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(71) Applicant (for all designated States except US): **WIS-**

CONSIN ALUMNI RESEARCH FOUNDATION
[US/US]; 614 Walnut Street, 13th Floor, Madison, WI
53726 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **MILETIC,**

Vjekoslav [US/US]; 7893 Serene Ct., Cross Plains, WI
53528 (US). **MILETIC, Gordana** [US/US]; 7893 Serene
Ct., Cross Plains, WI 53528 (US).

(74) Agents: **SARA, Charles, S.** et al.; DeWitt Ross & Stevens,

S.C., 2 East Mifflin Street, Suite 600, Madison, WI 53703-
2865 (US).

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(54) Title: METHOD TO INHIBIT NEUROPATHIC PAIN BY INTRATHECAL INJECTION OF PROTEIN PHOSPHATASES

(57) Abstract: A method and corresponding pharmaceutical composition to inhibit neuropathic pain by injecting intrathecally into a mammalian subject a neuropathic pain-inhibiting amount of a phosphatase falling within E.C. 3.1.3.x, more specifically a protein phosphatase falling within E.C. 3.1.3.16, and most specifically calcineurin.



METHOD TO INHIBIT NEUROPATHIC PAIN BY INTRATHECAL INJECTION
OF PROTEIN PHOSPHATASES

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FEDERAL FUNDING STATEMENT

This invention was made with government support under NS034870 and
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10 rights in this invention.

CROSS-REFERENCE TO RELATED APPLICATIONS

Priority is hereby claimed to provisional application Serial No. 61/498,796,
filed June 20, 2011, which is incorporated herein.

15

FIELD OF THE INVENTION

Described herein is a method to treat neuropathic pain by intrathecal injection
of phosphatases in general, protein phosphatases in particular, and calcineurin most
preferably.

20

BACKGROUND

Neuropathic pain is caused by damage or disease affecting the somatosensory
system. It is often associated with abnormal sensations referred to as dysesthesia.
These sensations are perceived by the subject as an unpleasant, abnormal sense of
25 touch, or other sensations such as burning, wetness, itching, electric shock, and pins
and needles. Neuropathic pain may also involve pain produced by normally non-
painful stimuli (allodynia). Neuropathic pain may chronic and continuous and/or
episodic. For example "phantom pain" from an amputated limb or from a part of the
body from which the brain no longer receives signals is a type of neuropathic pain.
30 The etiology of neuropathic pain is quite broad, and can be caused by traumatic
injuries, spinal cord injury, multiple sclerosis, stroke, diabetes and other metabolic
conditions, herpes zoster infection, HIV-related neuropathies, nutritional deficiencies,
toxins, remote manifestations of malignancies, immune-mediated disorders and
physical trauma to a nerve trunk.

Neuropathic pain is very difficult to treat. At present there is no effective treatment. Only about 40-60% of patients achieve partial relief via conventional therapies. See, for example, Dworkin RH, O'Connor AB, Backonja M, et al. (2007). "Pharmacologic management of neuropathic pain: evidence-based recommendations," Pain 132 (3): 237-51. Thus there remains a long-felt and unmet need for an effective method to treat neuropathic pain.

SUMMARY

Disclosed and claimed herein is a method to inhibit neuropathic pain. The method comprises injecting intrathecally into a mammalian subject a neuropathic pain-inhibiting amount of a phosphatase falling within E.C. 3.1.3.x, wherein x is an integer from 1 to 86. The subject may be a human being. The method also comprises injecting intrathecally into the mammalian subject a neuropathic pain-inhibiting amount of a protein phosphatase falling within E.C. 3.1.3.16. Again, the subject may be a human being. The method also comprising injecting intrathecally into the mammalian subject a neuropathic pain-inhibiting amount of calcineurin. Again, the subject may be a human being. The method may optionally comprise intrathecally injecting the phosphatase, protein phosphatase, or calcineurin in combination with a pharmaceutical delivery vehicle suitable for intrathecal injection. In a preferred version, the method comprises injecting intrathecally into the subject a dosage of about 0.2 μ g to about 200 μ g/kg body weight/day of the phosphatase, protein phosphatase, or calcineurin, administered in a single dose or in multiple doses.

Also disclosed herein is a pharmaceutical composition comprising a neuropathic pain-inhibiting amount of a phosphatase falling within E.C. 3.1.3.x, wherein x is an integer from 1 to 86, in combination with a pharmaceutical delivery vehicle suitable for intrathecal injection. In one version of the composition, the phosphatase contained within the composition is a protein phosphatase falling within E.C. 3.1.3.16. The most preferred phosphatase is calcineurin. The pharmaceutical composition may be in unit dosage form. The unit dosage may contain from about 10 μ g to about 1 g of the phosphatase. The unit dosage may contain from about 15 μ g to about 15 mg of the phosphatase. Dosages above and below these stated ranges are explicitly within the scope of this disclosure. The phosphatase within the pharmaceutical composition may be protein phosphatase falling within E.C. 3.1.3.16, including calcineurin. The unit dosage may contain from about 10 μ g to about 1 g of

the calcineurin. The unit dosage may contain from about 15 μ g to about 15 mg of the calcineurin.

Also disclosed herein is a phosphatase falling within E.C. 3.1.3.x, wherein x is an integer from 1 to 86, for use as a medicament to treat neuropathic pain via intrathecal injection. The phosphatase may be a protein phosphatase falling within E.C. 3.1.3.16. The phosphatase may be calcineurin. The use may include those in which the medicament is in unit dosage form. The unit dosage may contain from about 10 μ g to about 1 g of the phosphatase. The unit dosage may contain from about 15 μ g to about 15 mg of the phosphatase. The phosphatase in the unit dosage for the use may be a protein phosphatase falling within E.C. 3.1.3.16. The phosphatase in the unit dosage may be calcineurin. The unit dosage may contain from about 10 μ g to about 1 g of the calcineurin. The unit dosage may contain from about 15 μ g to about 15 mg of the calcineurin.

Additionally disclosed herein is a phosphatase falling within E.C. 3.1.3.x, wherein x is an integer from 1 to 86, for use in treating neuropathic pain via intrathecal injection. The phosphatase in the use may be a protein phosphatase falling within E.C. 3.1.3.16. The phosphatase may be calcineurin. The use may include those in which the phosphatase is in unit dosage form. The unit dosage for the use may contain from about 10 μ g to about 1 g of the phosphatase. The unit dosage for the use may contain from about 15 μ g to about 15 mg of the phosphatase. The phosphatase in the unit dosage for the use may be a protein phosphatase falling within E.C. 3.1.3.16. The phosphatase in the unit dosage may be calcineurin. The unit dosage may contain from about 10 μ g to about 1 g of the calcineurin. The unit dosage may contain from about 15 μ g to about 15 mg of the calcineurin.

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BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 depicts the mechanical stimulus threshold in control rats (-●-), CCI-altered rats (-■-) and sham-operated rats (-▲-) # = p < 0.005. See the examples for full experimental details.

FIG. 2 depicts the thermal latency threshold in control rats (-●-), CCI-altered rats

(-■-) and sham-operated rats (-▲-) $\# = p < 0.005$. See the examples for full experimental details.

FIG. 3 is a histogram depicting calcineurin activity in the PSD of control, sham-operated, and CCI-altered rats.

5 FIG. 4 is a histogram depicting calcineurin A α isoform content in the PSD of control, sham-operated, and CCI-altered rats.

FIG. 5 depicts the mechanical stimulus threshold in CCI-altered rats subject to a single intrathecal injection of calcineurin (-■-) or saline (-●-). $+ = p < 0.01$ when compared to pre-injection behavior. See the examples for full experimental details.

10 FIG. 6 depicts the thermal latency threshold in CCI-altered rats subject to a single intrathecal injection of calcineurin (-■-) or saline (-●-). $+ = p < 0.01$ when compared to pre-injection behavior. See the examples for full experimental details.

FIG. 7 is a histogram depicting calcineurin A α isoform content in the post-synaptic density of rats 90 minutes after intrathecal injection of either saline or a single dose of calcineurin. The inset shows the gel from which the histogram was generated.

FIG. 8 depicts the mechanical stimulus threshold in CCI-altered rats subjected to a single intrathecal injection of calcineurin (-■-) and control CCI-altered rats subjected to an injection of saline (-●-). *Significantly different from baseline.
20 #Significantly different from the pre-injection behavior. See the examples for full experimental details.

