether it is applied directly to the exposed spinal injury, or by

25 intravenous or subcutaneous injection, and (2) that a single subcutaneous injection of PEG approximately 6 hours after severe SCI is sufficient to induce a rapid reversal of functional losses in nearly all PEG-treated adult guinea pigs compared to the persistence of these deficits in nearly all sham-treated animals. The intravascular delivery of PEG for purposes of treating and repairing injured nerve tissue has also been investigated in a clinical setting using naturally

30 produced cases of paraplegia in dogs, as discussed hereinafter.

Drawing Fig. 4: Behavioral Model and Physiological Evaluation

This drawing shows the neural circuit of the Cutaneous Trunci Muscle (CTM) reflex, and its interruption by spinal injury. Nociceptive sensory receptors in the skin project their axons

into the spinal cord at each vertebral segment bilaterally via the Dorsal Cutaneous Nerves. These synapse within the spinal cord and project 2nd order ascending sensory nerves in the ventral funiculus of the white matter to the cervical region where these synapse on bilaterally organized constellations of CTM motor neurons. CTM motor neurons project their axons out of the cord on right and left sides via the brachial plexus, where these innervate the cutaneous muscle of the skin via the lateral thoracic branch of the plexus. When the spinal cord is intact, tactile stimulation of the back skin within the CTM receptive field causes a rippling contraction of the skin. Stimulation outside the receptive fields of back skin does not result in skin contractions. A spinal cord injury (drawn on only the left side of the cord for descriptive purposes) interrupts the ascending leg of this circuit producing a region of skin areflexia ipsilateral to the injury and on the same side. Tactile probing within this region does not produce CTM contractions, usually for the life of the animal. Stimulation of back skin above the level of this unilateral lesion, or on the right side produces CTM contractions, as these receptive fields remain unaffected by the unilateral injury to the left side of the spinal cord.

15

Methods

Animal surgery and spinal cord injury

Adult Guinea Pigs (< 300 gm) were anesthetized with an intramuscular injection of 100mg/kg ketamine HCL and 20 mg/kg xylazine and the spinal cord exposed by dorsal laminectomy. The midthoracic cord was crushed with special blunted forceps possessing a détente. This standardized, constant displacement injury [Moriarty, L.J., Duerstock, B.S., Bajaj, C.L., Lin, K., and Borgens, R.B. (1998) Two and three dimensional computer graphic evaluation of the subacute spinal cord injury, J. Neurologic. Sci., 155, 121-137] has produced more consistent anatomical injury to the cord and more consistent behavioral loss between animals than constant impact injuries (such as those produced by the various weight drop techniques). Animals were euthanized by deep anesthesia followed by perfusion/fixation. The localization of an FITC decorated PEG (F1-PEG) in spinal cord was determined by killing the animals for histological processing approximately 24 hours after the application or injection of F1-PEG. The spinal cords were dissected from the animals, and the segments of spinal cord containing the sites of injury and an intact, more rostral, segment were sectioned with a freezing microtome and evaluated with a fluorescent microscope. Histological cross sections were 5 µm thick, and observed on an Olympus™ Van Ox Fluorescent microscope using excitation wavelengths of 495 and 545 nm and barrier filters of 475 and 590 nm, respectively. Digital images were captured to the computer with an Optronics™ DEI 750 camera.

30

To test the effects of subcutaneous injections of PEG, adult guinea pigs were anesthetized and their mid-thoracic spinal cords were surgically exposed and then crushed by a standardized technique. [Blight, A.R. (1991): Morphometric analysis of a model of spinal cord injury in guinea pigs, with behavioral evidence of delayed secondary pathology, *J. Neurolog. Sci.*, 103: 156-171.] Twenty animals were divided into equal groups of 10. One group received a single subcutaneous injection of PEG (1400 MW) beneath the skin of the neck (0.5cc; 30% in sterile lactated Ringer's solution; SLR). The sham-treated control group received a single injection of the carrier, lactated Ringer's. Only this one subcutaneous injection per animal was made approximately 6 hours after the spinal cord injury. CTM testing and SSEP recordings were carried out on all 20 animals prior to spinal cord injury, 1 day, 1 week, 2 weeks, and 4 weeks post injury.

Tracing the distribution of PEG in injured spinal cord

The FITC decorated PEG (about 1400 Daltons; prepared by Molecular Probes, Chatsworth, Ca) was used to trace the distribution of PEG following different routes of administration. FI-PEG, 50% weight by weight in SLR was applied directly to exposed spinal cord injury site (with the dura removed) using a Pasteur pipette in two animals. As in prior experiments [Borgens, R. and Shi, R. (2000) Immediate recovery from spinal cord injury through molecular repair of nerve membranes with polyethylene glycol, *FASEB* 14, 27-35], PEG was removed by aspiration and the region irrigated with SLR two minutes later. Subcutaneous injection of 1 cc FI-PEG (30% w/w in SLR) was made beneath the skin of the neck in two spinal injured guinea pigs using a 22-gauge needle. For IV injection, the jugular vein was surgically exposed, and 1 cc of FL-PEG was injected using a 26-gauge needle. PEG, 30% in lactated Ringer's was also administered by intraperitoneal injection in one case.

In Vivo Conduction Studies

Functional deficits produced by SCI are largely caused by the loss of nerve impulse conduction through mechanically damaged tracts of nerve fibers in spinal cord white matter [Blight, A.R. (1993) Remyelination, Revascularization, and Recovery of Function in Experimental Spinal Cord Injury, *Advances in Neurobiology: Neural Injury and Regeneration* (Seil, F.J. Ed.), Vol. 59, pp. 91-103, Raven Press, New York]. Accordingly, the loss and recovery of compound action potential (CAP) conduction through the spinal cord injury was evaluated by evoked potential techniques (somatosensory evoked potential testing or SSEP). Stimulation of the Tibial nerve of the hind limb produced ascending volleys of nerve impulses recorded at the contralateral sensory cortex of the brain. These were eliminated between the site

of stimulation and recording by the spinal lesion - *immediately* abolishing the recording of these peaks (postcrush records). Each electrical record was comprised of a stimulus train of 200 stimulations (< 2mA square wave, 200 μ s duration at 3 HZ). Three sets of these recordings were made at each measurement period and averaged to produce the single waveform presented in the following data. The appearance of original records prior to computer averaging can be found in prior reports [Borgens, R. and Shi, R. (2000) Immediate recovery from spinal cord injury through molecular repair of nerve membranes with polyethylene glycol, *FASEB* 14, 27-35]. Conduction of nerve impulses through a median nerve circuit following stimulation of the median nerve of the forelimb (unaffected by the spinal cord injury at the midthoracic level) was a control procedure during SSEP recording. This control stimulation regimen was carried out in every circumstance where a failure to record evoked potentials at the cortex occurred in response to hind limb tibial nerve stimulation – to eliminate the possibility these failures were “false negatives”. SSEP recording and averaging was performed with a Nihon Kohden Neuropak 4 stimulator/recorder and a PowerMac™ G3 computer. Computation of the area beneath the early arriving SSEP peak (P1) was accomplished by scribing a reference line beneath the base of the peak, and determining the unit area contained within it as pixels using IP Lab Spectrum software.

Behavioral Studies

As an index of behavioral recovery, evaluations are made of a spinal cord dependent contraction of back skin in animals - the Cutaneous Trunci Muscle reflex (CTM)[Blight, A.R., McGinnis, M.E., and Borgens, R.B. (1990): Cutaneus trunci muscle reflex of the guinea pig, *J.Comp.Neurol.*, 296, 614-633; Borgens, R.B. (1992): Applied Voltages in Spinal Cord Reconstruction: History, Strategies, and Behavioral Models, in *Spinal Cord Dysfunction, Volume III: Functional Stimulation*, (Illis, L.S. ed.), Chapter 5, pp. 110-145, Oxford Medical Publications, Oxford]. The loss of CTM behavior following injury to the spinal cord is observed as a region of back skin, which no longer responds, by muscular contraction to local tactile stimulation [Blight, A.R., McGinnis, M.E., and Borgens, R.B. (1990): Cutaneus trunci muscle reflex of the guinea pig, *J.Comp.Neurol.*, 296, 614-633; Borgens, R.B. (1992): Applied Voltages in Spinal Cord Reconstruction: History, Strategies, and Behavioral Models, in *Spinal Cord Dysfunction, Volume III: Functional Stimulation*, (Illis, L.S. ed.), Chapter 5, pp. 110-145, Oxford Medical Publications, Oxford; Borgens, R.B., Blight, A.R., and McGinnis, M.E. (1990): Functional recovery after spinal cord hemisection in guinea pigs: The effects of applied electric fields, *J. Comp. Neurol.*, 296, 634-653; Borgens, R.B., Blight A.R., and McGinnis M.E. (1987): Behavioral recovery induced by applied electric fields after spinal cord hemisection in guinea pig, *Science*, 238, 366-369]. This areflexia does not recover for the life of the animal if the

relevant (and identified) ascending CTM tract is severed within the ventral funiculus as the complete neural circuit underlying this behavior has been identified [Blight, A.R., McGinnis, M.E., and Borgens, R.B. (1990): Cutaneus trunci muscle reflex of the guinea pig, J.Comp.Neurol., 296, 614-633]. Following a severe bilateral *crush* injury of the mid-thoracic spinal cord (such as used here), a bilateral region of areflexia of back skin is produced that still shows very limited ability to spontaneously recover [Borgens, R. and Shi, R. (2000): Immediate recovery from spinal cord injury through molecular repair of nerve membranes with polyethylene glycol, FASEB, 14, 27-35; Borgens, R.B. (1992): Applied Voltages in Spinal Cord Reconstruction: History, Strategies, and Behavioral Models, in Spinal Cord Dysfunction, Volume III: Functional Stimulation. (Illis, L.S. ed.), Chapter 5, pp. 110-145, Oxford Medical Publications, Oxford]. A variable region of back skin recovery occurs in response to crush injury in a relatively small proportion of spinal injured animals (we estimate < 15% rate of overall recovery in untreated animals based on over a decade of experience using this reflex as an index of white matter integrity). Furthermore, there is no compensatory sprouting of cutaneous innervation into non-functioning receptive fields which might mimic a centrally mediated recovery of CTM function as these regions of skin *are not denervated* [Blight, A.R., McGinnis, M.E., and Borgens, R.B. (1990): Cutaneus trunci muscle reflex of the guinea pig, J.Comp.Neurol., 296, 614-633; Borgens, R.B., Blight, A.R., and McGinnis, M.E. (1990): Functional recovery after spinal cord hemisection in guinea pigs: The effects of applied electric fields, J. Comp. Neurol., 296, 634-653]. Complete details of the anatomically identified circuit, its physiology, behavioral loss and monitoring, and other testing of the CTM as a spinal cord injury model can be found in previous reports [Blight, A.R., McGinnis, M.E., and Borgens, R.B. (1990): Cutaneus trunci muscle reflex of the guinea pig, J.Comp.Neurol., 296, 614-633; Borgens, R.B. (1992): Applied Voltages in Spinal Cord Reconstruction: History, Strategies, and Behavioral Models, in Spinal Cord Dysfunction, Volume III: Functional Stimulation, (Illis, L.S. ed.), Chapter 5, pp. 110-145, Oxford Medical Publications, Oxford; Borgens, R.B., Blight, A.R., and McGinnis, M.E. (1990): Functional recovery after spinal cord hemisection in guinea pigs: The effects of applied electric fields, J. Comp. Neurol., 296, 634-653; Borgens, R.B., Blight A.R., and McGinnis M.E. (1987): Behavioral recovery induced by applied electric fields after spinal cord hemisection in guinea pig, Science, 238, 366-369].

Evaluations were not made of walking, inclined plane performance, rope climbing, or other direct or indirect measures dependent on the functioning of hind limbs in spinal injured rodents. These tests are more subjective in interpretation, are not based on identified neural circuits, and cannot sufficiently discriminate movements dependent on intact bilateral hind limb reflexes from those based on restored functioning of damaged white matter tracts.

Statistics

Comparison of the proportions of animals in each group was carried out using Fisher's exact test, two tailed; and a comparison of means with Mann Whitney non parametric two tailed
 5 test on Instat software.

Results

FITC-labeled PEG in Spinal Cord

Very localized regions of spinal cord tissue surrounding blood vessels and capillaries
 10 were faintly marked in uninjured spinal cord rostral or caudal of the injury – nearly at the level of detection (FIG. 6A). This faint labeling was evident around larger vessels of the gray matter and those associated with the pial surface. Crushed regions of spinal cord were heavily labeled *in all animals* independent of the means of FI-PEG administration. Furthermore, this intense labeling of spinal cord parenchyma was confined to the region of contused gray and white matter but did
 15 not extend into adjacent, intact, spinal cord parenchyma (FIG. 6, B-D). In summary, PEG specifically labeled the spinal cord lesion but not undamaged tissues of adjacent regions.

PEG mediated recovery of conduction

Prior to the crush injury of the spinal cord, tibial nerve evoked SSEPs usually segregate into
 20 an early and late arriving peaks of CAPs recorded from the sensory cortex (P1 and P2) [Borgens, R. and Shi, R. (2000): Immediate recovery from spinal cord injury through molecular repair of nerve membranes with polyethylene glycol, FASEB, 14, 27-35]. As in prior experiments these peaks are completely eliminated following a severe constant displacement crush to the midthoracic spinal cord (FIG. 4).

25 During the 1 month of observation following a single injection of PEG or an injection of carrier in Control animals, *not one* control animal recovered the ability to conduct CAPs through the lesion as measured by SSEP recording compared to a variable recovery of CAP magnitudes recorded to arrive at the sensorimotor cortex in 100% of the PEG-treated animals ($P = 0.0001$; Fishers Exact two-tailed test; FIG. 7B; Table 1).

Table 1.

Treat-Ment	# of Anim als	% loss, area of Areflexia ¹	%CTM Recovered ²	# CTM Recoverd ²	# SSEP Recovered ³	Area:CAP(P1) pre-injury in pixels ⁴	Area:CAP P1), Post-injury in pixels ⁴	Stat ⁵
PEG	10	43.6 ± 0.03	32.7±7.5	7/10	10/10	17026 ± 258	11482 ± 144	P=0.14
Cont	10	42.5 ± 0.02		0/10	0/10			
		P=0.8 ⁶		P=0.003 ⁷	P=0.001 ⁶			

¹The % loss of the CTM receptive field = unit area of areflexia in mm²/total intact pre-injury receptive field in mm²

²The average percent (and SEM) of the former region of areflexia that recovered following PEG treatment at 1 month.

³Number of animals recovered/the total number of animals

⁴The unit area in pixels comprising the early arriving SSEP peak (see methods)

⁵Comparison of pre and post-injury CAP; Mann Whitney, two tailed test

⁶Fisher's exact test, two tailed

⁷Mann Whitney, two tailed test

A decrease in the amplitude and extended duration of the CAP is typical of recovering nerve impulses. Thus, it is both useful and possible to compare the change in CAP shape *before the injury* and *after* recovery to determine a relative index of the degree of CAP recovery. In this study, the area under the early arriving peak (P 1) was measured in pixels in only PEG-treated animals (since there were no recoveries of SSEPs in Control animals). If 100% of all single nerve fibers contributing to the CAP were once again recruited into conduction subsequent to the injury – but with a decreased amplitude and extended latency period – the normalized mean area under the curve (CAP above baseline) divided by the same pre-injury data should approach unity (1.0). In this experiment, integration of the magnitude (in mVs) and latency (in ms) of PEG-treated animal's SSEP P1 divided by the same pre-injury data equaled 0.88 (Preinjury Mean = 1706, SEM = 2583 pixels. Post-PEG mean = 11482, SEM = 1445 pixels, N = 10). Paired statistical comparison of these data also confirmed there was not a statistically significant difference in their means, further suggesting limited change in the CAP following PEG mediated recovery (P = 0.14, Students T test, paired two tailed comparison). Altogether these calculations suggest a significant recruitment of injured nerve fibers into CAP conduction following PEG treatment that would not have occurred otherwise.

Recovery of the CTM Reflex

The proportion of recovered and unrecovered animals, as well as the unit area of the recovered CTM receptive fields between controls and PEG- injected animals was quantitatively compared. The unit area of back skin that did not respond to CTM stimulation following the injury – *but before PEG treatment* - was statistically similar in both groups ($P = 0.81$; Mann Whitney, two tailed test; Table 1). Thus, the spinal injury produced a similar level of CTM behavioral loss in *all* animals. In the 10 PEG-treated animals, 3 recovered CTM function within 24 hours of the injection, 3 more within the first week of the treatment, and 7 by two weeks. The area of recovering backskin of these ten animals continued to increase in size to week four when the experiment was ended. The mean area of recovered CTM receptive fields was approximately 33%. Not one control animal of 10 showed spontaneous recovery of any portion of the CTM receptive field during the 1-month of observation (which was first observed at week 4). The difference in the frequency of recovery between PEG-injected and sham-injected animals was statistically significant ($P \leq 0.03$, Fishers Exact Test, two tailed). Similar results were also achieved in a smaller number of spinal animals in response to a single intraperitoneal injection of PEG (data not shown).

Discussion

PEG is well known to be able to fuse numerous single cells *in vitro* into one giant cell, as well as join the membranes of neurons and giant invertebrate axons [Bittner, G.D., Ballinger, M.L., and Raymond, M.A. (1986): Reconnection of severed nerve axons with polyethylene glycol, *Brain Research*, 367, 351-355; Davidson, R.L. and Gerald, P.S. (1976): Improved techniques for the induction of mammalian cell hybridization by polyethylene glycol, *Somat. Cell Genet.*, 2, 165-176; O'Laigue, P.H. and Hunter, S.L. (1980): Physiological and morphological studies of rat pheochromocytoma cells (PC12) chemically fused and grown in culture, *Proc. Nat. Acad. Sci. USA*, 77, 1701-1705]. As a "proof of concept" of the reparative capability of PEG application, variable amounts of completely *severed* guinea pig white matter axons were physiologically and anatomically reconnected in isolated spinal cord [Shi, R., Borgens, R.B., and Blight, A.R. (1999): Functional reconnection of severed mammalian spinal cord axons with polyethylene glycol, *J. Neurotrauma*, 16, 727-738]. This result is less relevant to clinical spinal cord injury since transections are rare – but set the stage for further testing of the usefulness of the polymer in severely crushed CNS and PNS nerve fiber tracts.