FIG. 9 depicts the thermal latency threshold in CCI-altered rats subjected to a single intrathecal injection of calcineurin (-■-) and control CCI-altered rats subjected to an injection of saline (-●-). *Significantly different from baseline. #Significantly different from the pre-injection behavior.
25

FIG. 10 depicts the mechanical stimulus threshold in unaltered rats subjected to a single intrathecal injection of calcineurin.

FIG. 11 depicts the thermal latency threshold in unaltered rats subjected to a single intrathecal injection of calcineurin.

30 FIG. 12 depicts the mechanical stimulus threshold in CCI-altered rats subjected to a single intrathecal injection of calcineurin and control CCI-altered rats subjected to an injection of saline. *Significantly different from baseline. #Significantly different from the pre-injection behavior. See the examples for full experimental details.

FIG. 13 depicts the thermal latency threshold in CCI-altered rats subjected to a single intrathecal injection of calcineurin and control CCI-altered rats subjected to an injection of saline. *Significantly different from baseline. #Significantly different from the pre-injection behavior.

5 FIG. 14 depicts the mechanical stimulus threshold in control rats injected with saline (-●-) and rats subjected to a single injection of FK-506 (-■-).

FIG. 15 depicts the thermal latency threshold in control rats injected with saline (-●-) and rats subjected to a single injection of FK-506 (-■-).

10 FIG. 16 is a histogram depicting calcineurin activity in the post-synaptic density in saline-injected rates and rats injected with FK-506.

DETAILED DESCRIPTION

Phosphatases/Protein Phosphatases:

15 Calcineurin (a protein phosphatase 3) plays a pivotal role in regulating activity-dependent synaptic plasticity in the brain. The present inventors have now found that phosphatases in general, protein phosphatases more specifically, and calcineurin most notably play a pivotal role in regulating the development and maintenance of neuropathic pain. As used herein, “phosphatase” generally refers to
20 any enzyme falling within the EC 3.1.3.x (where x is an integer from 1 to 86, *i.e.*, phosphoric monoester hydrolases). These enzymes include those falling within the following enzyme classification numbers:

	EC 3.1.3.1	alkaline phosphatase
	EC 3.1.3.2	acid phosphatase
25	EC 3.1.3.3	phosphoserine phosphatase
	EC 3.1.3.4	phosphatidate phosphatase
	EC 3.1.3.5	5'-nucleotidase
	EC 3.1.3.6	3'-nucleotidase
	EC 3.1.3.7	3'(2'),5'-bisphosphate nucleotidase
30	EC 3.1.3.8	3-phytase
	EC 3.1.3.9	glucose-6-phosphatase
	EC 3.1.3.10	glucose-1-phosphatase
	EC 3.1.3.11	fructose-bisphosphatase
	EC 3.1.3.12	trehalose-phosphatase

	EC 3.1.3.13	bisphosphoglycerate phosphatase
	EC 3.1.3.14	methylphosphothioglycerate phosphatase
	EC 3.1.3.15	histidinol-phosphatase
	EC 3.1.3.16	phosphoprotein phosphatase
5	EC 3.1.3.17	[phosphorylase] phosphatase
	EC 3.1.3.18	phosphoglycolate phosphatase
	EC 3.1.3.19	glycerol-2-phosphatase
	EC 3.1.3.20	phosphoglycerate phosphatase
	EC 3.1.3.21	glycerol-1-phosphatase
10	EC 3.1.3.22	mannitol-1-phosphatase
	EC 3.1.3.23	sugar-phosphatase
	EC 3.1.3.24	sucrose-phosphate phosphatase
	EC 3.1.3.25	inositol-phosphate phosphatase
	EC 3.1.3.26	4-phytase
15	EC 3.1.3.27	phosphatidylglycerophosphatase
	EC 3.1.3.28	ADP-phosphoglycerate phosphatase
	EC 3.1.3.29	N-acylneuraminate-9-phosphatase
	EC 3.1.3.30	Now included with EC 3.1.3.31
	EC 3.1.3.31	nucleotidase
20	EC 3.1.3.32	polynucleotide 3'-phosphatase
	EC 3.1.3.33	polynucleotide 5'-phosphatase
	EC 3.1.3.34	deoxynucleotide 3'-phosphatase
	EC 3.1.3.35	thymidylate 5'-phosphatase
	EC 3.1.3.36	phosphoinositide 5-phosphatase
25	EC 3.1.3.37	sedoheptulose-bisphosphatase
	EC 3.1.3.38	3-phosphoglycerate phosphatase
	EC 3.1.3.39	streptomycin-6-phosphatase
	EC 3.1.3.40	guanidinodeoxy-scyllo-inositol-4-phosphatase
	EC 3.1.3.41	4-nitrophenylphosphatase
30	EC 3.1.3.42	[glycogen-synthase-D] phosphatase
	EC 3.1.3.43	[pyruvate dehydrogenase (acetyl-transferring)]-phosphatase
	EC 3.1.3.44	[acetyl-CoA carboxylase]-phosphatase
	EC 3.1.3.45	3-deoxy-manno-octulosonate-8-phosphatase
	EC 3.1.3.46	fructose-2,6-bisphosphate 2-phosphatase

	EC 3.1.3.47	[hydroxymethylglutaryl-CoA reductase (NADPH)]- phosphatase
	EC 3.1.3.48	protein-tyrosine-phosphatase
	EC 3.1.3.49	[pyruvate kinase]-phosphatase
5	EC 3.1.3.50	sorbitol-6-phosphatase
	EC 3.1.3.51	dolichyl-phosphatase
	EC 3.1.3.52	[3-methyl-2-oxobutanoate dehydrogenase (2-methylpropanoyl- transferring)]- phosphatase
	EC 3.1.3.53	[myosin-light-chain] phosphatase
10	EC 3.1.3.54	fructose-2,6-bisphosphate 6-phosphatase
	EC 3.1.3.55	caldesmon-phosphatase
	EC 3.1.3.56	inositol-polyphosphate 5-phosphatase
	EC 3.1.3.57	inositol-1,4-bisphosphate 1-phosphatase
	EC 3.1.3.58	sugar-terminal-phosphatase
15	EC 3.1.3.59	alkylacetylglycerophosphatase
	EC 3.1.3.60	phosphoenolpyruvate phosphatase
	EC 3.1.3.61	deleted
	EC 3.1.3.62	multiple inositol-polyphosphate phosphatase
	EC 3.1.3.63	2-carboxy-D-arabinitol-1-phosphatase
20	EC 3.1.3.64	phosphatidylinositol-3-phosphatase
	EC 3.1.3.65	Now EC 3.1.3.64
	EC 3.1.3.66	phosphatidylinositol-3,4-bisphosphate 4-phosphatase
	EC 3.1.3.67	phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase
	EC 3.1.3.68	2-deoxyglucose-6-phosphatase
25	EC 3.1.3.69	glucosylglycerol 3-phosphatase
	EC 3.1.3.70	mannosyl-3-phosphoglycerate phosphatase
	EC 3.1.3.71	2-phosphosulfolactate phosphatase
	EC 3.1.3.72	5-phytase
	EC 3.1.3.73	adenosylcobalamin/ α -ribazole phosphatase
30	EC 3.1.3.74	pyridoxal phosphatase
	EC 3.1.3.75	phosphoethanolamine/phosphocholine phosphatase
	EC 3.1.3.76	lipid-phosphate phosphatase
	EC 3.1.3.77	acireductone synthase
	EC 3.1.3.78	phosphatidylinositol-4,5-bisphosphate 4-phosphatase

- EC 3.1.3.79 mannosylfructose-phosphate phosphatase
- EC 3.1.3.80 2,3-bisphosphoglycerate 3-phosphatase
- EC 3.1.3.81 diacylglycerol diphosphate phosphatase
- EC 3.1.3.82 D-glycero- β -D-manno-heptose 1,7-bisphosphate 7-phosphatase
- 5 EC 3.1.3.83 D-glycero- α -D-manno-heptose-1,7-bisphosphate 7-phosphatase
- EC 3.1.3.84 ADP-ribose 1"-phosphate phosphatase
- EC 3.1.3.85 glucosyl-3-phosphoglycerate phosphatase
- EC 3.1.3.86 phosphatidylinositol-3,4,5-trisphosphate 5-phosphatase

Preferred phosphatases are those falling within E.C. 3.1.3.16. Synonyms for
 10 this specific class of enzyme include protein phosphatase, phosphoprotein
 phosphatase, and phosphoprotein phosphohydrolase. The term "protein phosphatase"
 shall be used herein. As used herein, the phrase "protein phosphatase" encompasses
 any enzyme that catalyzes the reaction: phospho-protein + H₂O $\rightarrow$ a protein +
 phosphate. Thus, the term "protein phosphatase" as used herein, includes, but is not
 15 limited to protein phosphatase I, protein phosphatase IB, protein phosphatase II,
 protein phosphatase III, protein phosphatase IV, protein phosphatase 2A, protein
 phosphatase 2B, protein phosphatase-2C, protein phosphatase C-II, protein
 phosphatase H-II, protein phosphatase SP, protein D phosphatase, protein
 phosphatase, phosphopyruvate dehydrogenase phosphatase, phosphospectrin
 20 phosphatase, polycation modulated (PCM-) phosphatase, 3-hydroxy 3-methylglutaryl
 coenzymeA reductase phosphatase, *Aspergillus awamori* acid protein phosphatase,
 BCKDH phosphatase, branched-chain alpha-keto acid dehydrogenase phosphatase,
 calcineurin, casein phosphatase, and HMG-CoA reductase phosphatase.