In previous reports it has been shown that the reversal of conduction loss in injured spinal cord was associated with a PEG-mediated sealing of breaches in the nerve membrane produced

by mechanical damage [Shi, R and Borgens, R.B. (2001): Anatomical repair of nerve membranes in crushed mammalian spinal cord with polyethylene glycol, J Neurocytol, in press]. Breaches in nerve membrane allow the unregulated exchange of ions between the extracellular and intracellular compartments. This causes an immediate local collapse in membrane potential and the failure of nerve impulse conduction through this region of the axon. This initial conduction block accounts for the immediate functional loss following SCI, which becomes permanent due to progressive anatomical degeneration of injured nerve fibers and spinal parenchyma - so called "secondary injury" [Young, W. (1993): Secondary injury mechanisms in acute spinal cord injury, J. Emerg. Med., 11, 13-22; Tator, C.H. and Fehlings, M. G. (1991): Review of the secondary injury theory of acute spinal cord trauma with emphasis on vascular mechanisms, J. Neurosurg 75, 15-26]. The remarkable increases in cytosolic Na^+ and Ca^{++} moving down their concentration gradients into the cell (or local region of its process) through compromised membrane is implicated in the destruction of the cell's cytoskeleton and triggers a cascade of degenerative changes that unchecked, leads to axotomy, sometimes cell death [Borgens, R.B. (1988): Voltage gradients and ionic currents in injured and regenerating axons, Advances in Neurology, Vol. 47: Functional Recovery in Neurological Diseases, (Waxman, S.G., ed.), pp.51-66 Raven Press, New York; Maxwell, W.L. and Graham, D.I. (1997): Loss of axonal microtubules and neurofilaments after stretch-injury to guinea pig optic nerve fibers, J Neurotrauma, 14, 603-614]. There is clear evidence that PEG treatment intervenes in this process by sealing the membrane, quickly restoring its ability to propagate nerve impulses and inhibiting the progressive dissolution of cells of the spinal cord predicated on the breakdown of the membrane's barrier properties. This result was shown using a dye exclusion test where PEG treatment largely inhibited the uptake of a horseradish peroxidase (HRP; about 40,000 Daltons) marker into damaged axons of crushed guinea pig spinal cord. This effect was also independent of axon caliber [Shi, R. and Borgens, R.B. (2001): Anatomical repair of nerve membranes in crushed mammalian spinal cord with polyethylene glycol, J Neurocytol in press]. This seal produced by PEG is not perfect however, in spite of the recovery of membrane excitability. Reports have been made that local application of the fast potassium channel blocker 4-Aminopyridine nearly doubles the magnitude of the recovered CAP in vitro testing [Shi, R. and Borgens, R. (1999): Acute repair of crushed guinea pig spinal cord by polyethylene glycol, J. Neurophysiology, 81, 2406-2414] suggesting that the PEG-sealed region of membrane is still leaky to potassium.

Membrane breaches secondary to mechanical damage large enough to permit the uptake of large molecular weight intracellular labels such as horseradish peroxidase (HRP) – a common means to introduce such markers into neurons [Borgens, R.B., Blight, A.R. and Murphy, D.J.

(1986): Axonal regeneration in spinal cord injury: A Perspective and new technique, *J. Comp. Neurol.*, 250, 157-167; Malgrem, L. and Olsson, (1977): A sensitive histochemical method for light and electron microscope demonstration of horseradish peroxidase, *Y. J. Histochem. Cytochem.*, 25, 1280-1283] - likely progress on to such a size as to lead to secondary axotomy.

5 The destruction of the white matter has been implicated as producing a robust signal for the inflammatory processes which further destroy the cells and tissues of the spinal cord – essentially collateral damage to healthy cells. The histology of PEG-treated spinal cord lesions has been compared to controls by computer managed quantitative 3 D spinal cord reconstruction techniques [Duerstock, B.S., Bajaj, C.L., Pascucci, V., Schikore, D., Lin, K-N., and Borgens, R.B. (2000): Advances in three-dimensional reconstructions of the experimental spinal cord injury, *Computer Medical Imaging and Graphics*, 24 (6), 389-406]. In these studies a topical application of PEG produced 1-month-old spinal cord lesions of smaller volume, and possessing less cavitation than measured in control animals (to be reported elsewhere). These data strongly suggests that polymeric sealing of nerve cell membranes is also reflected in an overall reduction in spinal cord pathology which can be observed many weeks later.

Evaluation of the ability of this agent and other water-soluble membrane sealing polymers such as the poloxamers and poloxamines continues [Padanlam, J.T., Bischof, J.C., Cravalho, E.G., Tompkins, R.G., Yarmush, M.L. and Toner, M. (1994): Effectiveness of Poloxamer 188 in arresting calcein leakage from thermally damaged isolated skeletal muscle cells. *Ann N.Y. Acad. Sci.* 92, 111-123; Palmer, J.S., Cromie, W.J. and Lee, R.C. (1998): Surfactant administration reduces testicular ischemia-reperfusion injury, *J. Urology*, 159, 2136-2139; Lee, R., River, L.P., Pan, F.S., Wollmann, L. Jr. and R.L. (1992): Surfactant-induced sealing of electroporabilized skeletal muscle membranes in vivo, *Proc. Natl. Acad. Sci. U.S.A.*, 89, 4524-4528] as novel treatments for severe CNS and PNS injury, as well as head injury and stroke.

Since the PEG injection can be made many hours after injury, clinical testing of an intravenous (IV) PEG administration to severe, acute, natural cases of paraplegia in dogs has begun [Borgens, R.B., Toombs, J.P., Blight A.R., McGinnis M.E., Bauer, M.S., Widmer, W.R. and Cook Jr., W.R. (1993): Effects of applied electric fields on clinical cases of complete paraplegia in dogs, *J. Restorative Neurology and Neurosci.*, 5, 305-322; Borgens, R.B., Toombs, J.P., Breur, G., Widmer, W.R., Water, D., Harbath, A.M., March, P. and Adams, L.G. (1999): An imposed oscillating electrical field improves the recovery of function in neurologically complete paraplegic dogs, *J. of Neurotrauma*, 16, 639-657]. This means of clinical development is unique to this spinal research center and has been previously used to develop two other laboratory animal derived treatments for spinal injury [Borgens, R.B., Toombs, J.P., Breur, G.,

Widmer, W.R., Water, D., Harbath, A.M., March, P. and Adams, L.G. (1999): An imposed oscillating electrical field improves the recovery of function in neurologically complete paraplegic dogs, *J. of Neurotrauma*, 16, 639-657; Blight, A.R., Toombs, J.P., Bauer, M.S. and Widmer, W.R. (1991): The effects of 4-aminopyridine on neurological deficits in chronic cases of traumatic spinal cord injury in dogs: a phase I clinical trial, *J. Neurotrauma*, 8, 103-119] into human clinical testing. In this new trial, PEG administration is an adjunct to the routine management of neurologically complete spinal injured dogs since the polymer can be safely introduced in the IV fluids administered soon after their admission to the hospital. Though this clinical trial is not yet completed, preliminary observations are encouraging, and appear to show unexpected recoveries of varied functions within hours to a few days after PEG injections.

Example 3

Intravenous Hydrophilic Polymer Induces Rapid Recovery from Clinical Paraplegia in Dogs

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This example demonstrates a swift, striking, and statistically significant recovery of multiple functions in clinical cases of severe, acute, naturally occurring paraplegia in dogs. Recovery of function occurred in response to a combination of topically applied, and intravenously administered, polyethylene glycol (PEG). Recoveries of sensory and motor functions occurred rapidly and at all time points studied between 3 days and 6-8 weeks post-injury.

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Admission and Treatment

Dogs with spinal cord injuries were admitted to the emergency services of the University Veterinary Teaching Hospitals (UVTH) at Texas A&M University, College Station, Texas, and at Purdue University, West Lafayette, Indiana. An identical protocol for admission, neurological evaluation, treatment, and follow up (R.B. Borgens *et al.*, *J. Restorative Neurology and Neurosci.* 5, 305 (1993); R.B. Borgens *et al.*, *J. Neurotrauma* 16, 639 (1999)) was adhered to by each Research Center. In special circumstances, computerized x-ray tomography (CT) imaging was available in addition to routine radiography and myelography at the Texas Center, while electrophysiological study of nerve impulse conduction through the spinal cord lesion by evoked potential testing was performed at Purdue University.

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Each dog received a radiological examination (Figs. 9A-9D), and a thorough, videotaped, neurological examination (Fig. 10A) that included: 1) tests for deep pain in hind limbs and digits, 2) superficial pain appreciation below the level of the injury in flank, lower limbs and

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digits, 3) proprioceptive evaluation of the hind limbs (i.e. conscious proprioception), 4) evaluation of hind limb load-bearing and voluntary locomotion, and 5) spinal reflex testing (patellar, tibialis, cranialis, flexor withdrawal, and sciatic reflexes). Tests 1–4 were also used as functional measures of outcome and were quantitatively scored using previously reported techniques and methods (R.B. Borgens *et al.*, *J. Restorative Neurology and Neurosci.* 5, 305 (1993); R.B. Borgens *et al.*, *J. Neurotrauma* 16, 639 (1999)) (Fig. 10A). These data then provided a total neurological score (TNS) (R.B. Borgens *et al.*, *J. Restorative Neurology and Neurosci.* 5, 305 (1993); R.B. Borgens *et al.*, *J. Neurotrauma* 16, 639 (1999)) for each dog at each time point tested. Since neurological recovery is varied in its expression between animals, the most valid means to compare outcomes is by comparison of the TNS (R.B. Borgens *et al.*, *J. Restorative Neurology and Neurosci.* 5, 305 (1993)). All dogs admitted to the clinical trial possessed the worst clinical signs for spinal injury secondary to spinal cord compression characterized by complete paraplegia, urinary and fecal incontinence, and lack of deep pain response [grade 5 lesions (J.R. Coates, *Common Neurological Problems* 30, 77 (2000))]. These functional tests (and others, see below) were also used as exclusion criterion so that neurologically “incomplete” dogs were not included in the trial. Additionally, only paraplegic dogs with upper motor neuron syndrome - true spinal cord injuries - were study candidates. A persistent lack, or hyporeflexia of the lower limb(s) revealed segmental compromise of spinal cord circuitry or “lower motor neuron sequela”. This was sufficient to exclude animals from the study (R.B. Borgens *et al.*, *J. Restorative Neurology and Neurosci.* 5, 305 (1993); R.B. Borgens *et al.*, *J. Neurotrauma* 16, 639 (1999)). During the initial clinical evaluation, owners were asked to review a document concerning the experimental treatment, and then requested to sign an informed consent should they wish to participate in the study.

Next, paraplegic dogs received the first of two intravenous injections of PEG. Later, but within 24 hours of admission, the location of the lesion was determined by survey radiography and myelography (Figs. 9A-9D). The latter examination insured that myelomalacia was limited to less than 1 vertebral segment. All dogs received an injection of methylprednisilone sodium succinate (30mg/kg body weight), underwent general anesthesia, and taken to surgery. All injured dogs received *standard – of – care* veterinary management of these injuries, including surgical decompression of the affected site and fixation of the vertebral column when required. The dura was removed during decompressive surgery, exposing the spinal cord lesion, and about 1cc of the PEG solution (about 2000daltons, 50% W/W in sterile saline; 150mg/kg body weight) was layered onto the injury site. The polymer was aspirated from the surface of the exposed cord within 2 min of application, next the region lavaged with sterile Ringer’s solution, and these fluids aspirated as well. A fat pad graft was placed superficially, the incision closed, and the

animals taken to the Intensive Care Unit (ICU) for recovery. Within 24 hours of surgery, a second injection of PEG, identical to the first, was performed usually in ICU. Animals were monitored within the hospital for 7–10 days, and a full neurological exam, videotaped as was the original, was performed approximately 3 days (74 ± 9 hours) post surgery, approximately 1 week post surgery at discharge ($6.8 \text{ days} \pm 1.2 \text{ days}$) and at 6–8 weeks post surgery at recheck. As in past clinical trials, owners were provided detailed instructions concerning care of their animals (i.e. bladder expression, skin care, etc.) and, initially, use of a wheeled cart (K–9 Carts, Montana) to aid in the dog's rehabilitation (R.B. Borgens *et al.*, *J. Restorative Neurology and Neurosci.* 5, 305 (1993); R.B. Borgens *et al.*, *J. Neurotrauma* 16, 639 (1999)). However this latter practice was discontinued after only 3 admissions because the recovery of function was so rapid (see below) such as to make the use of the cart unnecessary.

Control Dogs

During the development of the experimental protocol, paraplegic dogs recovered rapidly and unexpectedly within a few days after PEG administration. Participating neurosurgeons believed it unethical to carry out a control procedure (intravenous injection of the solvent for PEG - sterile saline) knowing full well these client-owned animals would sustain variable, but severe, life long behavioral losses (R.B. Borgens *et al.*, *J. Neurotrauma* 16, 639 (1999)). Given the ca. 48-hour window in treatment, it was also not possible to perform a single cross-over study. Thus a medical decision was made to use historical controls rather than inject such severely injured animals with sterile salt water. Relevant historical control data was obtained for sham-treated dogs from recent peer reviewed and published studies performed at the Indiana Center (R.B. Borgens *et al.*, *J. Restorative Neurology and Neurosci.* 5, 305 (1993); R.B. Borgens *et al.*, *J. Neurotrauma* 16, 639 (1999)). These control dogs were 1) also admitted to veterinary clinical trials restricted to only neurologically complete cases of acute canine paraplegia, 2) received *identical* conventional management as described above, 3) were neurologically evaluated by *identical* methods, and excluded from the studies by *identical* exclusion criteria (R.B. Borgens *et al.*, *J. Restorative Neurology and Neurosci.* 5, 305 (1993); R.B. Borgens *et al.*, *J. Neurotrauma* 16, 639 (1999)) (see FIGS. 9A-9E and FIGS. 11A and 11B) were evaluated at the same time points, and 5) in most cases, their neurological scores were derived by the same investigators participating in this trial (R.W., G.B., J.T., R.B). It is important to emphasize that all investigators were completely blinded to the experimental or control status of all dogs recruited into these previous trials. The use of these identical procedures in recruitment and particularly in the scoring of neurological functions yielded little to no variation between the multiple investigators when their individual scores were compared (R.B. Borgens *et al.*, *J. Restorative Neurology and Neurosci.* 5, 305 (1993); R.B. Borgens *et al.*, *J. Neurotrauma* 16, 639

(1999)). The validity of this comparison appears to be eminently greater than data gathered from the veterinary literature. These latter investigations do not: i) use multiple neurological functions as exclusion criteria to limit the possibility that evaluations would include neurologically incomplete dogs, ii) report a complete axis of neurological behavior including the function of relevant lower spinal reflexes, iii) use the outcome measures used here, or iv) evaluate animals at the same post-surgical time points post surgery.

For our comparison complete medical records, score sheets, and video tapes were available for 14 control (sham-treated) dogs from 1993 (R.B. Borgens *et al.*, *J. Restorative Neurology and Neurosci.* 5, 305 (1993)) and 11 control dogs from 1999 (3) – 25 dogs total for comparison to 20 PEG-treated dogs. Moreover, in the latter clinical trial (R.B. Borgens *et al.*, *J. Neurotrauma* 16, 639 (1999)), the experimental application (oscillating field stimulation) was delayed in 12 experimental dogs for about 96 hours after surgery to determine what, if any, early functional recovery could be associated with surgery and steroid treatment alone. The neurological status of this subset of dogs was reported (R.B. Borgens *et al.*, *J. Neurotrauma* 16, 639 (1999)). These data then, provided a total of 37 control dogs to compare to 20 PEG-treated dogs at the 3 day time point, and 25 control dogs for comparison at the 1 week and 6-8 week neurological checkups.

Clinical Responses to Polymer Administration in Paraplegic Dogs

The most sensitive indicator of early functional recovery in clinical cases of neurologically complete canine paraplegia is the reappearance of deep pain response in hind limbs and digits (R.B. Borgens *et al.*, *J. Restorative Neurology and Neurosci.* 5, 305 (1993); R.B. Borgens *et al.*, *J. Neurotrauma* 16, 639 (1999); J.R. Coates, *Common Neurological Problems* 30, 77 (2000)). This was evaluated in 17 of the 20 acutely injured PEG-treated dogs approximately 3 days after surgery (approximately 48 hours after the second injection of PEG (Fig. 10A). During this time, 4 of the 17 PEG-treated dogs recovered deep pain, while only one of the 37 control dogs had ($P=0.03$; Fisher's exact test, two tailed, in this and all subsequent comparison of proportions).

Comparison of the mean TNS at this time, a numbers derived largely from recoveries in deep and superficial pain, was markedly statistically significantly improved in the PEG-treated group compared to controls ($P = 0.009$; comparison of means here and below were made using a Students' T test, two tailed, or the Welch variation, FIGS. 9A-9E).

Though more than half of the population of PEG-treated dogs had recovered deep pain responses by 1 week post-treatment, improvements in deep pain in 25 control dogs eliminated significance in this one functional comparison between the groups at this time point ($P = 0.2$).

However, recoveries in proprioception, improvement in load bearing in hind limbs and voluntary walking in 8 PEG-treated dogs of 20 at this time were *unmatched by such improvements in control dogs*. Analysis revealed marked statistically significant improvement in the TNSs of PEG-treated dogs at this time point ($P = 0.007$; FIGS. 9A-9E).

5 The total neurological scores of control dogs showed modest and progressive improvement by the 6-week recheck, however, this remained manifest as mainly improvements *in the quality of pain appreciation* (R.B. Borgens *et al.*, *J. Restorative Neurology and Neurosci.* 5, 305 (1993); R.B. Borgens *et al.*, *J. Neurotrauma* 16, 639 (1999)). Thus, there was no significant difference between the PEG-treated and control dogs when the proportions of
10 animals with positive deep and superficial pain responses were compared ($P = 0.1$ and 0.6 , respectively). However, this improvement in pain appreciation was not matched by any of the other outcome measures evaluated in control dogs. Thirty-five percent (7 of 20) of the PEG-treated dogs recovered measurable proprioception by 6 weeks, while only 1 of 25 (4%) of control animals had improved proprioception ($P = 0.01$). Fully 70% of all PEG-treated dogs (14 of 20)
15 could ambulate voluntarily, compared to only 28% (7 of 25) of controls ($P = 0.007$). Furthermore, the overall quality of functional recovery secondary to PEG administration at the 6 week recheck (as given by the total neurological score) was strikingly improved by PEG treatment relative to controls ($P \leq 0.0008$; FIGS. 9A-9E).

Qualitatively, the two groups appeared quite different in a manner masked by the dry
20 recitation of quantitative neurological scores and proportions. The possible range of an individual dog's total neurological score was 4 (a totally paraplegic dog) to 20 (a totally normal dog (see FIGS. 9A-9E). Fifteen of the 25 control dogs (60%) remained neurologically complete paraplegics 6–8 weeks after decompressive surgery and corticosteroid treatment, all were individually assessed a neurological score of 4. The best performing control dog scored 11 at
25 this time point (R.B. Borgens *et al.*, *J. Neurotrauma* 16, 639 (1999)). However, this animal remained seriously impaired; locomotion alone was evaluated as only a score of 2. PEG treatment resulted in 35% (7 dogs of the 20) individually scoring 13 to 16. By the 6–8 week recheck some dogs had made a such a striking recovery – to the extent any remaining functional loss could only be determined by a thorough neurological examination. Only 3 of 20 PEG-
30 treated dogs (15%) remained paraplegic at the end of the 6–8 week period of observation (a highly significant comparison to controls, $P = 0.003$).