Calcineurin is a protein phosphatase within E.C. 3.1.3.16, also known as
 25 protein phosphatase 3, PPP3CA, and calcium-dependent serine-threonine
 phosphatase, and formerly known as protein phosphatase 2B (PP2B). It activates the
 T cells of the immune system and can be blocked by various inhibiting drugs,
 including the immune system suppressants cyclosporine, pimecrolimus, and
 tacrolimus.

30 Thus, described herein is a method to inhibit neuropathic pain comprising
 injecting intrathecally into a subject a neuropathic pain-inhibiting amount of a protein
 phosphatase, more preferably a protein phosphatase 3 and most preferably
 calcineurin.

While not being bound to any underlying mechanism or pathway, nerve injury may give rise to neuropathic pain at least in part as a result of the loss of calcineurin-mediated dephosphorylation in the DH. With no dephosphorylation to prevent a remodeling of the PSD which favors synaptic enhancement, only exaggerated evoked
5 responses would be elicited by primary afferent activity. This enhanced sensory input manifests then as neuropathic pain.

Pharmaceutical Compositions:

Included herein are pharmaceutical compositions comprising one or more
10 protein phosphatases or a pharmaceutically acceptable salt thereof together with a pharmaceutically acceptable carrier therefor and, optionally, other therapeutically active substances in addition to the protein phosphatase or salt thereof. The pharmaceutical compositions comprise an amount of one or more protein phosphatases or a pharmaceutically acceptable salt thereof that is effective to treat
15 neuropathic pain in a mammal suffering therefrom, to which the composition is administered. In a pharmaceutical composition of the invention, the carrier must be pharmaceutically acceptable in the sense of being compatible with other ingredients in the particular composition and not deleterious to the recipient thereof. The compositions include those suitable for parenteral administration in general (including
20 subcutaneous, intramuscular, intradermal, intravenous, and intrathecal) and intrathecal administration in particular.

A “pharmaceutically suitable salt” or “pharmaceutically acceptable salt” (the two phrases are synonymous) is any acid or base addition salt whose counter-ions are non toxic to the patient in pharmaceutical doses of the salts, so that the beneficial
25 effects inherent in the free base or free acid are not vitiated by side-effects ascribable to the counter-ions. A host of pharmaceutically suitable salts are well known in the art. For basic active ingredients, all acid addition salts are useful as sources of the free base form even if the particular salt, per se, is desired only as an intermediate product as, for example, when the salt is formed only for purposes of purification, and
30 identification, or when it is used as intermediate in preparing a pharmaceutically-suitable salt by ion exchange procedures. Pharmaceutically-suitable salts include, without limitation, those derived from mineral acids and organic acids, explicitly including hydrohalides, *e.g.*, hydrochlorides and hydrobromides, sulphates, phosphates, nitrates, sulphonates, acetates, citrates, lactates, tartrates, malonates,

oxalates, salicylates, propionates, succinates, fumarates, maleates, methylene bis b
hydroxynaphthoates, gentisates, isethionates, di p toluoyltartrates, methane
sulphonates, ethanesulphonates, benzenesulphonates, p toluenesulphonates,
cyclohexylsulphamates, quinate, and the like. Base addition salts include those
5 derived from alkali or alkaline earth metal bases or conventional organic bases, such
as triethylamine, pyridine, piperidine, morpholine, N methylmorpholine, and the like.
Other suitable salts are found in, for example, Handbook of Pharmaceutical Salts,
P.H. Stahl and C.G. Wermuch, Eds., © 2002, Verlag Helvetica Chemica Acta (Zurich,
Switzerland) and S.M. Berge, et al., "Pharmaceutical Salts," *J. Pharm. Sci.*, 66: p.1-
10 19 (January 1977), both of which are incorporated herein by reference.

In a particular aspect, the pharmaceutical compositions comprise the active
ingredient (one or more protein phosphatases or a pharmaceutically acceptable salt
thereof) presented in unit dosage form. The term "unit dosage" or "unit dose" is
denoted to mean a predetermined amount of the active ingredient sufficient to be
15 effective to treat neuropathic pain. Preferred unit dosage formulations are those
containing a daily dose, daily sub-dose, or an appropriate fraction thereof, of the
administered active ingredient.

The pharmaceutical compositions may be prepared by any of the methods well
known in the art of pharmacy. All methods include the step of bringing the active
20 ingredient into association with a carrier which constitutes one or more accessory
ingredients. In general, the compositions are prepared by uniformly and intimately
bringing the active ingredient into association with a liquid or solid carrier and then, if
necessary, shaping the product into the desired unit dosage form. Clearly, for
intrathecal injection, the protein phosphatase is formulated into a suitable liquid form.

25 Thus, pharmaceutical compositions suitable for parenteral administration,
including intrathecal injection, conveniently comprise a sterile injectable preparation
of the active ingredient in, for example, a solution which is preferably isotonic with
the blood or cerebrospinal fluid of the recipient. Useful formulations also comprise
concentrated solutions or solids containing the active ingredient which upon dilution
30 with an appropriate solvent give a solution suitable for parenteral (including
intrathecal) administration. The parenteral compositions include aqueous and non-
aqueous formulations which may contain conventional adjuvants such as buffers,
bacteriostats, sugars, thickening agents and the like. The compositions may be

presented in unit dose or multi-dose containers, for example, sealed ampules and vials.

In addition to the aforementioned ingredients, the compositions of this invention may further include one or more optional accessory ingredients(s) utilized in the art of pharmaceutical formulations, *e.g.*, diluents, buffers, colorants, binders, surfactants, thickeners, lubricants, suspending agents, preservatives (including antioxidants) and the like.

The amount of active ingredient required to be effective for alleviating neuropathic pain will, of course, vary with the individual mammal being treated and is ultimately at the discretion of the medical or veterinary practitioner. The factors to be considered include the species and sex of the mammal, the condition being treated, the route of administration, the nature of the formulation, the mammal's body weight, surface area, age and general condition, and the particular protein phosphatase(s) to be administered.

In general, the pharmaceutical compositions of this invention contain from about 10 µg to about 1 g and, preferably, from about 15 µg to about 15 mg of the active ingredient, preferably in a unit dosage form. However, a suitable effective dose is in the range of about 0.2 µg to about 200 µg/kg body weight/day, preferably in the range of about 0.2 µg to about 100 µg/kg body weight/day, or about 0.2 µg to 10 µg/kg body weight/day or about 0.2 µg/kg body weight/day to about 5 µg/kg body weight/day calculated as the non-salt form of the phosphatase. The total daily dose may be given as a single dose, multiple doses, *e.g.*, two to six times per day, or by intrathecal infusion for a selected duration. Dosages above or below the range cited above are within the scope of the disclosed method and composition and may be administered to the individual patient if desired and necessary.

For example, for a 75 kg mammal, a dose range would be about 15 µg to about 15 mg per day.

Methods to Treat Neuropathic Pain:

The processing of sensory information in the spinal dorsal horn may change significantly following peripheral nerve injury or inflammation. Ultimately this changed processing may lead to the development of neuropathic pain. For example, a short but intense period of acute injury to the sciatic nerve may lead to persistent pain that far outlasts the initial insult. Much progress has been made in elucidating some of

the mechanisms underlying the development of neuropathic pain. Yet many gaps in our knowledge remain and as a result neuropathic pain continues to be inadequately treated. There remains a long-felt and unmet need for a method to alleviate neuropathic pain.