Electrophysiology and Bladder Management

Evoked potential testing [Somatosensory Evoked Potential or SSEP(R.B. Borgens *et al.*,
35 *J. Restorative Neurology and Neurosci.* 5, 305 (1993); R.B. Borgens *et al.*, *J. Neurotrauma* 16,

639 (1999)), FIGS. 10A-10C] was performed on 11 of the 12 PEG-treated dogs recruited to the Purdue Center to determine if nerve conduction through the lesion had been restored (FIGS. 10A-10C). Somatosensory Evoked Potential recordings could not usually be obtained prior to the first PEG injection and surgery due to the need to move these animals through the battery of evaluations and on to surgery as soon as possible after admission. In addition, most dogs were unable to be sedated for such tests in the first few hours after admission due to food intake and other complicating factors. Of the 11 dogs on which electrophysiological tests of conductance were performed at more than two recheck periods, 7 were recorded to have positive SSEPs, while 4 did not demonstrate evidence of nerve impulse conductance through the lesion. All four dogs scoring above the median TNS of 12 showed a clear recovery of conductance through the lesion. Furthermore, one severely injured animal (fracture/dislocation and subluxation of the vertebral column) was accessible for SSEP testing during the second of two PEG injections. This animal showed a progression from a negative SSEP (flatline) to low amplitude, long duration, cortical potentials during the 30 min period of injection and observation of the sedated animal (FIGS. 11A, 11B).

In contrast, of the 11 control dogs from the 1993 study, none recovered SSEP conduction by even 6 months post injury [refer to page 313 (R.B. Borgens *et al.*, *J. Restorative Neurology and Neurosci.* 5, 305 (1993))]. Only two control dogs of 14 recovered measurable conduction by 6–8 months in the 1999 clinical trial [refer to page 649 (R.B. Borgens *et al.*, *J. Neurotrauma* 16, 639 (1999))]. This proportion of PEG-treated dogs (7 of 11) that recovered ascending electrophysiological conduction through the lesion was highly statistically significant compared to the relative lack of recorded evoked potentials in control dogs ($P=0.001$).

The status of bladder continence due to paraplegia is problematic in dogs just as in man. We have found that electrophysiological measurements of micturation (urethral pressure profilemetry and cystometry) while providing data relevant to isolating cases of lower motor neuron syndrome, do not correlate highly with observations of recovery of urinary continence particularly by owners (R.B. Borgens *et al.*, *J. Neurotrauma* 16, 639 (1999)). Incontinence is not easily confused by owners, since it represents a major behavioral loss in the dog's "house training" and is the most common reason given for euthanasia of their pets. Moreover, a consistent failure of owners to manually express the bladder of incontinent dogs leads to readmission for urinary tract infection. Of the 20 PEG-treated dogs, owners reported all but 6 were continent, and did not require bladder expression. These latter animals were of a group of PEG treated dogs exhibiting the least recovery at the end of the study. We offer these facts as additional but modest evidence that recovery from paraplegia mediated by PEG likely improved or eliminated at least urinary incontinence as well.

Paraplegia in Laboratory Animals, Dogs, and Man

The history of spinal cord injury research can be characterized in some part by the quest for standardized injury methods and credible means to assay behavioral loss and recovery in laboratory animals – usually adult guinea pigs, rats, or cats. There has always been debate and controversy concerning both of these quests. In the former, the difficulty centers on various different techniques used to induce injury to the exposed spinal cord. For example, constant impact [usually standardized weight drop techniques (S.K. Somerson, and B.T. Stokes, *Exp. Neurol.* 96, 82 (1987))], constant compression of the spinal cord [using specially fabricated clips (A.S. Rivlin, C.H. Tator, *Surg. Neurol.* 10, 39 (1978)) or forceps (A.R. Blight, *J. Neurologic. Sci.* 103, 156 (1990))] and partial or complete transection (R.B. Borgens, A.R. Blight, D.J. Murphy, *J. Comp. Neurol.* 250, 157 (1986)), of the spinal cord have been employed and contrasted (R.B. Borgens, A.R. Blight, D.J. Murphy, *J. Comp. Neurol.* 250, 157 (1986)). With the exception of the latter technique, an important goal of these methods has been to reduce the variability in lesions, and to produce a central hemorrhagic injury typical of clinical injury in man (A.R. Blight, *J. Neurologic. Sci.* 103, 156 (1990); A.R. Allen, *J. Am. Med. Assoc.* 57, 878 (1911); C.H. Tator, M.J. Fehlings, *Neurosurg.* 75, 15, (1991)). While the successes of the different approaches can be debated relative to these goals, there is no question that modern laboratory injuries are made to the surgically exposed spinal cords of anesthetized animals producing an initially dorsal locus of injury. This is inconsistent with most clinical injuries where the initial site of SCI (“spinal cord injury”) injury is anterior (ventral), and the impact is to the trunk of the body or neck (so called “closed” injuries). Moreover, during experimental insult to the cord, anesthesia provides neuroprotection (S.K. Salzman, M.M. Mendez, S. Sabato, et al., *Brain Res.* 521, 33 (1990)), and is a complicating factor. Thus, naturally-occurring injuries in dogs provide a more direct comparison to clinical injuries in man (R.B. Borgens, in *Spinal Cord Dysfunction, Volume III: Functional Stimulation*, L.S. Illis, Ed. (Oxford Medical Publications, Oxford, 1992), chap 5).

There have also been numerous and varied attempts to measure and/or quantitate the behavioral recovery from SCI in laboratory models of spinal injury. Measurement of hindlimb locomotion (M.D. Basso, M. Beattie, J.D. Bresnahan, *J. Neurotrauma* 12, 1 (1995)), or some form of it (A.S. Rivlin, C.H. Tator, *J. of Neurosurgery* 47, 577-581 (1977)), has dominated rodent studies of SCI – usually because of the underlying notion that the results might be relevant to lower limb locomotion in man even though there is no evidence to support such a view. Humans are the only obligatory bipedal mammals, and upright walking is completely dominated by supraspinal control (S. Mori, K. Matsuyama, E. Miyashita, K. Nakajima, M.

Asanome, *Folia Primatologica* 66 , 192 (1996)). In experimental SCI models, locomotion is dominated by locally controlled and generated stepping (S. Rossignol, R. Dubuc, *Curr. Opin. Neurobiol.* 4, 894-902 (1994); A. Naito, Y. Shimizu, Y. Handa, *Neurosci Res* 8, 281 (1990)). Such walking behavior is often called "spinal walking" to separate it from walking behavior that requires the restored transmission of nerve impulses through the spinal cord lesion from higher centers. Because restored nerve impulse traffic through the lesion is not required for voluntary ambulation in animals, walking behavior by itself does not represent a valid behavioral recovery with which to infer restored conduction through the lesion. This requires use of kinestheseological methods confirming fore limb and hind limb coordination during voluntary locomotion.

For all of the above reasons, no attempt has been made to develop even more complicated systems to grade walking behavior associated with clinical paraplegia in dogs. Instead, reliance is placed on a simple 5-point score that provides a reliable, precise reflection of increasing capabilities in ambulation, but without additionally attempting to indicate the neural mechanisms of action underlying it (R.B. Borgens *et al.*, *J. Restorative Neurology and Neurosci.* 5, 305 (1993); R.B. Borgens *et al.*, *J. Neurotrauma* 16, 639 (1999)).

In the present example, whatever the mechanism underlying the return of voluntary walking – a strikingly significant number of dogs walked with superior capability than occurred in controls. Moreover, the statistically significant improvement in TNS is a clear indication of substantive, and meaningful recovery in several other clinically relevant areas of function, including: recovery in the neurological appreciation of both deep and superficial pain, recovery of ascending nerve impulse conductance through the lesion, recovery of conscious proprioception, as well as substantial load bearing and voluntary walking.

The strengths of these methods as applied to naturally occurring paraplegia are that they provide real potential for assessing the clinical importance of experimental therapies for human SCI (R.B. Borgens *et al.*, *J. Neurotrauma* 16, 639 (1999); A.R. Blight, J.P. Toombs, M.S. Bauer, W.R. Widmer, *J. Neurotrauma* 8, 103-119 (1991)). The weakness of this SCI model is that little is learned about the biological basis for the response to treatment. This is more easily achieved in laboratory models where invasive physiological testing and anatomical techniques can be applied (R.B. Borgens, in *Spinal Cord Dysfunction, Volume III: Functional Stimulation*, L.S. Illis, Ed. (Oxford Medical Publications, Oxford, 1992), chap 5).

Polymer Application in Experimental SCI

Both topical and/or intravascular administration of polyethylene glycol (2000-3000 Daltons, approximately 30–50% W/W in water) has been documented to induce:

- 1) Rapid (minutes) anatomical fusion of severed white matter axons (R. Shi, R.B. Borgens, A.R. Blight, *J. Neurotraum* 16, 727 (1999)) and rapid sealing of anatomic breaches in both myelinated and unmyelinated axons of guinea pig ventral white matter (R. Shi, R.B. Borgens, *J. Neurophysiology* 81, 2406 (1999)). In both cases neural tissue was maintained and evaluated *in vitro* in a double sucrose gap recording chambers (R. Shi, A.R. Blight, *Neuroscience* 77, 553-562 (1997)).
- 2) Rapid (minutes) recovery of nerve impulse conduction through the lesion in these same studies (R. Shi, R.B. Borgens, A.R. Blight, *J. Neurotraum* 16, 727 (1999); R. Shi, R.B. Borgens, *J. Neurophysiology* 81, 2406 (1999)) – or through severe and standardized crush injuries to the guinea pig spinal cord *in vivo*, measured by SSEP testing (R.B. Borgens, R. Shi, *FASEB* 14, 27 (2000); R.B. Borgens, D.M. Bohnert, *J. Neurosci. Res.* 66, 1179 (2001); R.B. Borgens, R. Shi, D.M. Bohnert, *J. Exp. Bio.* 205, 1 (2002)).
- 3) Rapid (hours to days) recovery of long-tract dependent spinal cord reflex (the cutaneous trunci muscle or CTM reflex) (R.B. Borgens, R. Shi, *FASEB* 14, 27 (2000); R.B. Borgens, D.M. Bohnert, *J. Neurosci. Res.* 66, 1179 (2001); R.B. Borgens, R. Shi, D.M. Bohnert, *J. Exp. Bio.* 205, 1 (2002)), which is totally dependent on the integrity of an identified white matter column of axons within the ventral funiculus of the guinea pig (A.R. Blight, M.E. McGinnis, R.B. Borgens, *J. Comp. Neurol.* 296, 614-633 (1990)) and rat (E. Thierault, J. Diamond, *J. Neurophysiol.* 60, 446-447 (1988)) spinal cord.

A variable level of recovery of the CTM reflex (produced by compression of the spinal cord) occurred in > 90% of PEG-treated guinea pigs, compared to a range of 0–17% in sham-treated control populations in three separate studies (R.B. Borgens, R. Shi, *FASEB* 14, 27 (2000); R.B. Borgens, D.M. Bohnert, *J. Neurosci. Res.* 66, 1179 (2001); R.B. Borgens, R. Shi, D.M. Bohnert, *J. Exp. Bio.* 205, 1 (2002)). The recovery of cortical potentials was documented as restored volleys of SSEPs measured to arrive at the sensory motor cortex following electrical stimulation of the tibial nerve of the hind limb. In all (100%) of the control guinea pigs, such nerve impulse conduction through the lesion was eliminated for the 1 month of observation. In PEG-treated animals, SSEPs recovered in 100% of the population in these same three investigations (R.B. Borgens, R. Shi, *FASEB* 14, 27 (2000); R.B. Borgens, D.M. Bohnert, *J. Neurosci. Res.* 66, 1179 (2001); R.B. Borgens, R. Shi, D.M. Bohnert, *J. Exp. Bio.* 205, 1 (2002)).

Mechanisms of polymer based therapy for neurological injuries

The molecular mechanisms of action of, for instance, surfactants and tri-block polymers in sealing or fusing cell membranes have been reviewed in the literature. (R. B. Borgens,

Neurosurgery 49, 370-379 (2001); B.R. Lentz, *Chem.Phys.Lipid* 73, 91 (1994); J. Lee, B.R. Lentz, *Biochemistry* 36, 6251 (1997); J.M. Marks, C-Y. Pan, T. Bushell, W. Cromie, R.C. Lee *FASEB J* 15,1107 (2001).) Briefly: an initial mechanism common to all hydrophilic surfactants that may be beneficial to soft tissue trauma is the formation of a chemical film sealing defects in the cell membranes at the site of mechanical damage. However, it is the watery-hungry character of this class of hydrophilic polymers (PEG, EPAN, and some dextrans) that is believed to instantly dehydrate the membrane locally. Furthermore, either removal or rearrangement of water molecules in the vicinity of membrane breach permits the lipid core of the intact membrane surrounding the breach – and perhaps the structural elements suspended in it – to merge into each other. When the polymer is removed, or in lowered concentration, variable amounts of structural self-assembly occur in response to reintroduction of the aqueous phase of the membrane. Triblock polymers such as poloxamers are comprised largely of PEG – yet they also possess a hydrophobic component (polypropylene oxide) which may actually target breaches in membranes – inserting into the breach where the hydrophobic core of the membrane is exposed (J.M. Marks, C-Y. Pan, T. Bushell, W. Cromie, R.C. Lee *FASEB J* 15,1107 (2001)). The long PEG side chains likely contribute to sealing in the fashion described above. We have tested poloxamer 188 in a spinal injury model in guinea pigs and have found no difference in the physiological and behavioral recoveries in response to PEG as described above. These findings suggest various polymers may prove beneficial for application to soft tissue trauma and other injuries to the nervous system (J.M. Marks, C-Y. Pan, T. Bushell, W. Cromie, R.C. Lee *FASEB J* 15,1107 (2001); J. Donaldson, R. Shi, R. Borgens, *Neurosurgery* 50, 147-157 (2002)).

Likely any large molecular polymer like PEG or poloxamers, introduced to the blood supply, will target only regions of tissue trauma where there is a loss of vascular integrity. We have demonstrated this by observing accumulation of a fluorescently labeled PEG in crushed guinea pig spinal cord – comparing intravenous, subcutaneous, and peritoneal administration with a topical application of the polymer to the exposed lesion (R.B. Borgens, D.M. Bohnert, *J. Neurosci. Res.* 66, 1179 (2001)). Labeling was barely detectable or non-existent in intact regions of the spinal cord in these same animals.

Of the putative mechanisms of action for PEG, formal proof of its membrane sealing properties have been demonstrated. The uptake of extracellular applied labels such as horseradish peroxidase (HRP), ethidium bromide, or the leakage of lactic dehydrogenase into the extracellular space, are excellent indices of cell membrane compromise (R. Shi, R.B. Borgens, *J. Neurocytology* 29, 633-643 (2000)). Both uptake of, and leakage of, these intracellular labels from injured white matter of the spinal cord is strikingly reduced or eliminated by PEG

administration. Furthermore, the susceptibility for axonal sealing is equal across a broad range of axon calibers (R. Shi, R.B. Borgens, *J. Neurocytology* 29, 633-643 (2000)).

5 We hypothesized this inhibition of leakage of the nerve fiber membrane reduces the opportunity for secondary axotomy to occur. This is consistent with the observation that PEG-treated cords are more intact, possess greater amounts of intact white matter, and a reduced lesion volume than in untreated guinea pig spinal cord as shown by quantitative comparison of three dimensional reconstructions of these spinal cords (B.S. Duerstock, R.B. Borgens, *J. Exp. Biol.* 205, 13 (2002)).

10 In summary, intravenous and topical administration of a hydrophilic polymer in clinical cases of acute neurologically complete spinal cord injury in dogs results in an unexpected, rapid recovery of multiple measures of functional outcome. Such a rapid and complete clinical recovery is not observed in response to conventional clinical/surgical management of neurologically complete injuries, including the administration of steroids, and decompressive surgery (J.R. Coats et. al., *Veterinary Surgery* 24, 128-139 (1995)).

15

While the invention has been illustrated and described in detail in the drawings and foregoing description, the same is to be considered as illustrative and not restrictive in character, it being understood that only the preferred embodiment has been shown and described and that all changes and modifications that come within the spirit of the invention are desired to be
20 protected.

THE EMBODIMENTS OF THE INVENTION FOR WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. Use of an effective amount of a composition comprising an effective amount of a biomembrane fusion agent formulated for parenteral delivery and a pharmaceutical carrier to treat an injury to nerve tissue of a mammalian patient, wherein the biomembrane fusion agent is an amphipathic polymer.
2. Use of a composition comprising an effective amount of a biomembrane fusion agent formulated for parenteral delivery and a pharmaceutical carrier in the manufacture of a medicament to treat an injury to nerve tissue of a mammalian patient, wherein the biomembrane fusion agent is an amphipathic polymer.
3. Use of an effective amount of a composition comprising an effective amount of a biomembrane fusion agent formulated for parenteral delivery and a pharmaceutical carrier to increase the conduction of action potentials in injured nerve tissue of a mammalian patient's central nervous system, wherein the biomembrane fusion agent is an amphipathic polymer.
4. Use of a pharmaceutical composition comprising an effective amount of a biomembrane fusion agent formulated for parenteral delivery and a pharmaceutical carrier in the manufacture of a medicament to increase conduction of action potentials in injured nerve tissue of a mammalian patient's central nervous system, wherein the biomembrane fusion agent is an amphipathic polymer.
5. A pharmaceutical composition comprising an effective amount of a biomembrane fusion agent formulated for topical delivery and a pharmaceutical carrier; wherein the biomembrane fusion agent is an amphipathic polymer.
6. Use of a pharmaceutical composition comprising an effective amount of a biomembrane fusion agent formulated for topical delivery and a pharmaceutical carrier to

increase conduction of action potentials in injured nerve tissue of a mammalian patient's central nervous system; wherein the biomembrane fusion agent is an amphipathic polymer.

7. Use of a pharmaceutical composition comprising a biomembrane fusion agent formulated for topical delivery and a pharmaceutical carrier in the manufacture of a medicament to increase conduction of action potentials in injured nerve tissue of a mammalian patient's central nervous system; wherein the biomembrane fusion agent is an amphipathic polymer.

8. Use of a pharmaceutical composition comprising an effective amount of a biomembrane fusion agent formulated for topical delivery and a pharmaceutical carrier to treat an injury to nerve tissue of a mammalian patient's central nervous system; wherein the biomembrane fusion agent is an amphipathic polymer.