5 Preliminary studies by the present inventors suggested that the loss of calcium phosphatases in general, and more specifically protein phosphatase 3, also known as calcineurin, at synapses of spinal dorsal horn neurons may lead to the development of neuropathic pain following sciatic nerve injury. As shown in the Examples below, a single intrathecal injection of exogenous calcineurin alleviated well-established
10 neuropathic pain for four days after the injection. This was intriguing and unexpected because a method of effectively relieving or diminishing pain in patients suffering from well-established neuropathic pain is unknown.

Thus, described herein is a method of alleviating, reducing, diminishing or otherwise attenuating neuropathic pain by administering a protein phosphatase
15 intrathecally into a mammal in need of pain relief. The preferred protein phosphatase is calcineurin.

Protein phosphatases in general and calcineurin (protein phosphatase 3, also previously known as protein phosphatase 2B) in particular play a pivotal role in regulating activity-dependent synaptic plasticity in the brain. The development of
20 neuropathic pain appears dependent upon some of the same mechanisms that underlie brain synaptic plasticity. Thus, the inventors hypothesized that protein phosphatases such as calcineurin may similarly play an important role in long-lasting injury-elicited plasticity in the spinal dorsal horn.

Preliminary studies by the present inventors indicated that the loss of
25 calcineurin in the post-synaptic density (PSD) of spinal dorsal horn neurons may lead to the development of neuropathic pain following sciatic nerve injury. The studies (described in the Examples) also demonstrated that a single intrathecal injection of exogenous calcineurin alleviated well-established neuropathic pain for four days after the injection.

30 While not being limited to any specific underlying mechanism of action, the inventors postulate that the loss of protein phosphatase activity in the PSD critically permits nerve injury-elicited afferent activity to remodel the synapses of spinal dorsal horn neurons in such a way as to allow for the transition to long-lasting plasticity and thus the development of neuropathic pain. In other words, nerve injury may give rise

to neuropathic pain at least in part as a result of the loss of protein phosphatase-mediated dephosphorylation in the spinal dorsal horn. With no dephosphorylation to prevent the nerve injury-elicited remodeling of the PSD which favors synaptic enhancement, only exaggerated evoked responses would be elicited by primary
5 afferent activity. This enhanced sensory input manifests then as neuropathic pain.

Treatment of neuropathic pain remains elusive. Several promising drugs have proven ineffective in human clinical trials despite their demonstrated effectiveness in animals. Consequently, the need for novel therapeutic approaches to treat neuropathic pain remains great. These approaches should be based on individualized treatment
10 regimens to maximize analgesic effectiveness. An advantage of protein phosphatase therapy is that protein phosphatases are endogenous substances. Although this does not eliminate all toxic potential, it does reduce the possibility of long-term harm especially when coupled with judicious dosing.

The balance between protein kinase and protein phosphatase activity at the
15 synapse can critically determine overall synaptic strength. As a result, the loss of either one of these activities has the potential to engender long-lasting changes in synaptic function, *i.e.*, long-lasting plasticity. More than a decade ago, Kandel and colleagues described how the interplay between protein kinase A (PKA) and calcineurin was essential in initiating and maintaining long-lasting enhancement of
20 synaptic function in *Aplysia*, *Drosophila*, mice and rats.

On one hand, activation of PKA by cyclic AMP, and the subsequent phosphorylation of target proteins, resulted in long-term memory storage. On the other hand, activation of calcineurin led to the dephosphorylation of these target proteins to prevent the transition from short to long-term memory. Later studies in
25 other brain areas confirmed the general role of calcineurin in negatively constraining the acquisition of spatial or aversive memory, or of long-lasting plasticity in ocular dominance, cocaine addiction, and vestibular compensation.

It appears that injury-elicited plasticity accompanies peripheral nerve injury
30 and that this significant alteration in sensory processing in the spinal dorsal horn may ultimately contribute to the development of neuropathic pain. Plasticity allows for enhanced neuronal communication. In the brain, it enables learning and memory, but in the spinal dorsal horn it may transform the essential but rapidly terminated

sensation of acute pain into unproductive persistent, chronic pain (*i.e.*, neuropathic pain).

The post-synaptic density (PSD) is especially deserving of attention with respect to neuropathic pain because its function may represent a final common reflection of the many injury-elicited changes in receptors, pathways, transcription factors and genes that have been cataloged since the introduction of chronic pain models two decades ago. As detailed in the Examples, the inventors have now shown that the development of chronic pain is a pivotal consequence of the loss of protein phosphatase activity in the PSD of spinal dorsal horn neurons.

The data gathered by the inventors (see Examples) confirmed an association between calcineurin and neuropathic pain. There was significantly lower calcineurin activity and content in the ipsilateral PSD of spinal dorsal horn neurons in CCI animals exhibiting neuropathic pain. The pain behavior was attenuated by an intrathecal injection of exogenous calcineurin which accumulated in the PSD. (See Figs. 1 and 2). The analgesic action of calcineurin was long-lasting as a single intrathecal injection provided pain relief for four days. An intrathecal application of the calcineurin inhibitor FK-506 elicited pain behavior in control, uninjured animals. The same injection significantly reduced calcineurin activity in the PSD. (See Figs. 7, 8, and 9). For all figures error bars represent the SEM.

EXAMPLES

The following examples are included solely to provide a more complete description of the invention disclosed and claimed herein. The examples do not limit the scope of the invention in any fashion.

Summary of Testing Protocol:

- Perform behavioral tests (mechanical thresholds, thermal latencies).
- Anesthetize animal. Perform chronic constriction injury (CCI) surgery on sciatic nerve.
- Recover the animal and return to animal quarters.
- Re-test the behavior seven (7) days-post surgery.
- Anesthetize the animal.
- Perform intrathecal injection of protein phosphatase (calcineurin was used in the examples).

Recover the animal and return to animal quarters.

Re-test behavior 2, 4 and 6 days after intrathecal calcineurin injection.

5 Animals:

Male Sprague-Dawley rats (200-250g, Harlan Laboratories, Madison, WI) were used as experimental animals. Water and food was provided *ad libitum*. All experiments were conducted in accordance with guidelines accepted by the International Association for the Study of Pain (Zimmermann, 1983). The animal
10 protocol (M02113) was approved by the Animal Care Committee of the School of Medicine and Public Health at the University of Wisconsin-Madison.

Mechanical Withdrawal Threshold:

An automated instrument was used for assessing touch sensitivity. An animal
15 was placed inside a plastic box on a raised plasticized wire-mesh surface. The animal is free to move within the confines of the box. The animal acclimates to the apparatus, and a touch stimulator unit was then slid under the animal to position a stainless steel filament (0.5 mm diameter) under the plantar surface of a paw. A button press causes the filament to move perpendicularly to the tested paw. The
20 filament exerts an increasing force, starting below the threshold of detection and increases until the animal withdraws the paw. The force applied to the filament that results in paw withdrawal was recorded.

More specifically, a dual channel scale (Incapacitance Meter™, Stoelting, Chicago, IL), which separately measures the weight borne by each hind limb, was
25 used for the weight-bearing tests. (See Miletic and Miletic, 2008). The dual-channel scale separately measures the weight the animal distributes to each hind limb. The force exerted by each limb (measured in grams) is averaged over a user-selectable period, thus indicating any tendency for the animal to shift its weight from one limb to the other. While normal rats distribute weight about equally, animals with a unilateral
30 injury will shift the ratio of weight distribution between an injured and non-injured limb. The difference in weight distribution is taken as a measure of the level of discomfort in the injured limb. In other words, the less weight placed on an injured limb, the greater the level of discomfort. The weight distribution of each hind limb was measured over a 1s period, and the measurement repeated 20 times to obtain an

averaged left/right ratio. An important advantage of this test is that it allows assessing (at least to some extent) spontaneous pain behavior. The animal stands and makes its natural adjustment to the degree of pain.