9. Use of a pharmaceutical composition comprising a biomembrane fusion agent formulated for topical delivery and a pharmaceutical carrier in the manufacture of a medicament to treat an injury to nerve tissue of a mammalian patient's central nervous system; wherein the biomembrane fusion agent is an amphipathic polymer.

10. The composition of claim 5, wherein said composition further comprises a dextran.

11. The use according to any one of claims 1 to 4 or 6 to 9, wherein said composition further comprises a dextran.

12. The composition of claim 5, wherein said amphipathic polymer comprises a block copolymer.

13. The use according to any one of claims 1 to 4 or 6 to 9, wherein said amphipathic polymer comprises a block copolymer.

14. The composition of claim 12, wherein said block copolymer is taken from the group consisting of polyalkylene glycol block copolymers, mixtures of polyalkylene glycol block copolymers, and mixtures of polyalkylene glycols and polyalkylene glycol block copolymers.

15. The use according to claim 13, wherein said block copolymer is taken from the group consisting of polyalkylene glycol block copolymers, mixtures of polyalkylene glycol block copolymers, and mixtures of polyalkylene glycols and polyalkylene glycol block copolymers.

16. The composition of claim 12, wherein said block copolymer comprises a compound taken from the group consisting of polymethylene glycol, polyethylene glycol, polypropylene glycol, polybutylene glycol, polypentylene glycol, polyhexylene glycol, polyheptylene glycol, polyoctylene glycol, polynonylene glycol, and polydecylene glycol.

17. The use according to claim 13, wherein said block copolymer comprises a compound taken from the group consisting of polymethylene glycol, polyethylene glycol, polypropylene glycol, polybutylene glycol, polypentylene glycol, polyhexylene glycol, polyheptylene glycol, polyoctylene glycol, polynonylene glycol, and polydecylene glycol.

18. The composition of claim 12, wherein said amphipathic polymer is taken from the group consisting of polyethylene glycol/polypropylene glycol block copolymers, and mixtures of polyethylene glycol, polypropylene glycol, and polyethylene glycol/polypropylene glycol block copolymers.

19. The use according to claim 13, wherein said amphipathic polymer is taken from the group consisting of polyethylene glycol/polypropylene glycol block copolymers, and mixtures of polyethylene glycol, polypropylene glycol, and polyethylene glycol/polypropylene glycol block copolymers.

20. The composition of any one of claims 5, 10, 12, 14, 16 or 18, wherein said amphipathic polymer is taken from the group consisting of poloxamers and poloxamines.

21. The use according to any one of claims 1 to 4, 6 to 9, 11, 13, 15, 17 or 19, wherein said amphipathic polymer is taken from the group consisting of poloxamers and poloxamines.
22. The use according to any one of claims 1 to 4, 6 to 9, 11, 13, 15, 17, 19 or 21, wherein the nerve tissue is spinal cord tissue.
23. The use according to any one of claims 1 to 4, 6 to 9, 11, 13, 15, 17, 19, 21 or 22, wherein the injury is a mechanical injury.
24. The use according to any one of claims 1 to 4, 6 to 9, 11, 13, 15, 17, 19, 21, 22 or 23, wherein administration of said amphipathic polymer is effected using a technique selected from the group consisting of intravascular, intramuscular, subcutaneous and intraperitoneal injection.

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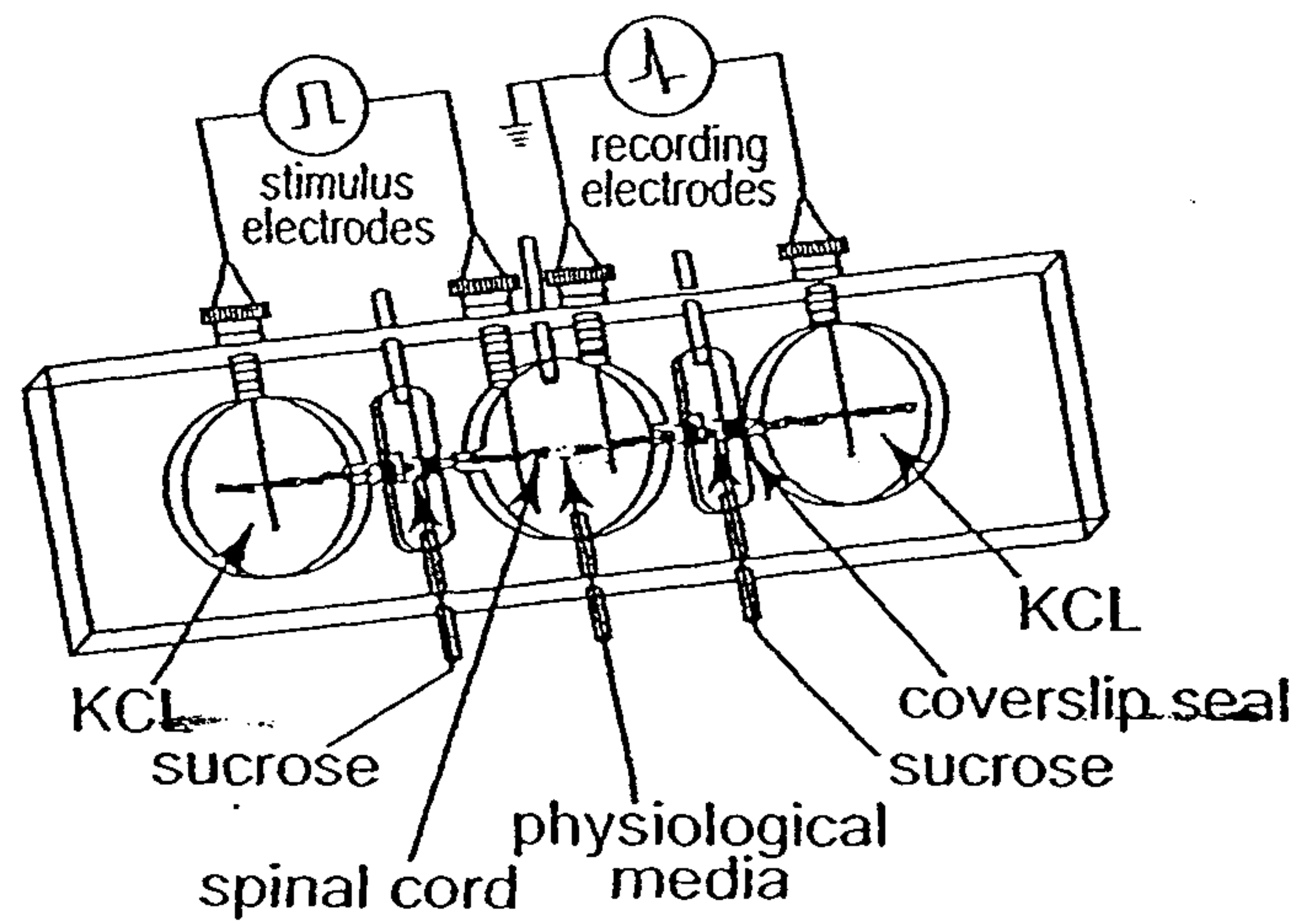


Fig. 1A

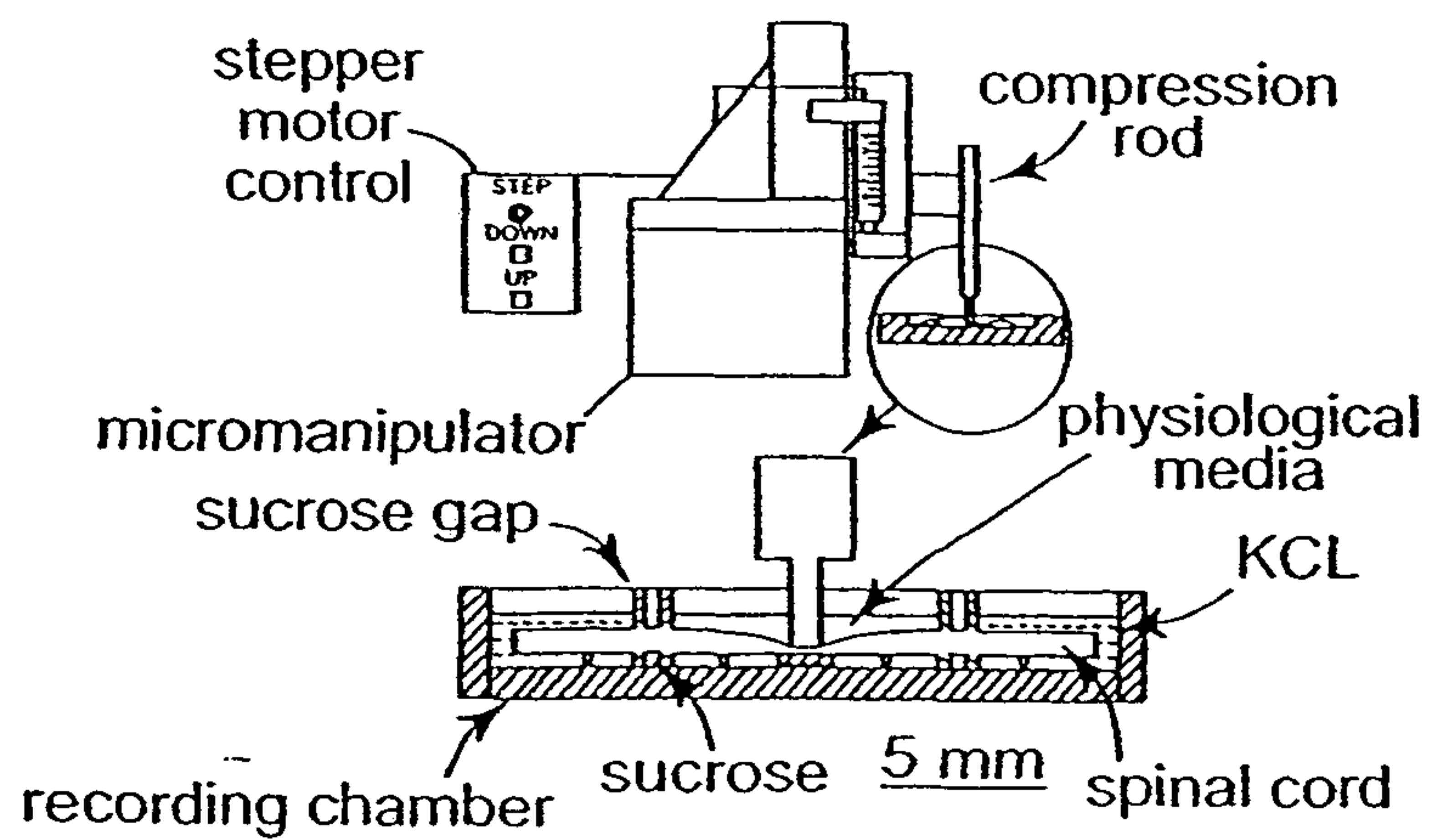
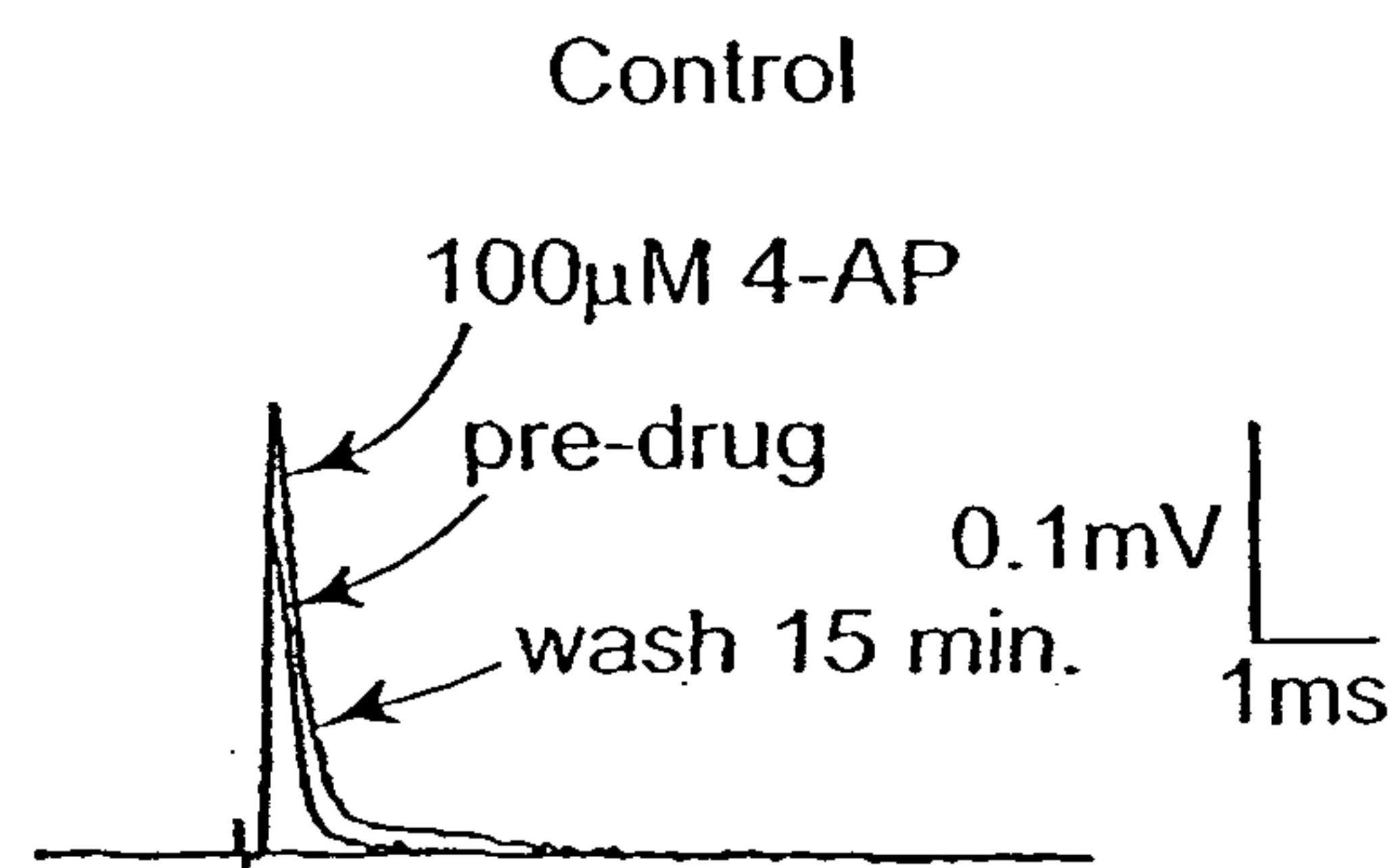
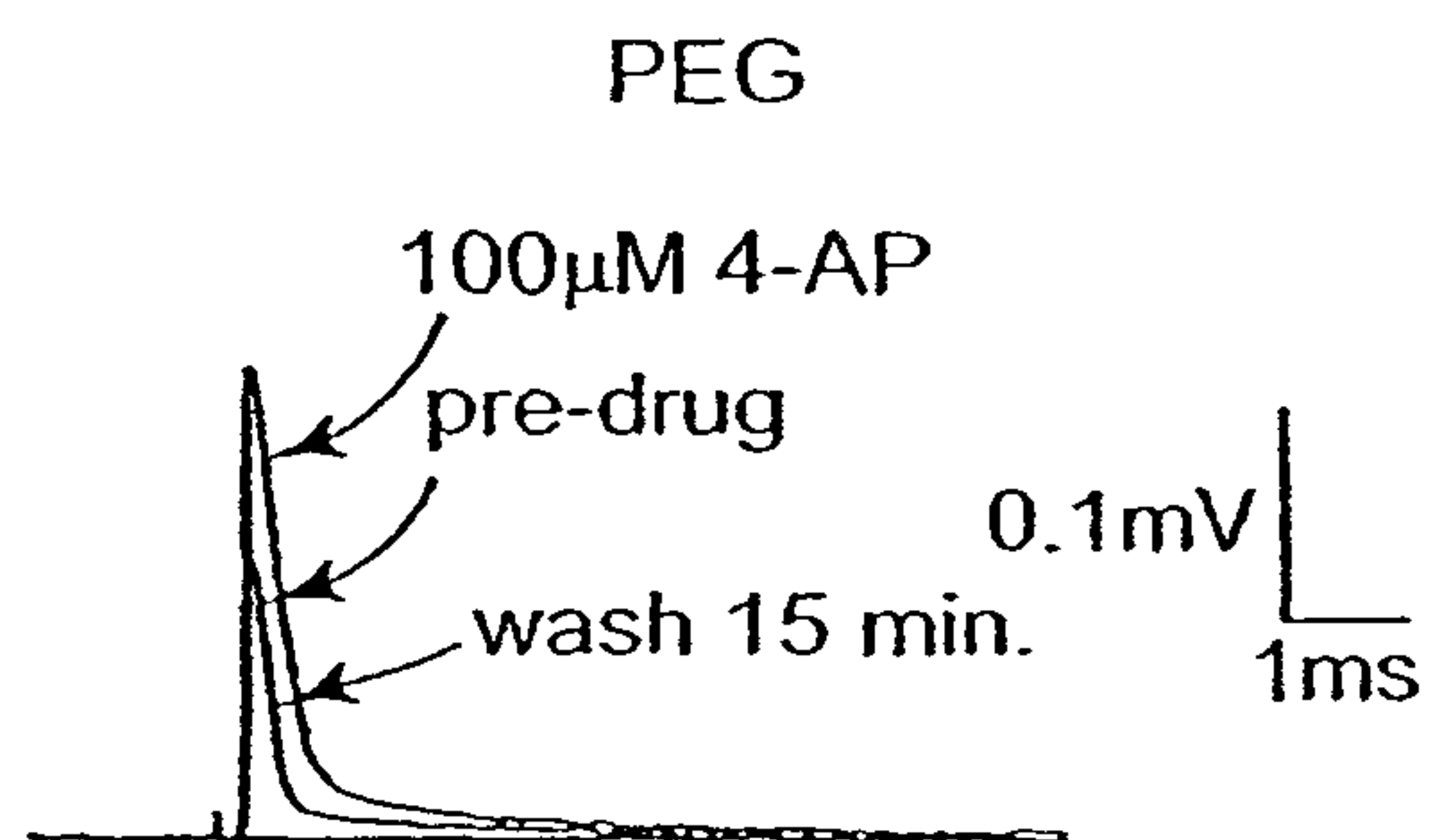
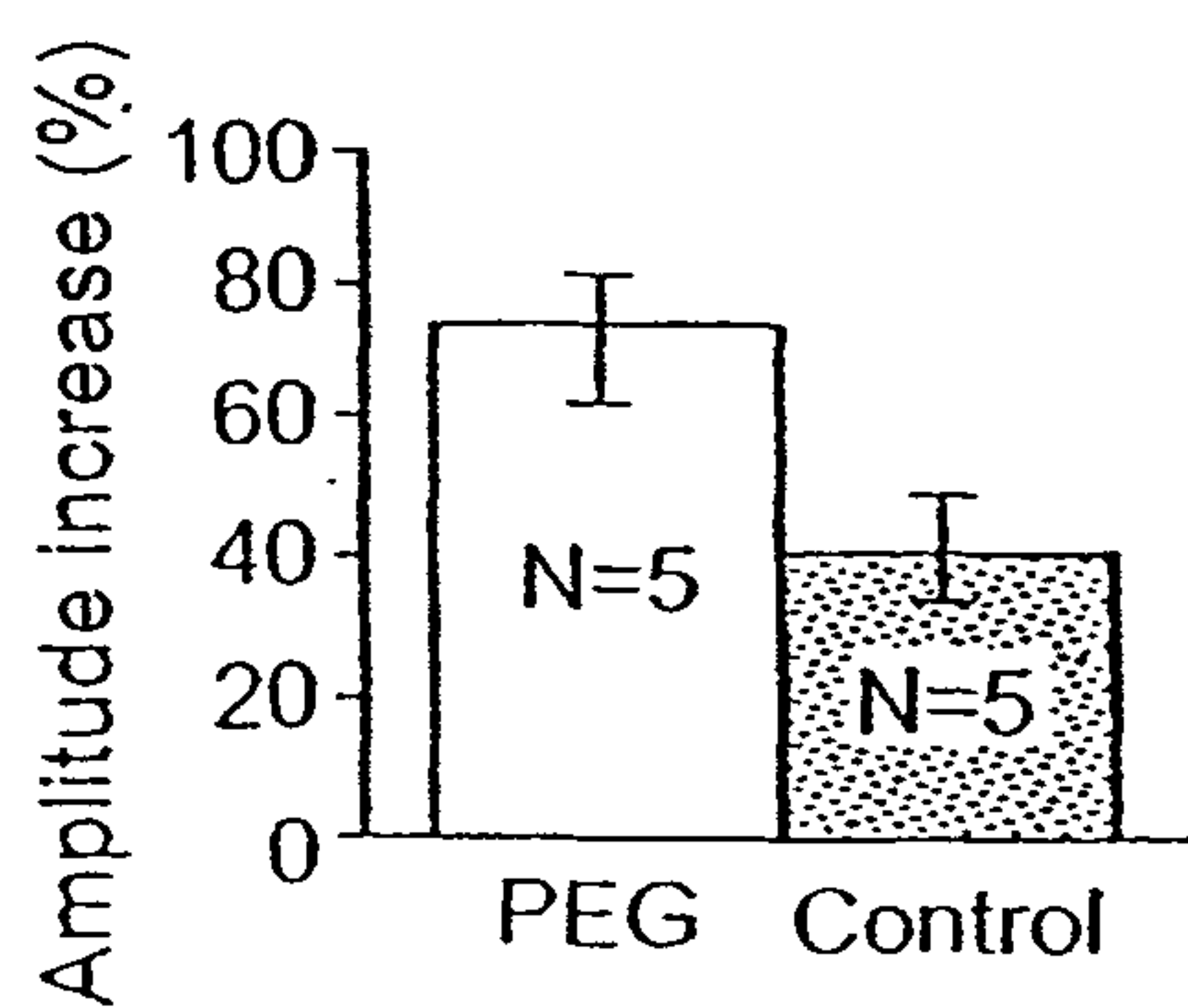