5 Thermal Withdrawal Latency:

Thermal sensitivity is assessed using the hindpaw withdrawal test to a thermal noxious stimulus. An animal was placed inside a plastic box on a raised glass surface. The animal is free to move within the confines of the box. A movable, focused beam of radiant heat was placed under the glass on which the animal is resting a paw. The
10 glass is progressively heated until a noxious threshold was reached to elicit paw withdrawal. As soon as the animal moved its paw, a photocell in the metal enclosure containing the heat source turned off the light. The latency (in sec) for the animal to withdraw its paw was recorded by a digital automatic display.

For both behaviors, the ipsilateral, injured, paw was tested four times to obtain
15 an average value. Each test is separated by 5 min. Persons performing behavioral tests are blinded to treatment whenever possible.

Anesthesia:

Animals were anesthetized with isoflurane using an anesthesia machine (4%
20 induction, 2-2.5% maintenance). Body temperature was kept at 37°C with a homeothermic blanket system. Anesthesia was sufficiently deep to prevent arousal, but light enough to permit spontaneous respiration. Surgical anesthetic depth was determined by the loss of corneal and pedal reflexes.

25 Chronic Constriction Injury (CCI, Loose Ligation of the Sciatic Nerve):

Animals were anesthetized with isoflurane and prepared for surgery by clipping the hair over the mid-thigh area of the left hind leg, scrubbing the clipped area with povidone iodine and alcohol (repeated three times), and lubricating the eyes with sterile ophthalmic ointment. The sciatic nerve was exposed at mid-thigh level by
30 blunt dissection through the biceps femoris muscles. The nerve was freed of adherent tissue, and four ligatures (4.0 chromic gut) were spaced about 1 mm apart. Care was taken to tie the ligatures so that the nerve trunk is just barely constricted when viewed with a dissecting microscope at 40x. This degree of constriction retards, but does not

arrest, circulation through the superficial epineural vasculature. The incision was closed in layers using chromic gut sutures for muscle and silk sutures for skin.

Calcineurin Solution:

5 A stock solution of calcineurin enzyme at a concentration of 100 units/microliter is commercially available (Enzo Life Sciences, Plymouth Meeting, PA, Cat. No. BML-SE163-5000). The stock solution was diluted 1:10 with saline (1 part of calcineurin stock solution + 9 parts of saline) to make a diluted calcineurin solution of 10 units/microliter. Ten microliters of the diluted calcineurin solution was
10 then injected intrathecally. In other words the dose is 100 units of calcineurin per animal. Animal weights were approximately 200 to 250 g. This equates to a dosage of roughly 3 μ g/kg body weight (in the rat).

Intrathecal Injection:

15 The animals were anesthetized with isoflurane and securely held in the sternal position. A 25 μ l Hamilton-brand microsyringe was fitted with a 25-gauge, 1-inch hypodermic needle and the needle inserted between the dorsal aspects of L5 and L6 perpendicular to the vertebral column. This site corresponds to the region where the spinal cord ends and the cauda equina begins and was chosen to reduce the possibility
20 of spinal damage. The needle was advanced until a slight but noticeable movement of the tail or the leg was observed. This indicates that the needle has entered into the intrathecal space. Drugs were injected in a volume of 10 μ l and the syringe was held in place for a few seconds before being progressively and slowly withdrawn to avoid drug outflow. The rat then recovers from the anesthesia. Any animal that showed
25 signs of motor impairment or other neurological disorders was immediately euthanized with an intracardiac injection of super-saturated potassium chloride solution (>350 mg/ml) after the animal had been first anesthetized with isoflurane.

30

Tissue Collection for Biochemical Assays:

Animals were anesthetized with isoflurane and while still deeply anesthetized were euthanized with an intracardiac injection of super-saturated potassium chloride (>350 mg/ml). A laminectomy was used to rapidly (< 2 min) expose the lumbar spinal

cord at L5. About 1 cm of the cord was excised and placed into a Petri dish containing dry-ice-cold saline or homogenizing buffer. Subsequently, the cord was cut under a dissecting microscope into dorsal and ventral halves, and the dorsal half further divided into ipsilateral and contralateral quadrants. All tissues were immediately
5 placed into dry-ice cooled collecting tubes, and stored at -80°C until used.

Tissue Collection for Double Immunofluorescence:

Animals were first rendered unconscious with isoflurane and then deeply anesthetized with sodium pentobarbital (60 mg/kg). Subsequently, the animals were
10 injected intracardially with 0.5 ml heparin (1000 iu/ml), and perfused intracardially with 4% paraformaldehyde in 0.2M phosphate buffered-saline (PBS). The fixed lumbar spinal cord (~L5) was removed and cryoprotected in 30% sucrose and 5% glycerin in 0.1M PBS, and stored for a few days before sectioning.

15 RT-PCR, Enzyme Activity Assay and Immunoblots:

Calcineurin mRNA levels were determined by monitoring in real-time the increase in fluorescence of SYBR-GREEN dye with the ABI Prism 7300 Sequence Detection System (Applied Biosystems, Foster City, CA) as detailed previously (Miyabe et al., 2006). Relative expression levels of calcineurin in each sample were
20 determined using a standard curve of 3-fold serial dilutions. Average fold induction relative to control animals was determined after normalizing to the amount of 18S rRNA in each sample. A 2-fold or greater change was considered significant. Primer sequences not shown.

Calcineurin activity was obtained with a commercial kit (Enzo Life Sciences,
25 Plymouth Meeting, PA) and expressed as nmol of phosphate released/min/mg of protein. Immunoblots were performed as described previously (Miletic et al., 2002). Calcineurin isoform A α (Millipore, Billerica, MA) was used at a dilution of 1:1000. Developed membranes were stripped and re-probed with beta III tubulin (1:1000; Promega, Madison, WI) as the loading control. Protein levels were estimated with the
30 BioSpectrum 500 Image Analysis System (UVP, Upland, CA). The calcineurin A α content within a gel was expressed over the beta III tubulin content, and then the content in sham-operated or CCI animals was normalized to those in control animals. The same normalization procedure was used for the enzyme activity assay.

Statistical Analysis:

ANOVA was used for the statistical data analysis. The main emphasis was on detecting differences in behavior, calcineurin message, activity, or protein content between control, sham-operated and CCI animals, or vehicle and drug-treated animals. Significant effects were further analyzed with Scheffe's post-hoc test. Statistical difference was inferred at $p \leq 0.05$. All data are expressed as mean $\pm$ SEM.

Lower Calcineurin Activity and Content in PSD of Spinal Dorsal Horn Neurons in CCI Animals Exhibiting Neuropathic Pain:

There was significantly lower calcineurin activity and calcineurin content in the ipsilateral PSD of spinal dorsal horn neurons in CCI animals exhibiting neuropathic pain. See Figs. 1, 2, 3, and 4. As shown in these four figures, loss of calcineurin activity and protein content in the PSD of spinal dorsal horn neurons was associated with pain behavior. Here, CCI animals were tested for mechanical threshold (Fig. 1) and thermal threshold (Fig. 2) and then assayed for calcineurin activity (Fig. 3) and calcineurin content in the PSD of spinal dorsal horn neurons (Fig. 4). Mechanical and thermal hypersensitivity was observed seven (7) days post-CCI (Figs 1 and 2, respectively) and were accompanied by a significant loss of calcineurin activity (Fig. 3) and content of the A α isoform (~60kDa) in the ipsilateral PSD (Fig. 4). In contrast, calcineurin activity or content in the contralateral PSD of CCI animals or either side in sham-operated animals was not different from controls. See Figs. 3 and 4, respectively. Neither control uninjured nor sham-operated animals exhibited pain behavior. For each group n=3. In Figs. 1, 2, 3, and 4, CaN = calcineurin. # $p < 0.005$, * $p < 0.001$.

Intrathecal Injection of Exogenous Calcineurin Alleviated Mechanical and Thermal Hypersensitivity Following CCI:

Here, animals were subjected to the CCI surgery and tested for pain behavior. CCI animals exhibited typical pain behavior 7 days post-ligation. See Figs. 5 and 6. Saline (n=3) or calcineurin (100 unit total dose in 10 μ l vehicle) (n=3) were then injected intrathecally and the animals were behaviorally retested 90 min after the injection. Both the mechanical and the thermal hypersensitivity were significantly attenuated in the calcineurin injected animals. Again, see Figs. 5 and 6. In these same

animals there was significantly more calcineurin A α in their ipsilateral PSD. See Fig. 7; * $p < 0.01$ when compared to the pre-injection behavior; * $p < 0.001$ vs. control.

This example shows that adding exogenous phosphatase enzyme by intrathecal injection alleviates pain behavior. This may be a result of the phosphatase's accumulation in the PSD.