Fig. 1B

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**Fig. 2A****Fig. 2B****Fig. 2C**

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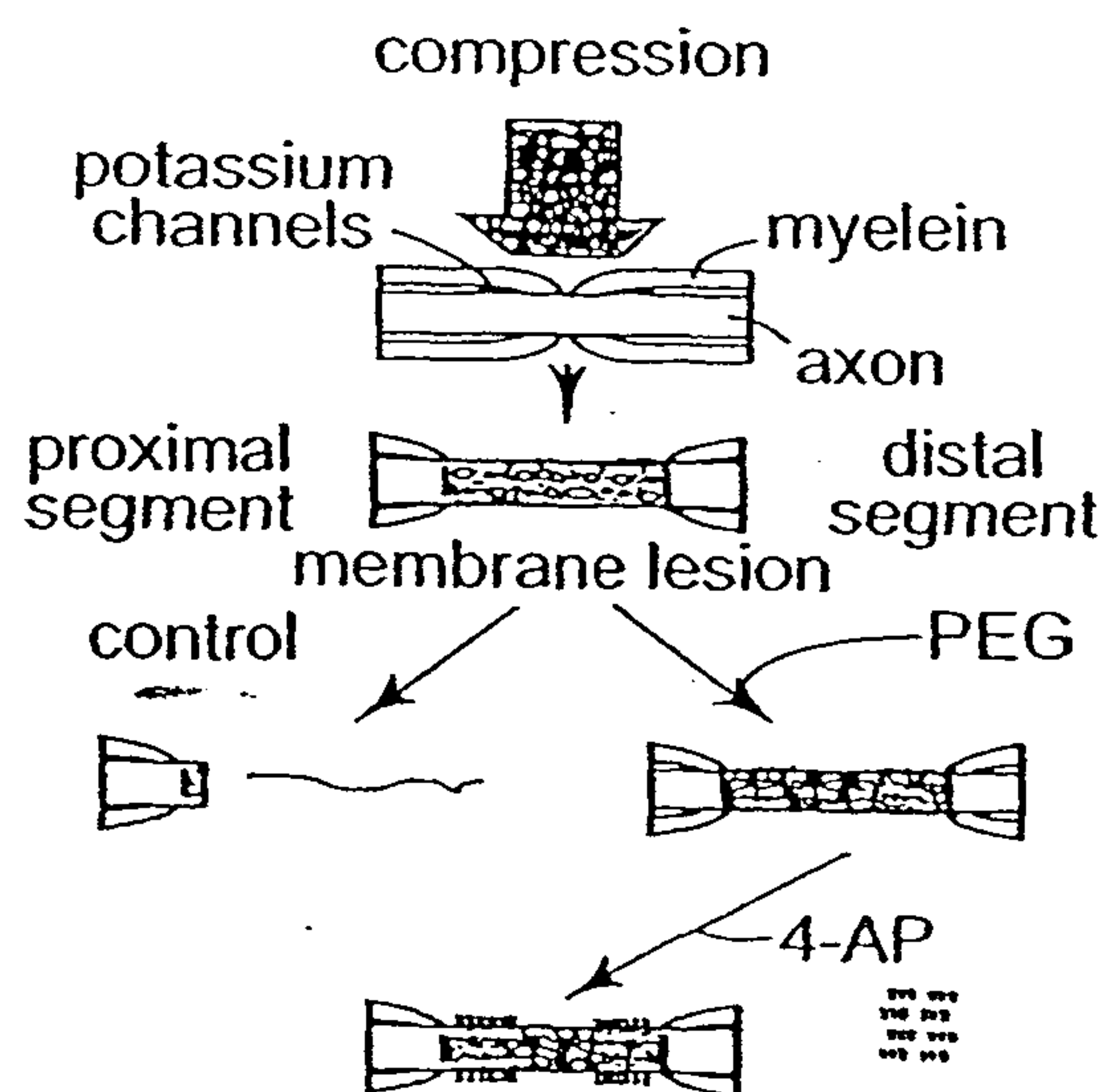


Fig. 3

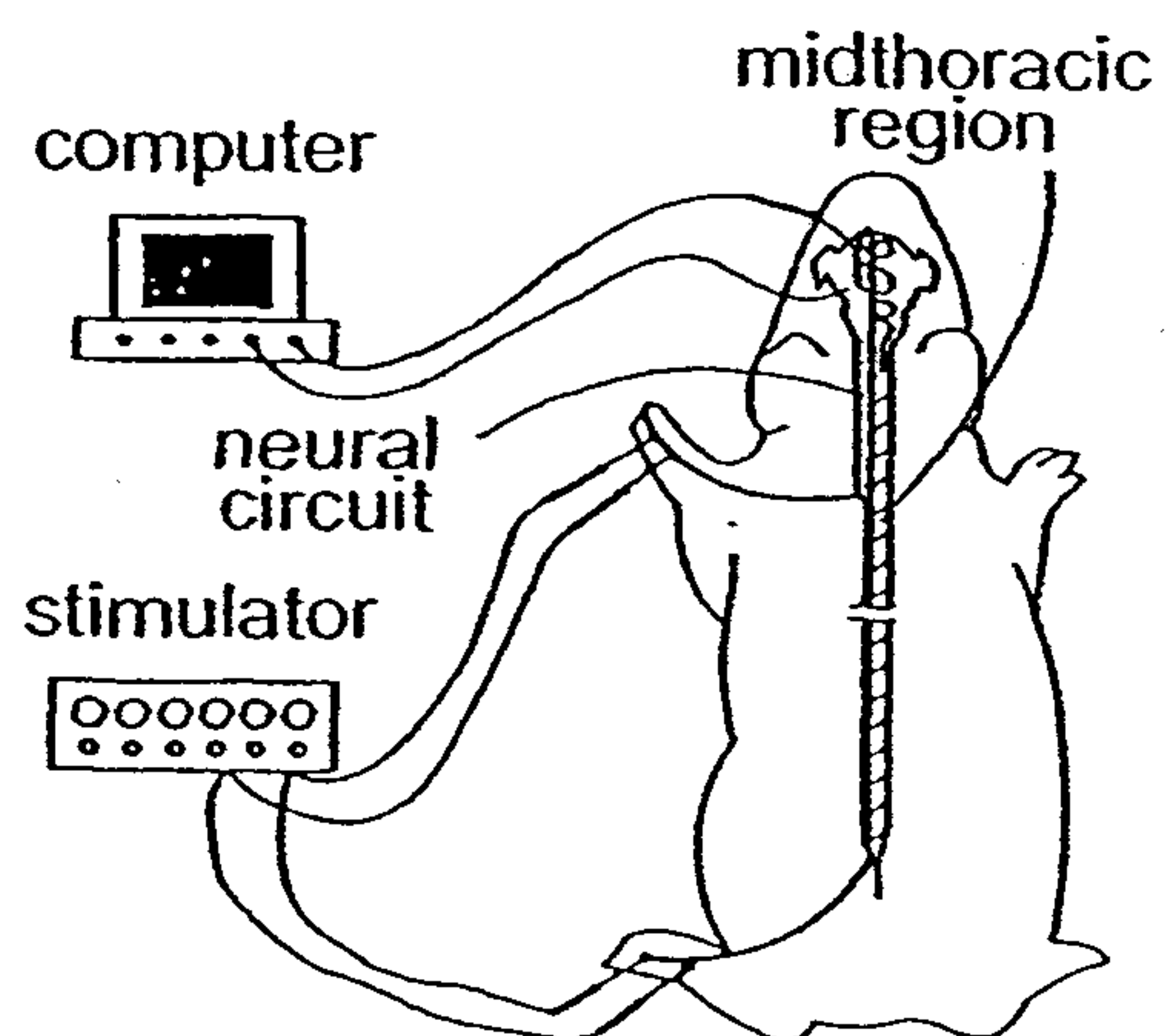
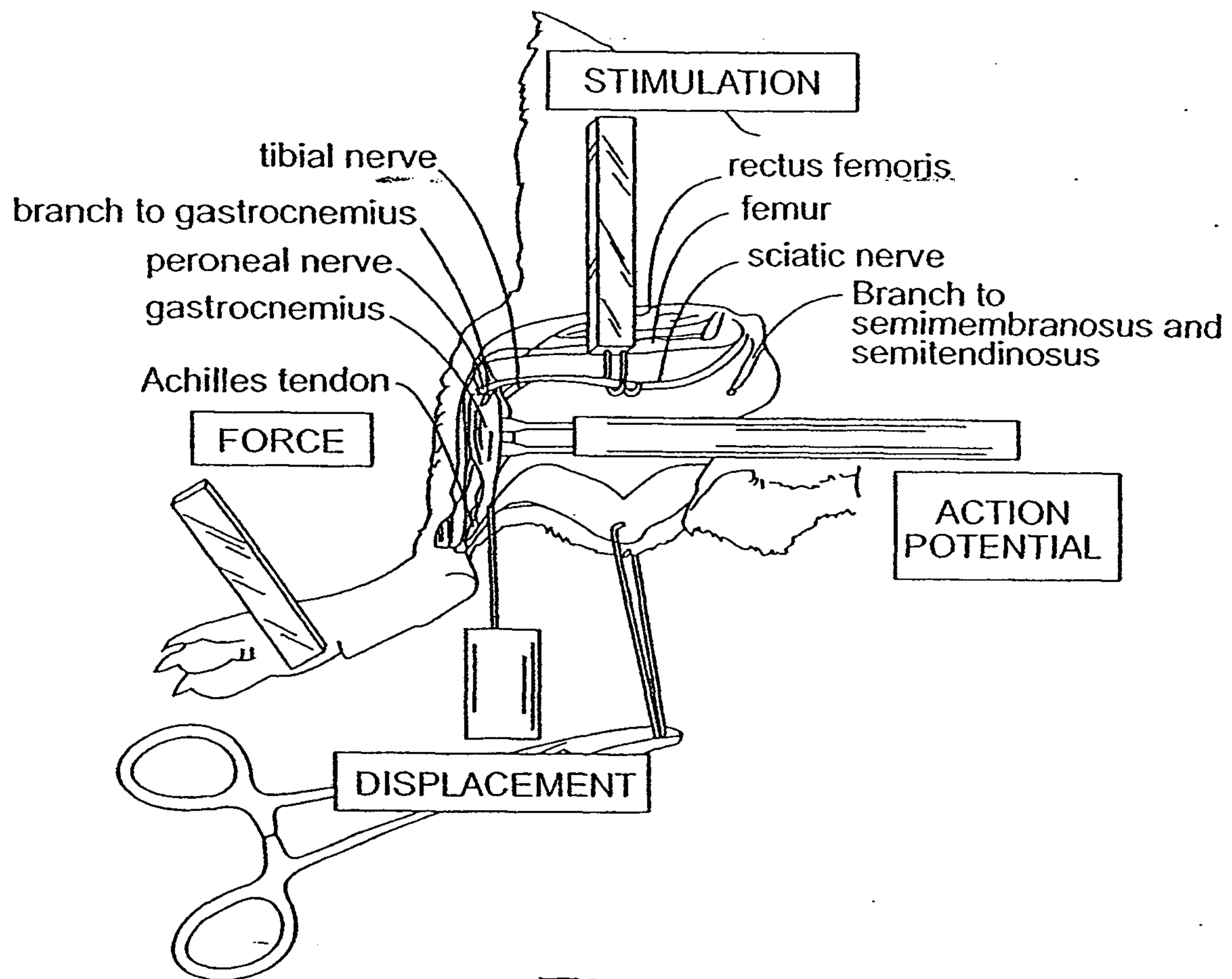


Fig. 4

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**Fig. 5**

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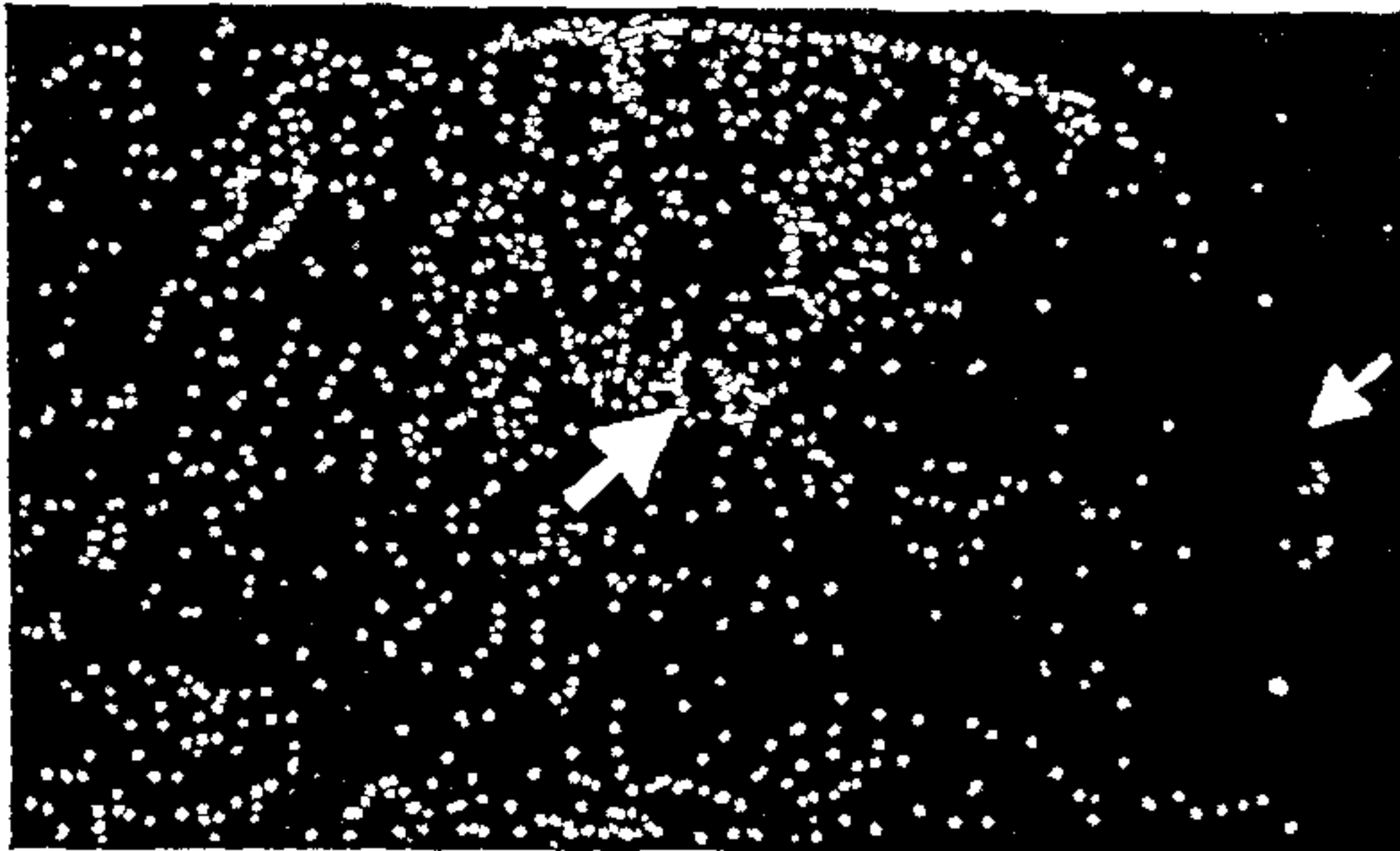