Duration of Analgesia from a Single Intrathecal Injection of Calcineurin:

To examine the duration of the analgesic action of calcineurin, the behavior of animals with chronic constriction injury (CCI) of the sciatic nerve was tested 2, 4 and 6 days after a single injection of calcineurin (10 units/microliters). The goal of this example was to investigate the longevity of the analgesic action of calcineurin by performing an experiment in chronic constriction injury (CCI) animals and repeatedly testing the behavior two, four and six days after the calcineurin injection.

As expected, the CCI animals exhibited both mechanical and thermal hypersensitivity seven (7) days after the ligation. These animals were then anesthetized and injected intrathecally with saline (n=3) or calcineurin (n=6). Two days later the animals were behaviorally retested. In the calcineurin-treated animals both the mechanical and thermal hypersensitivity were significantly attenuated. See Figs. 8 and 9, respectively. The calcineurin-treated CCI animals continued to exhibit analgesia when they were behaviorally retested four (4) days after the intrathecal injection. It was only six (6) days after the injection that the calcineurin-treated CCI animals exhibited once again mechanical and thermal hypersensitivity suggesting that calcineurin analgesia had ended. Saline-treated CCI animals exhibited typical pain behavior throughout the testing period. See Fig. 10 (mechanical threshold in the control animals) and Fig. 11 (thermal latency in the control animals).

These data establish that a single intrathecal injection of calcineurin provides continual pain relief for at least four (4) days.

As shown in Figs. 8 and 9, the CCI animals exhibited both mechanical hypersensitivity (Fig. 8, -●-) and thermal hypersensitivity (Fig. 9, -●-) as signs of pain behavior seven days post-CCI. These animals were then anesthetized and injected intrathecally with calcineurin (n=4) or saline vehicle (n=3). In the calcineurin-treated animals both the mechanical hypersensitivity (Fig. 8, -■-) and thermal hypersensitivity (Fig. 9, -■-) were attenuated two (2) and four (4) days after the injection. It was only six (6) days after the injection that the calcineurin-treated

animals exhibited pain behavior suggesting that the calcineurin analgesia had ended. Vehicle-treated CCI animals exhibited typical pain behavior which persisted throughout the testing period. See Figs. 8 and 9, -●-.

5 The behavior of three control, uninjured, animals was not modified by the intrathecal injection of calcineurin. Fig. 10 depicts the results in the control animals for mechanical stimulation; Fig. 11 depicts the results for the control animals for thermal stimulation.

Repeated measures ANOVA confirmed that the difference between the saline treatment and the calcineurin treatment was statistically significant.

10 Thus, as shown by this example, a single intrathecal application of exogenous calcineurin elicited prolonged analgesia in neuropathic animals. CCI-altered animals exhibited typical pain behavior seven (7) days post-CCI. After the behavioral testing, saline vehicle (10 μ l) or calcineurin (100 units in 10 μ l) were injected intrathecally and the animals were behaviorally retested 2, 4, and 6 days after the single injection. Both
15 the mechanical and the thermal hypersensitivity were significantly attenuated in the calcineurin injected animals two (2) and four (4) days post-injection. By six (6) days post-injection, the pain behavior returned. Saline-injected animals exhibited pain behavior throughout the repeated testing. These data indicate that a single intrathecal injection of calcineurin provided pain relief for at least four (4) days. Control,
20 uninjured animals did not show pain behavior and were unaffected by the calcineurin injection. See Figs. 10 and 11. The results depicted in Figs. 1 and 2 were significantly different from the pre-injection behavior (# $p < 0.005$) and also significantly different from the baseline (* $p < 0.001$ vs. control).

25 Biochemistry with Behavior Study:

The goal of this example was to investigate whether the prolonged analgesic action of calcineurin (10 units/ μ l, 10 μ l injection, 100 units total dose) was associated with the restoration of activity or protein content of the enzyme in the postsynaptic density of spinal dorsal horn neurons. The example analyzed data from 18 animals.

30 Baseline behavior was first established. Then the chronic constriction injury (CCI) surgery was performed the same day. Seven (7) days later behavior was re-tested. The animals were then assigned to individual groups depending on the duration of the post-injection period (*i.e.*, 2, 4, 6 days). After the last behavioral test the animals were euthanized and their lumbar spinal dorsal horn tissues collected for

the calcineurin activity and protein content assays. These data are called “group” data to differentiate them from the “continuous” data in which the same animal was tested at 2, 4, and 6 days, post-injection.

As can be seen from Figs. 12 and 13, all of the behavioral results confirmed the data from the continuous calcineurin study depicted in Figs. 8 and 9. In other words, a single intrathecal injection of calcineurin provided analgesia in both the 2- and 4-day post-injection animal groups. The analgesic effect of calcineurin disappeared in the 6-day post-injection group. Saline-injected CCI animal groups exhibited typical pain behavior at all testing time points.

Figs. 12 and 13 show that a single intrathecal application of exogenous calcineurin (10 units/microliter, 100 unit total dose) provided prolonged analgesia in neuropathic animals. All 18 CCI animals exhibited typical pain behavior 7 days post-ligation. After the behavioral testing, half of the animals were injected intrathecally with saline (n=9) and the other half with calcineurin (n=9). The individual animal groups were then behaviorally retested 2, 4, or 6 days after the single injection (n=3 in each group). The results depicted in Figs. 5 and 6 were significant: # $p < 0.005$ when compared to the pre-injection behavior; * $p < 0.001$ vs. control.

Intrathecal Injection of FK-506 Elicited Mechanical and Thermal Hypersensitivity in Control, Uninjured Animals:

Here, control animals were given an injection of FK-506, a calcineurin inhibitor. Thus, the purpose of this Example was to assess whether the loss of calcineurin activity in the spinal dorsal horn would elicit pain behavior in control, uninjured animals. Ninety (90) minutes after an intrathecal injection of the calcineurin inhibitor FK-506 (n=3) there was a significant decrease in mechanical withdrawal thresholds (Fig. 14) and thermal latencies (Fig. 15) in control, uninjured animals (-■-) as compared to control, uninjured animals injected with saline (-●-). In these same animals there was also a significant decrease in calcineurin activity in their PSD. See Fig. 16; # $p < 0.005$, * $p < 0.001$.

These data show that inhibition of calcineurin activity in the PSD leads to pain behavior.

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CLAIMS

What is claimed is:

1. A method to inhibit neuropathic pain comprising injecting intrathecally into a mammalian subject a neuropathic pain-inhibiting amount of a phosphatase falling within E.C. 3.1.3.x, wherein x is an integer from 1 to 86.
2. The method of Claim 1, wherein the subject is a human being.
3. The method of Claim 1, comprising injecting intrathecally into the mammalian subject a neuropathic pain-inhibiting amount of a protein phosphatase falling within E.C. 3.1.3.16.
4. The method of Claim 3, wherein the subject is a human being.
5. The method of Claim 1, comprising injecting intrathecally into the mammalian subject a neuropathic pain-inhibiting amount of calcineurin.
6. The method of Claim 5, wherein the subject is a human being.
7. The method of Claim 1, comprising intrathecally injecting the phosphatase in combination with a pharmaceutical delivery vehicle suitable for intrathecal injection.
8. The method of Claim 7, comprising injecting intrathecally into the subject a dosage of about 0.2 μg to about 200 $\mu\text{g/kg}$ body weight/day of the phosphatase, protein phosphatase, or calcineurin, administered in a single dose or in multiple doses.
9. A pharmaceutical composition comprising a neuropathic pain-inhibiting amount of a phosphatase falling within E.C. 3.1.3.x, wherein x is an integer from 1 to 86, in combination with a pharmaceutical delivery vehicle suitable for intrathecal injection.