Fig. 6A



Fig. 6B

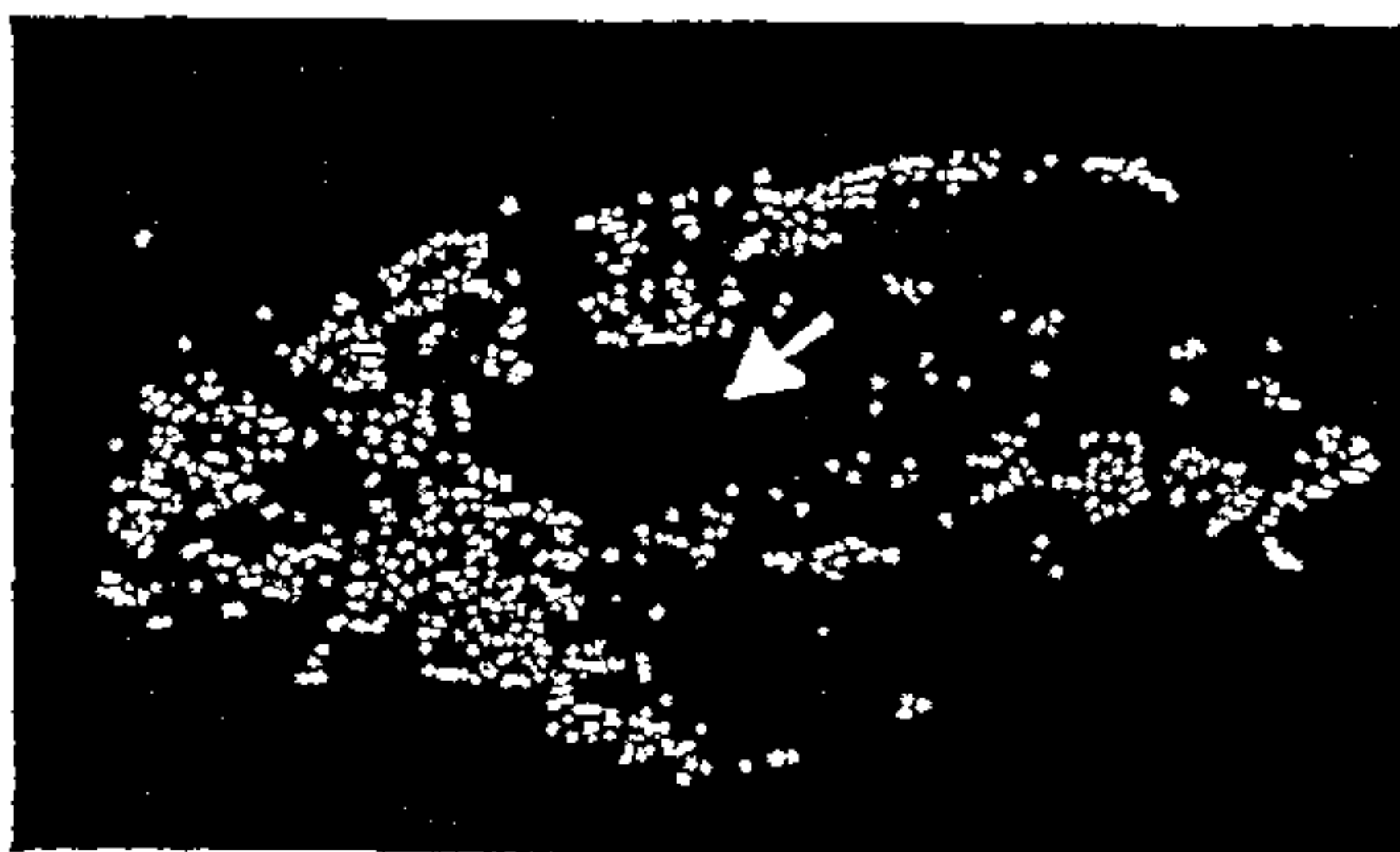


Fig. 6C

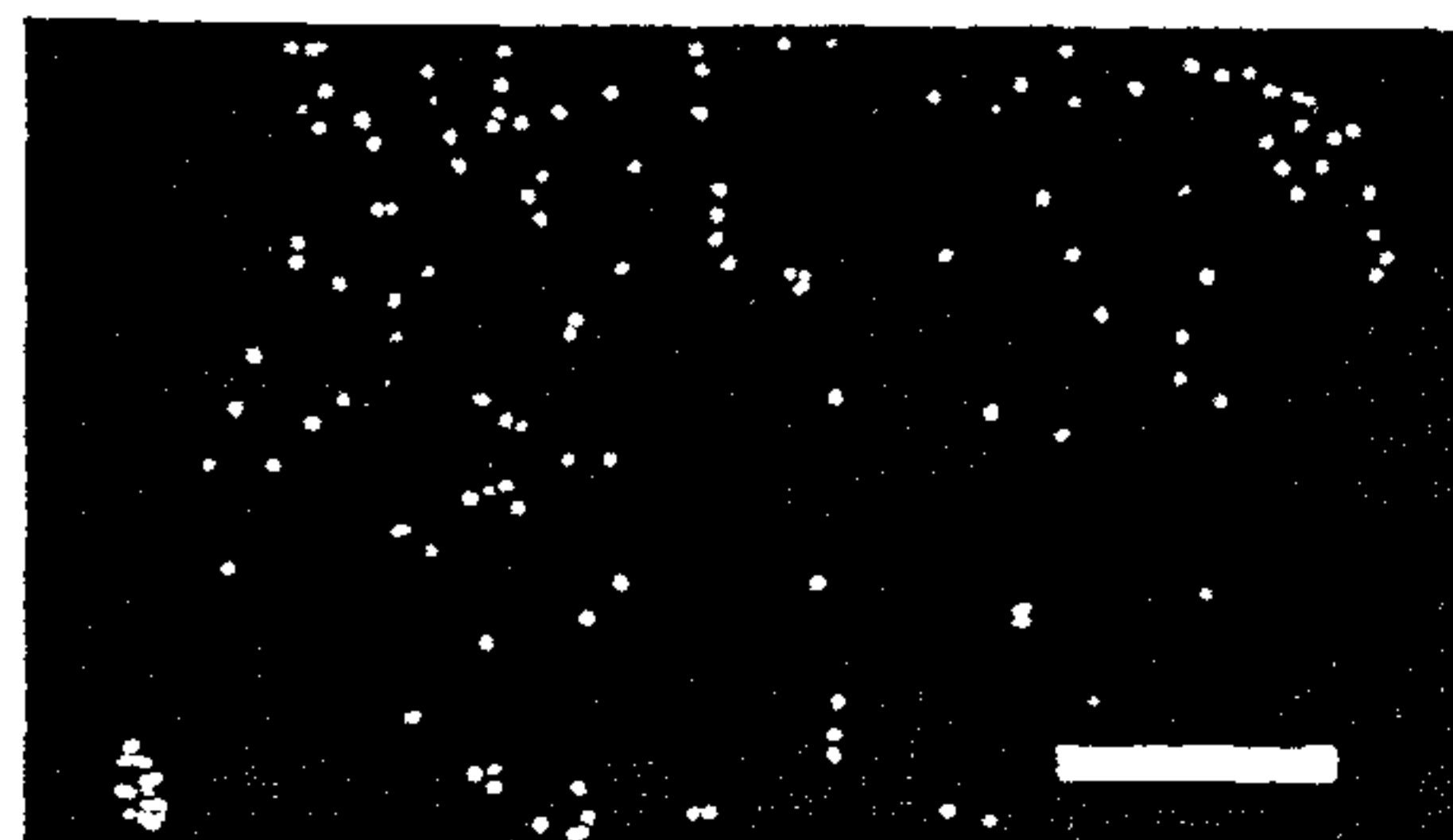


Fig. 6D

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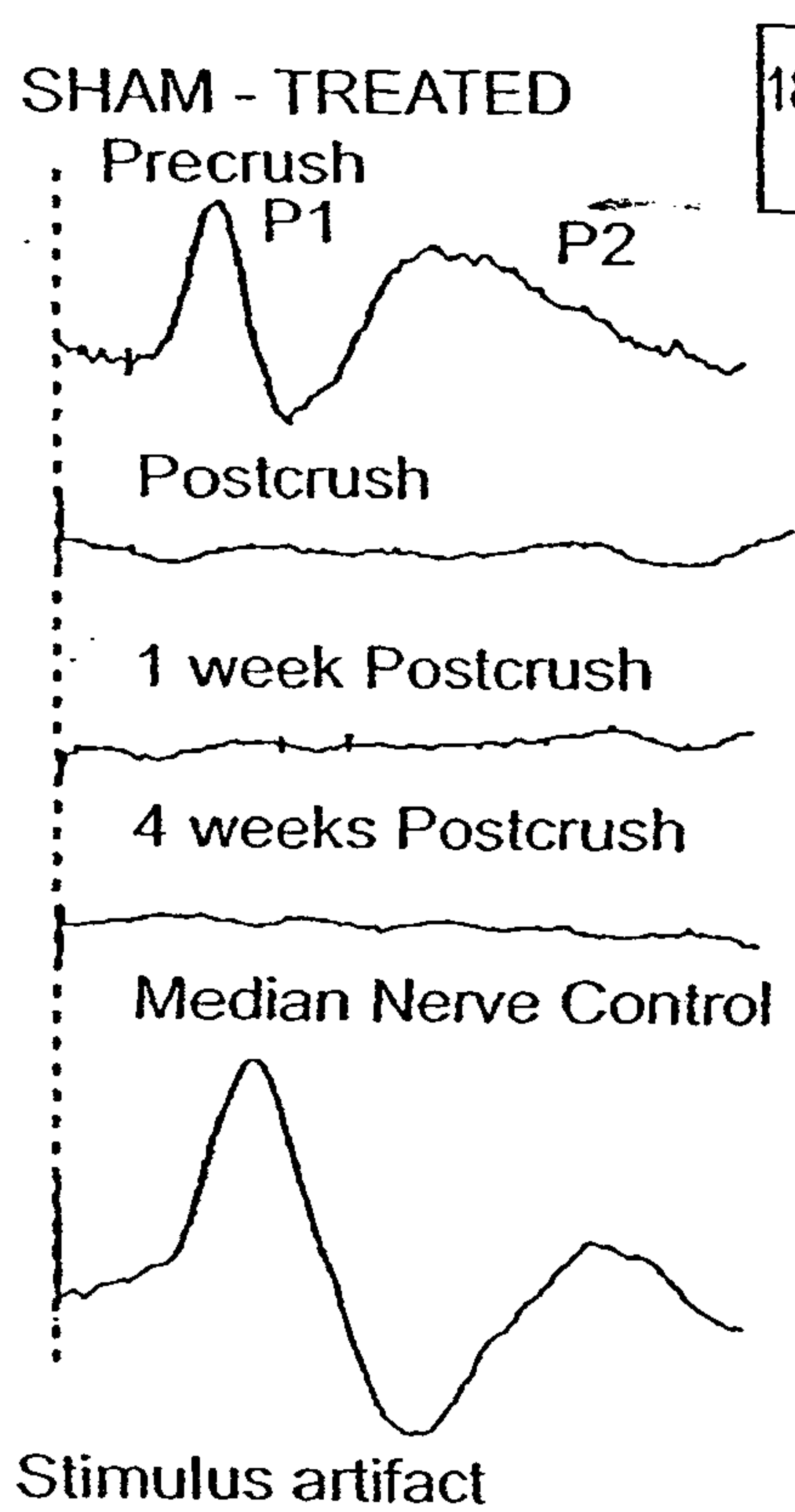


Fig. 7A

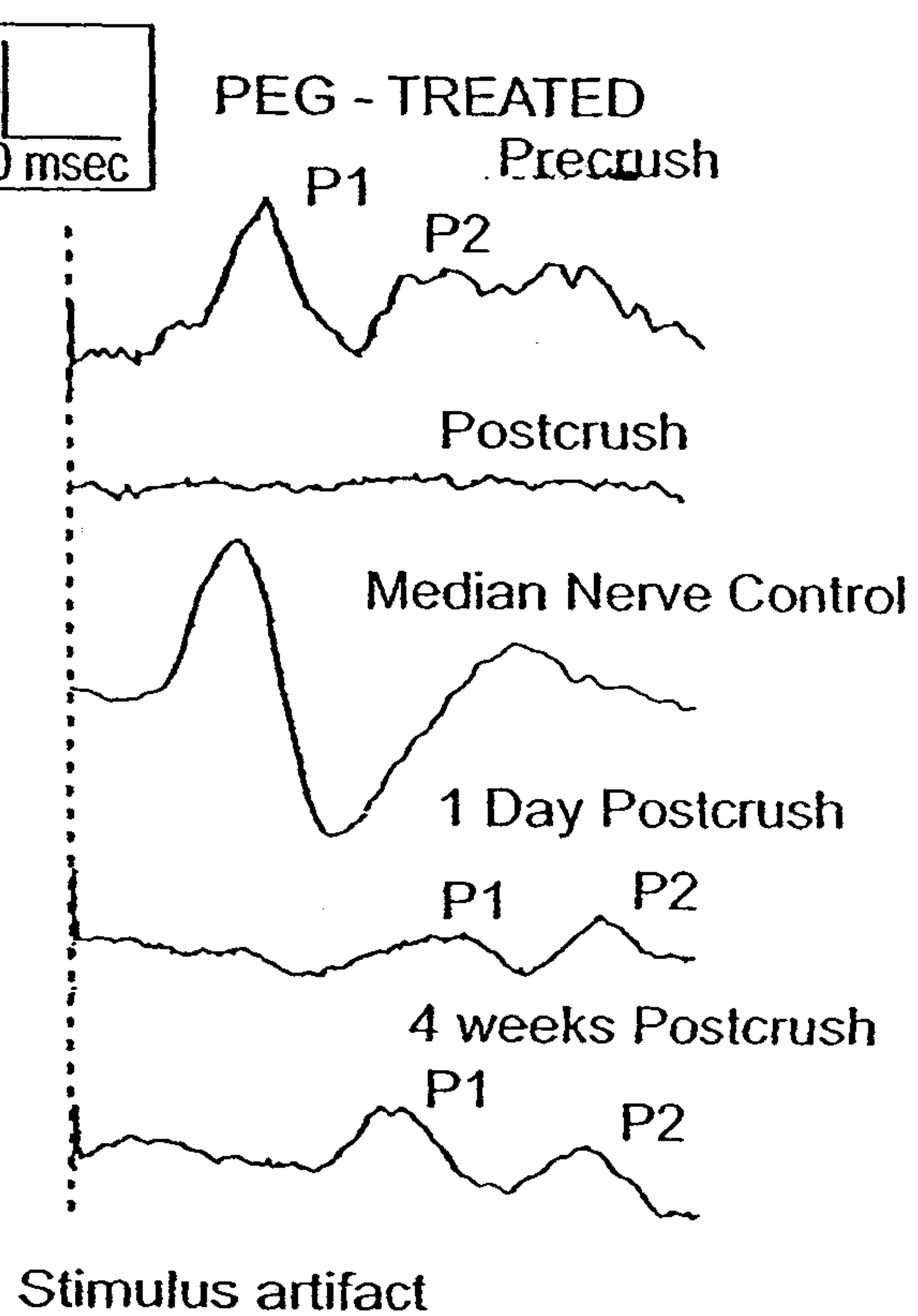
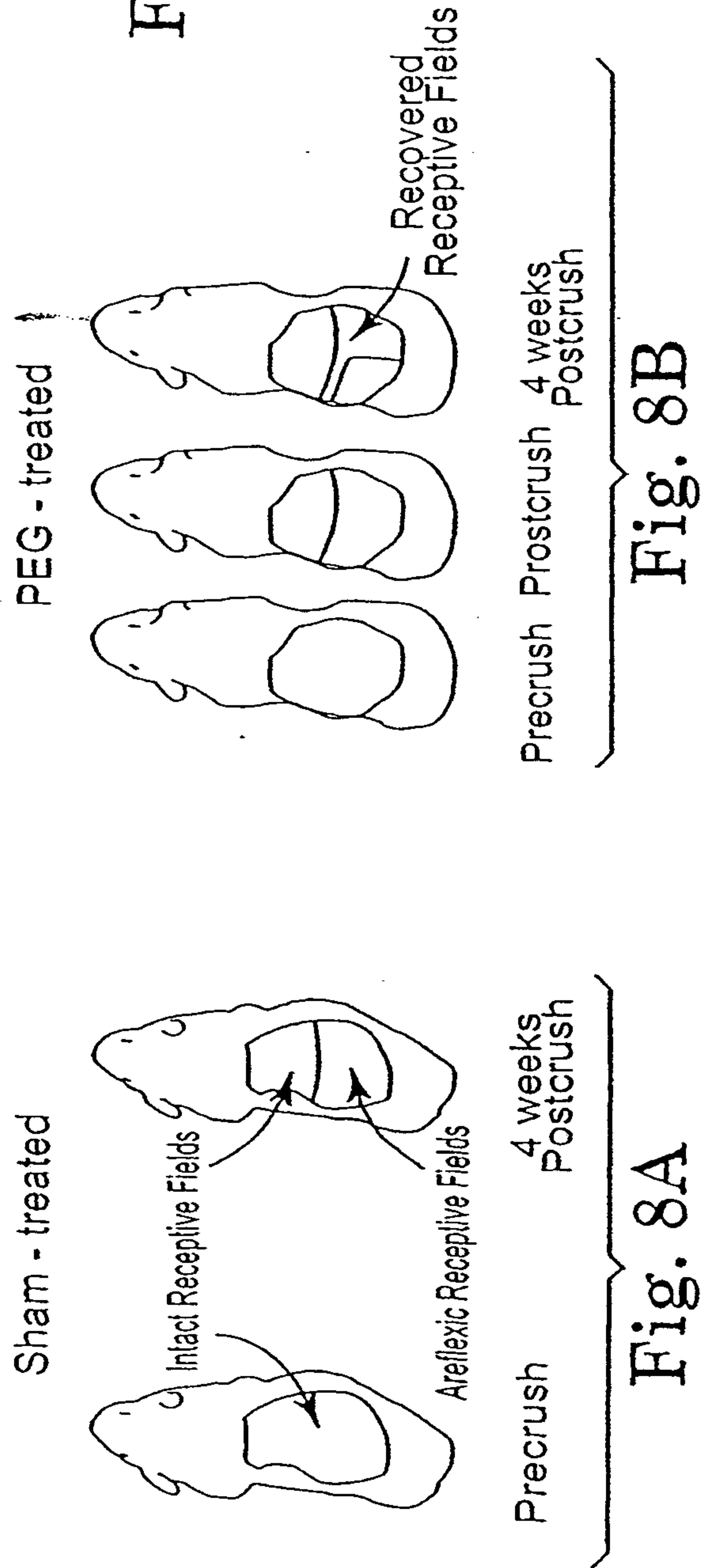


Fig. 7B



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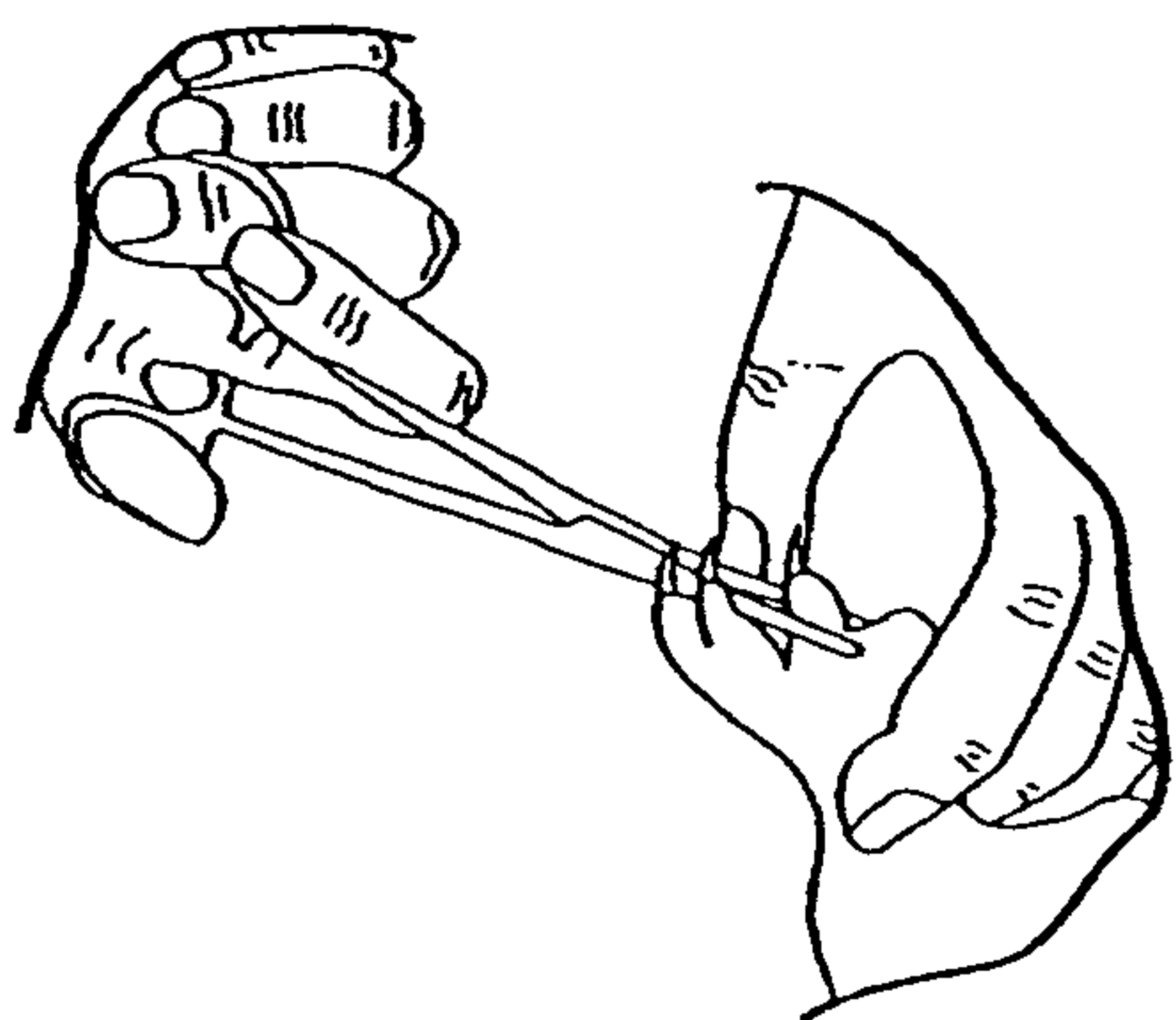


Fig. 9B

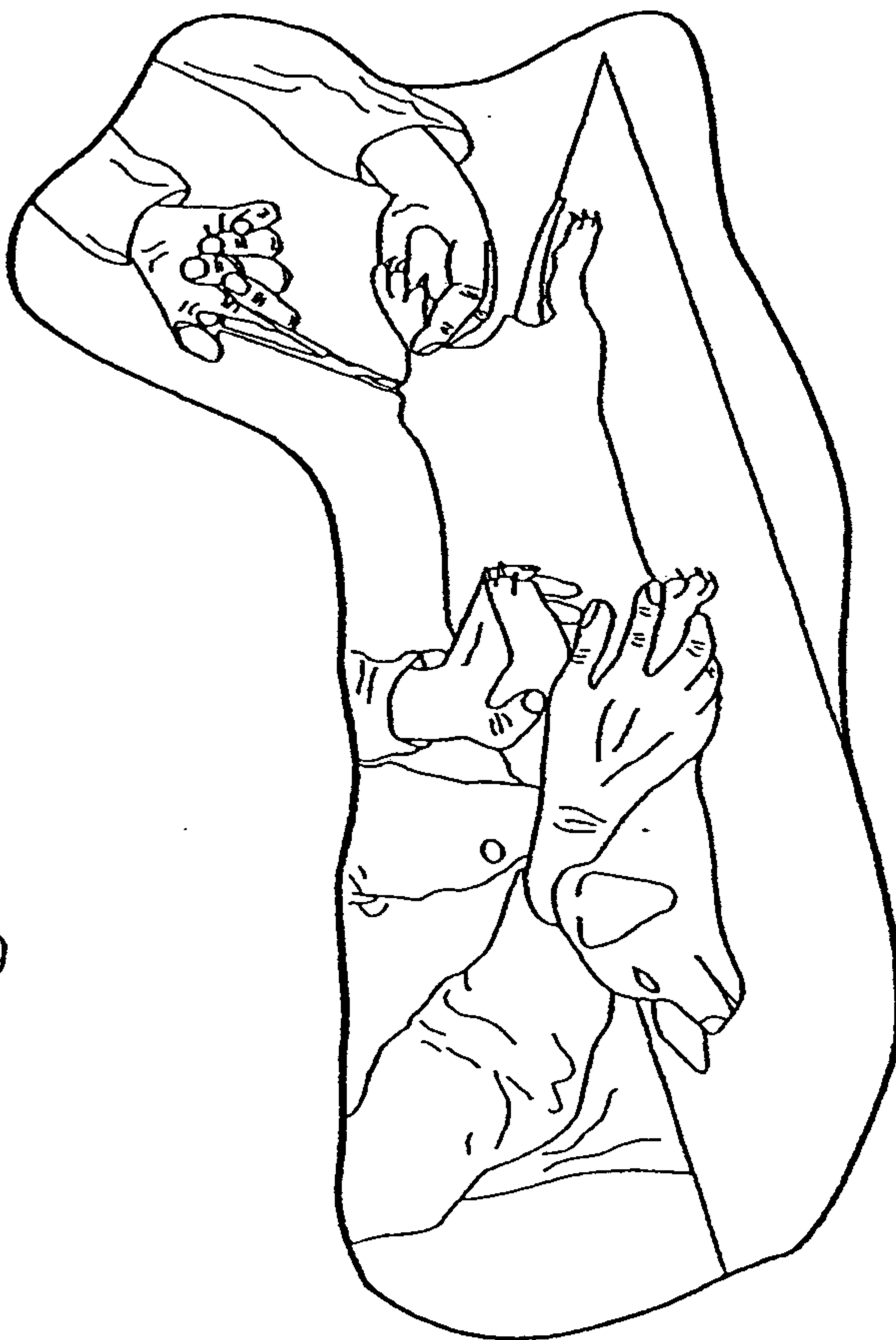


Fig. 9A

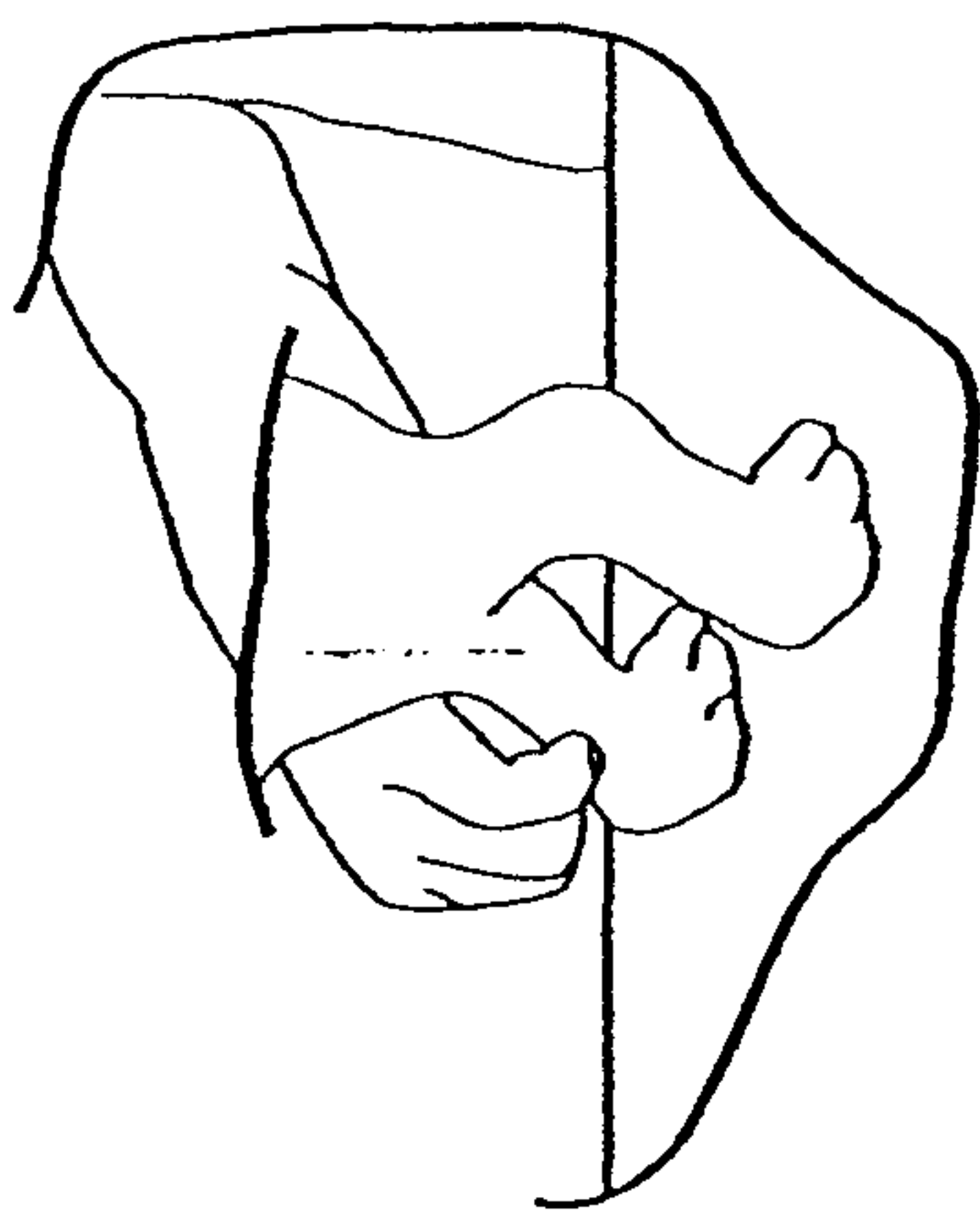


Fig. 9C

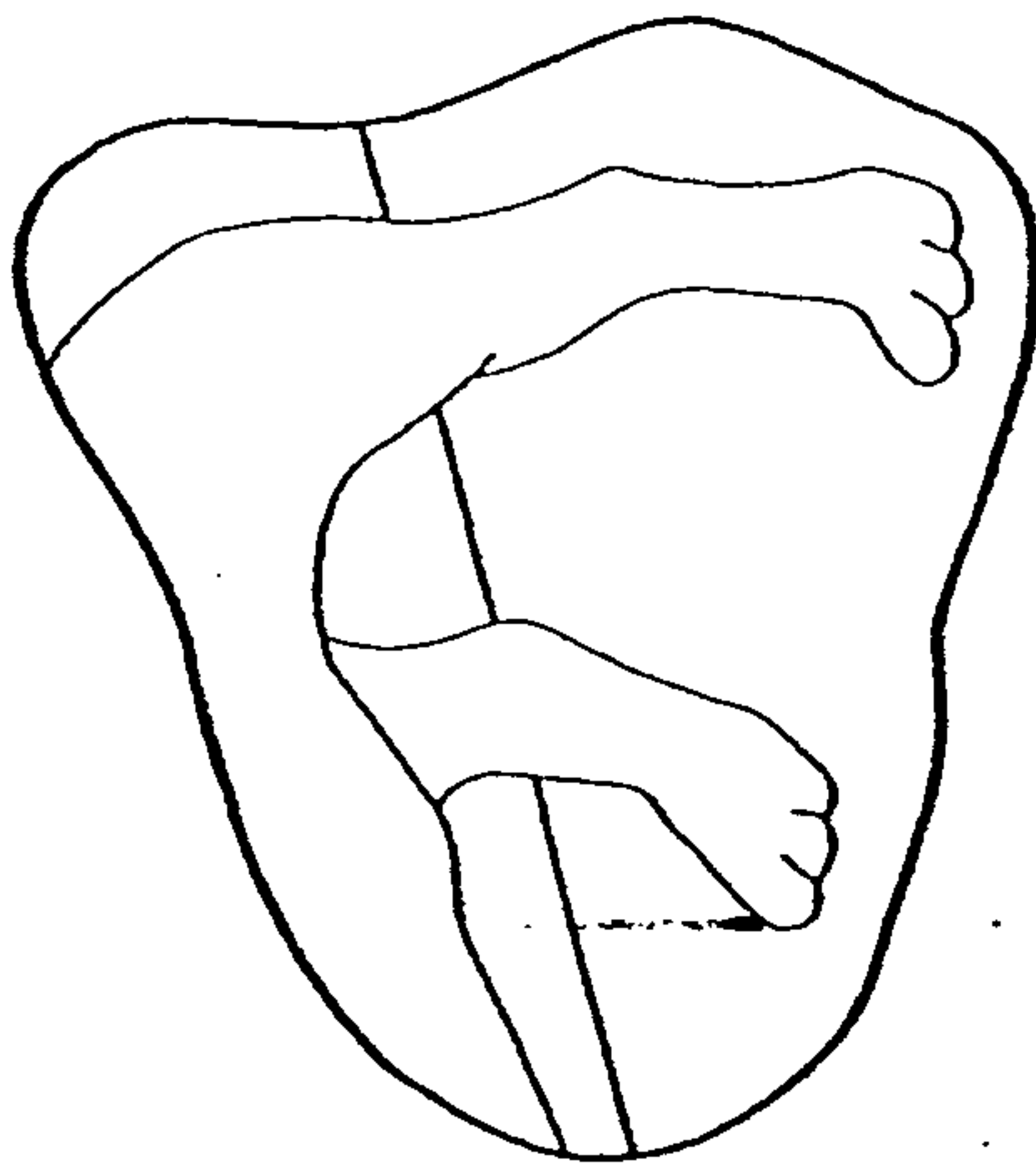


Fig. 9D

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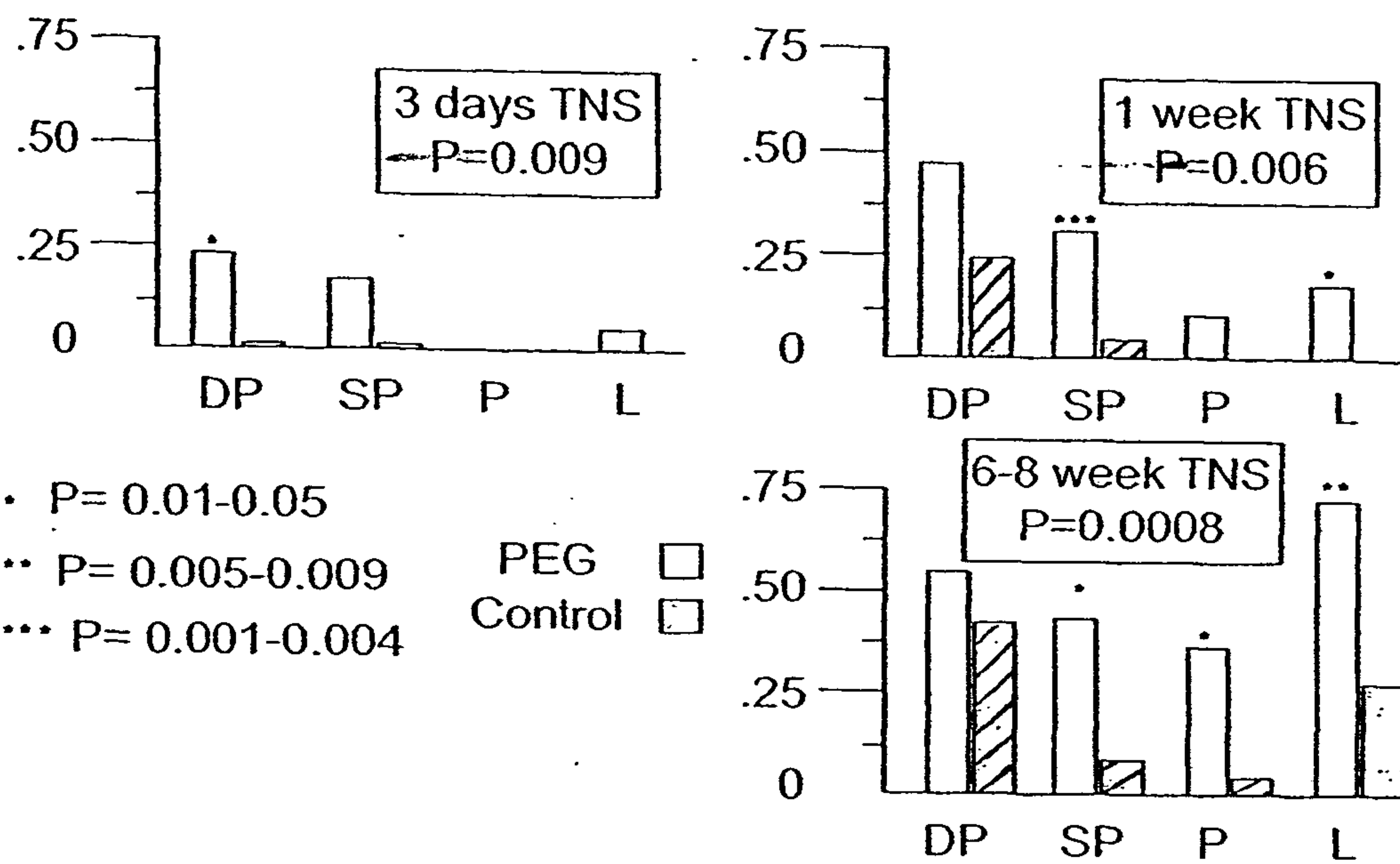