10. The pharmaceutical composition of Claim 9, wherein the phosphatase is a protein phosphatase falling within E.C. 3.1.3.16.
11. The pharmaceutical composition of Claim 9, wherein the phosphatase is calcineurin.
12. The pharmaceutical composition of Claim 9, in unit dosage form.
13. The pharmaceutical composition of Claim 12, wherein the unit dosage contains from about 10 µg to about 1 g of the phosphatase.
14. The pharmaceutical composition of Claim 12, wherein the unit dosage contains from about 15 µg to about 15 mg of the phosphatase.
15. The pharmaceutical composition of Claim 12, wherein the phosphatase is a protein phosphatase falling within E.C. 3.1.3.16.
16. The pharmaceutical composition of Claim 15, wherein the phosphatase is calcineurin.
17. The pharmaceutical composition of Claim 16, wherein the unit dosage contains from about 10 µg to about 1 g of the calcineurin.
18. The pharmaceutical composition of Claim 16, wherein the unit dosage contains from about 15 µg to about 15 mg of the calcineurin.
19. A phosphatase falling within E.C. 3.1.3.x, wherein x is an integer from 1 to 86, for use as a medicament to treat neuropathic pain via intrathecal injection.
20. The use of Claim 19, wherein the phosphatase is a protein phosphatase falling within E.C. 3.1.3.16.
21. The use of Claim 19, wherein the phosphatase is calcineurin.

22. The use of Claim 19, wherein the medicament is in unit dosage form.
23. The use of Claim 22, wherein the unit dosage contains from about 10 μg to about 1 g of the phosphatase.
24. The use of Claim 22, wherein the unit dosage contains from about 15 μg to about 15 mg of the phosphatase.
25. The use of Claim 22, wherein the phosphatase is a protein phosphatase falling within E.C. 3.1.3.16.
26. The use of Claim 22, wherein the phosphatase is calcineurin.
27. The use of Claim 26, wherein the unit dosage contains from about 10 μg to about 1 g of the calcineurin.
28. The use of Claim 26, wherein the unit dosage contains from about 15 μg to about 15 mg of the calcineurin.
29. A phosphatase falling within E.C. 3.1.3.x, wherein x is an integer from 1 to 86, for use in treating neuropathic pain via intrathecal injection.
30. The use of Claim 29, wherein the phosphatase is a protein phosphatase falling within E.C. 3.1.3.16.
31. The use of Claim 29, wherein the phosphatase is calcineurin.
32. The use of Claim 29, wherein the phosphatase is in unit dosage form.
33. The use of Claim 32, wherein the unit dosage contains from about 10 μg to about 1 g of the phosphatase.
34. The use of Claim 32, wherein the unit dosage contains from about 15 μg to about 15 mg of the phosphatase.

35. The use of Claim 32, wherein the phosphatase is a protein phosphatase falling within E.C. 3.1.3.16.

36. The use of Claim 32, wherein the phosphatase is calcineurin.

37. The use of Claim 36, wherein the unit dosage contains from about 10 μg to about 1 g of the calcineurin.

38. The use of Claim 36, wherein the unit dosage contains from about 15 μg to about 15 mg of the calcineurin.

FIG. 1

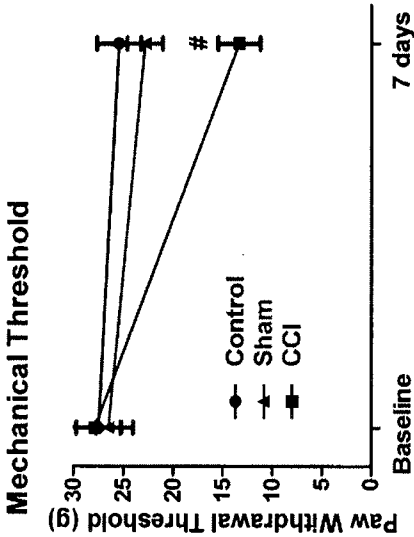


FIG. 2

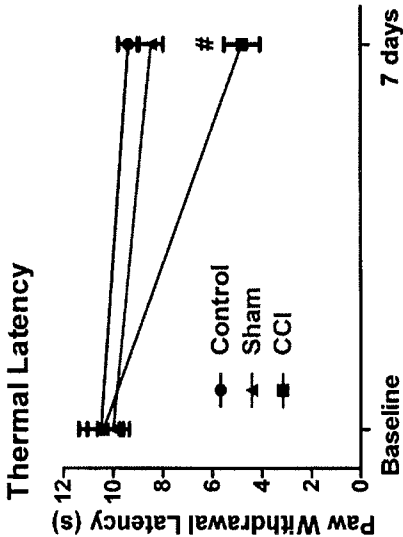


FIG. 3

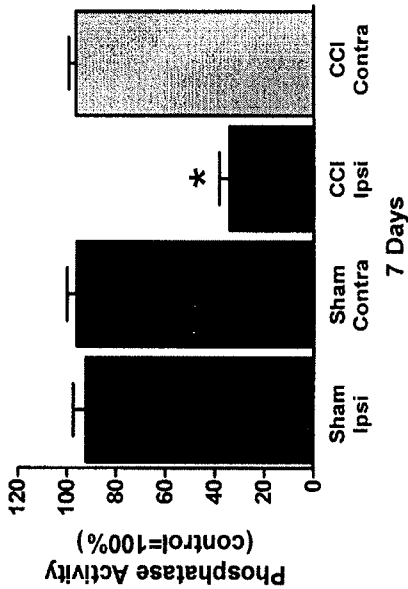
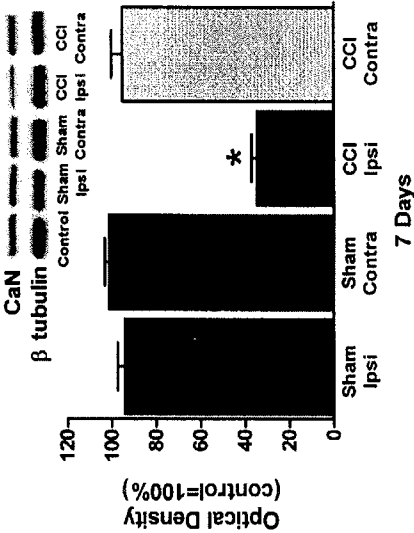