Fig. 9E

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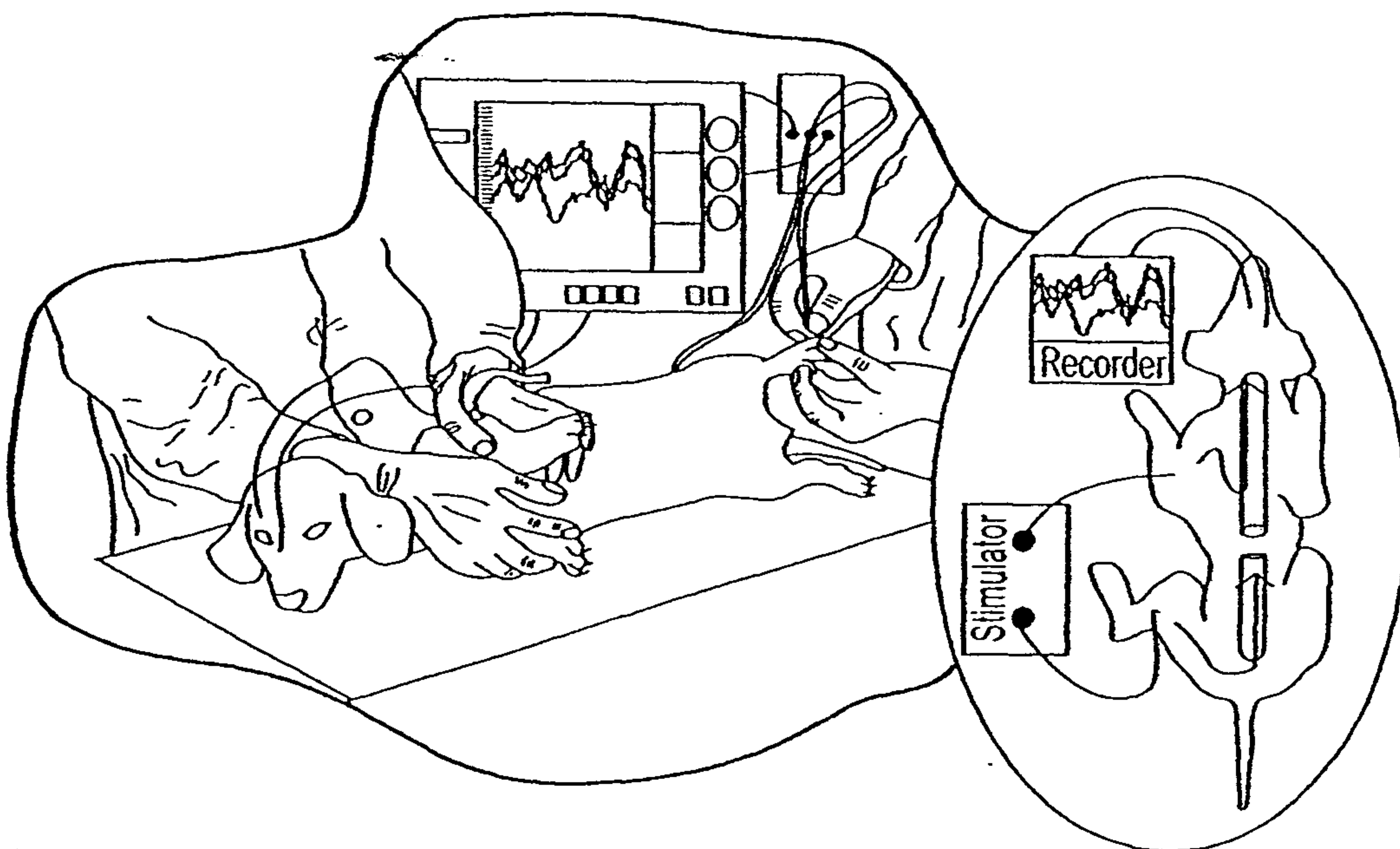


Fig. 10A

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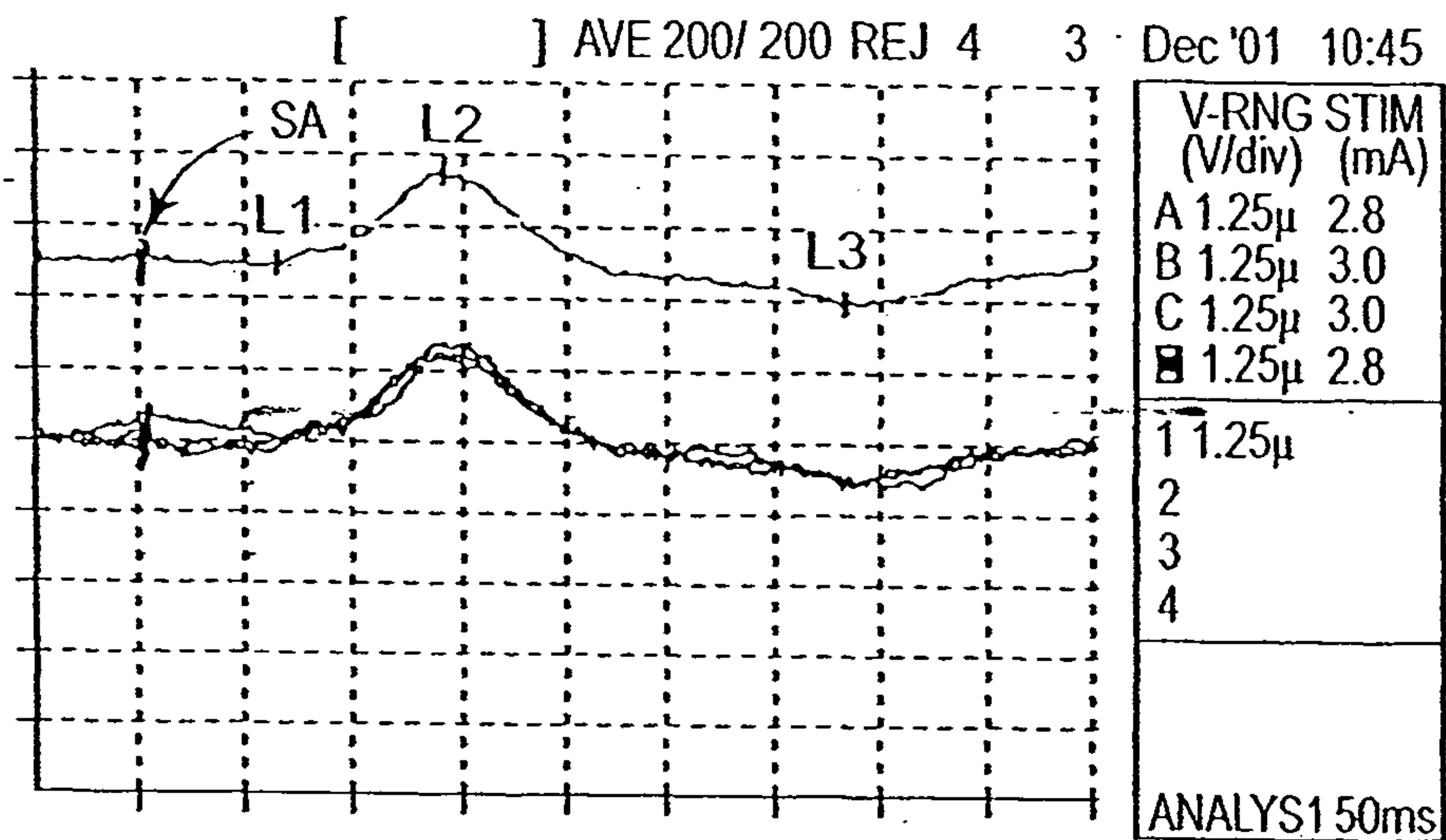


Fig. 10B

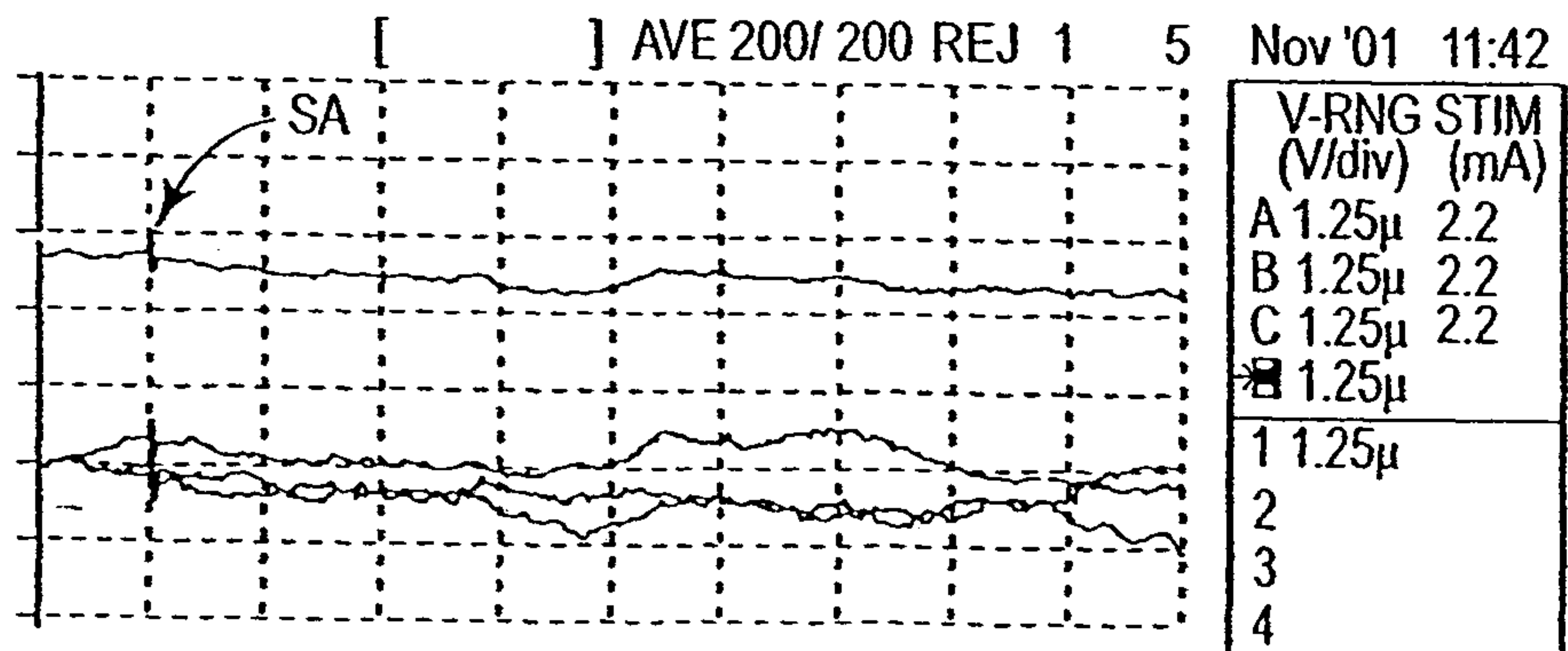


Fig. 10C

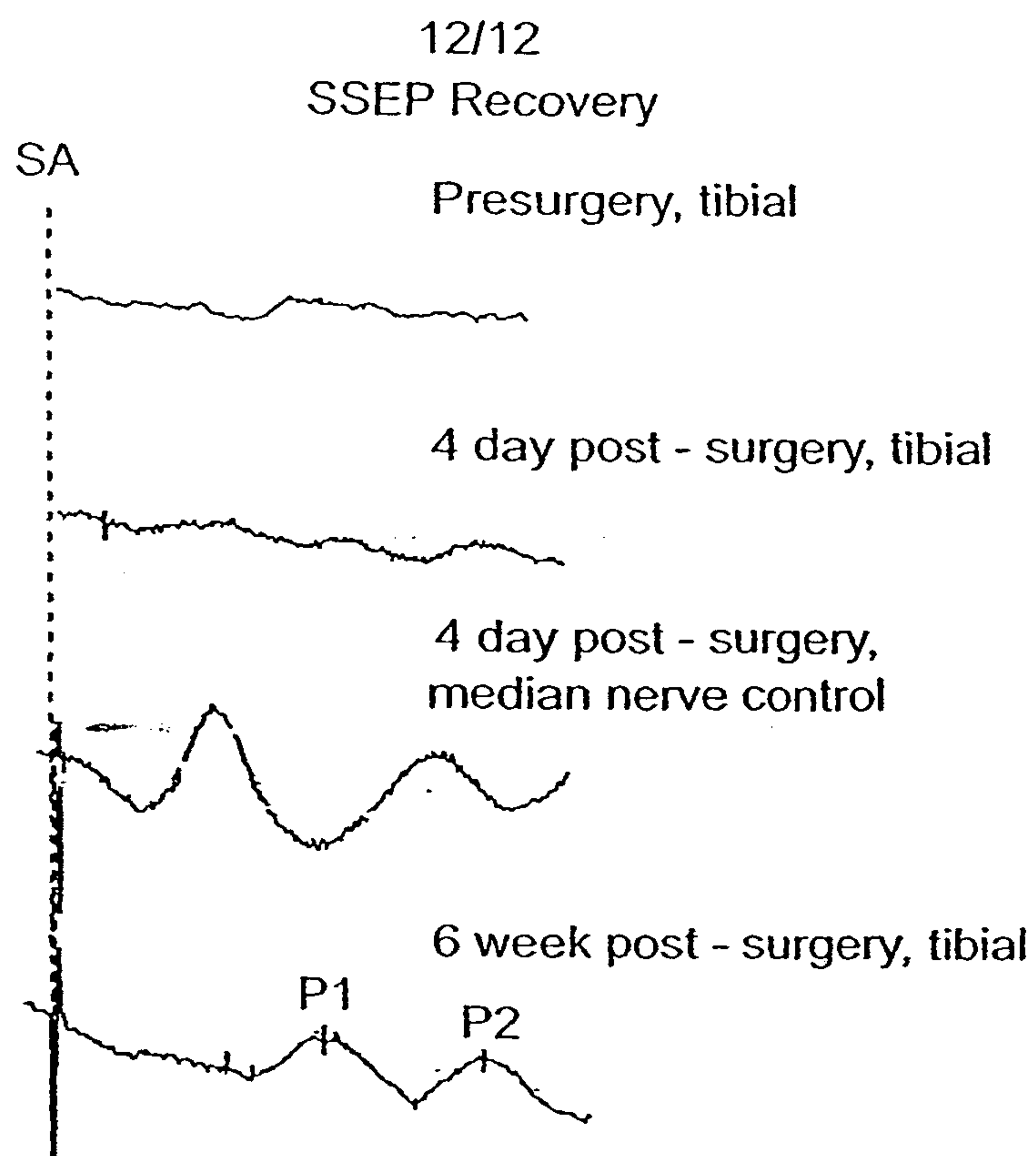


Fig. 11A

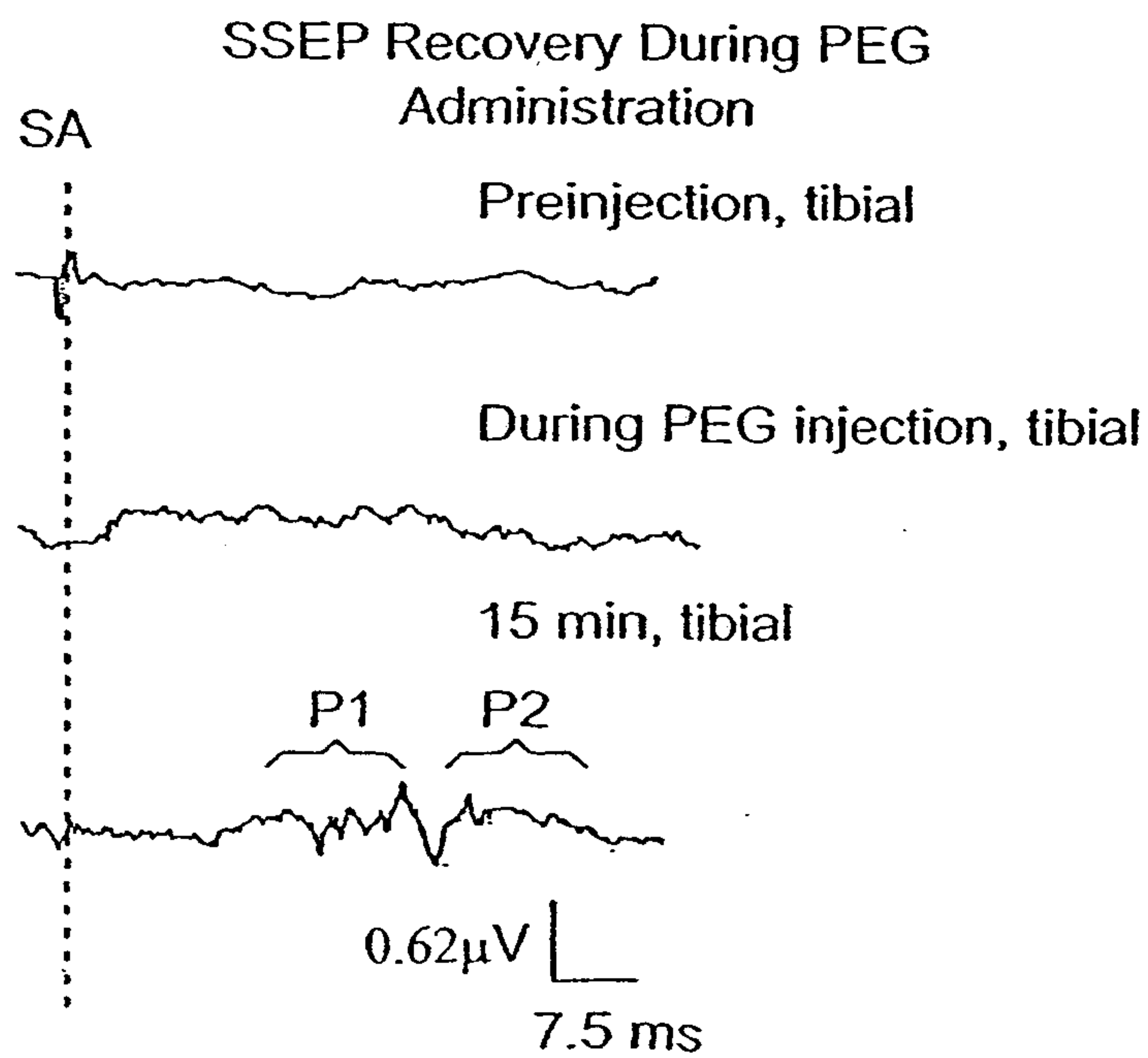


Fig. 11B