FIG. 4

FIG. 4



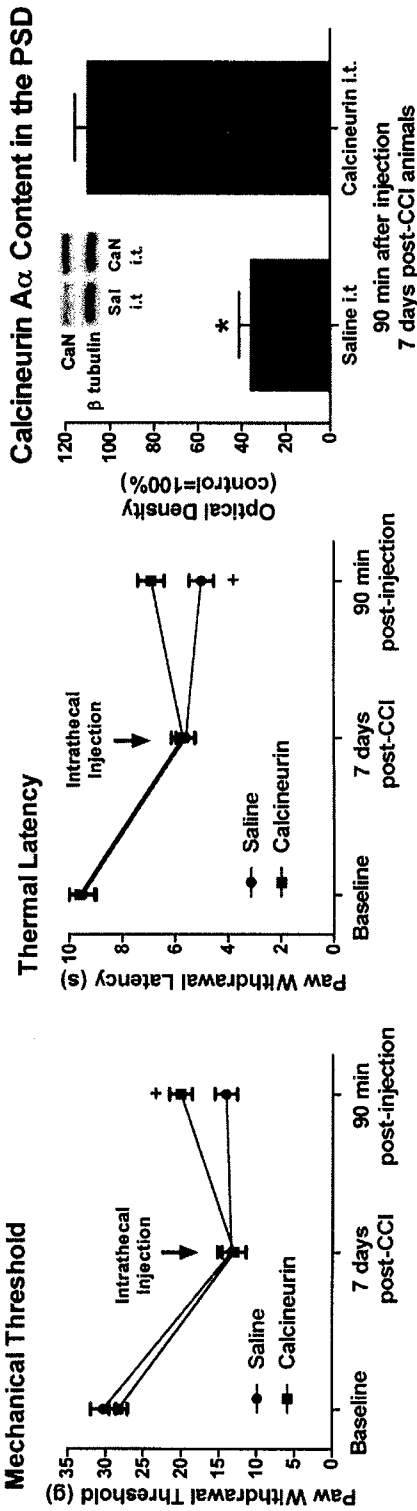


FIG. 7

FIG. 6

FIG. 5

FIG. 9

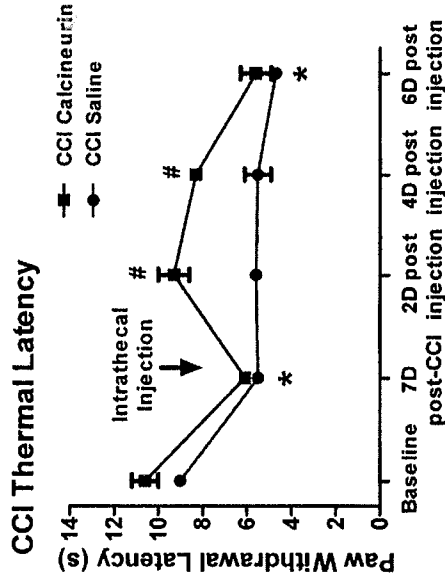
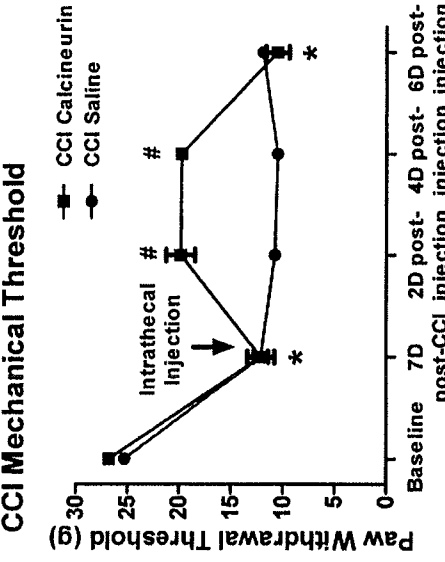
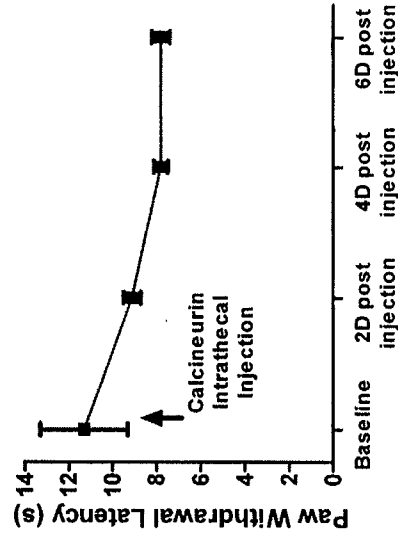


FIG. 8



Control Thermal Latency



Control Mechanical Threshold

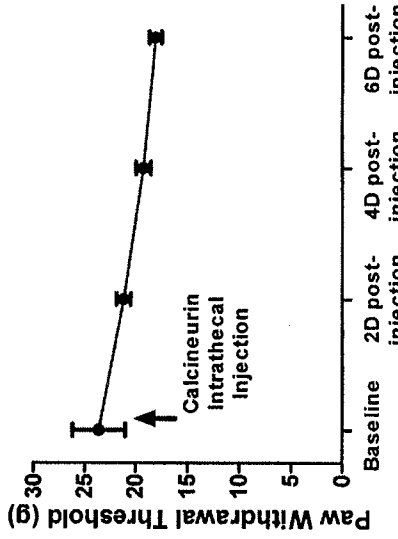


FIG. 11

FIG. 10

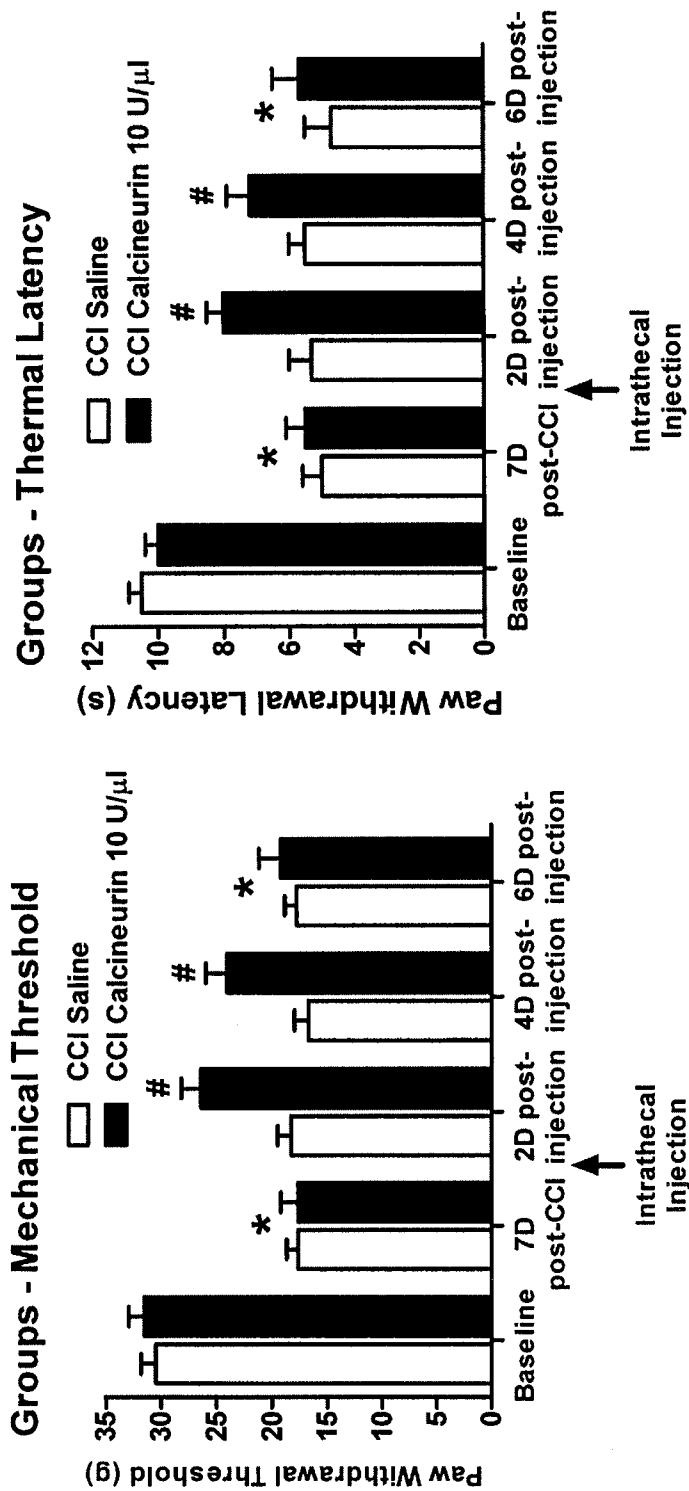


FIG. 12

FIG. 13

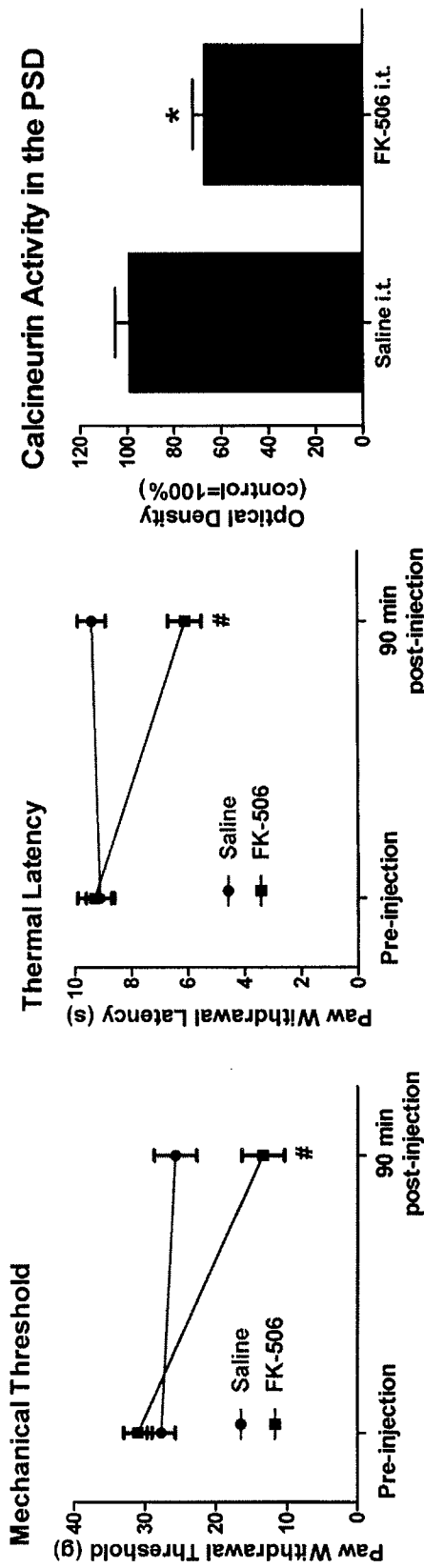


FIG. 14

FIG. 15

FIG. 16

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/042606

A. CLASSIFICATION OF SUBJECT MATTER INV. A61K38/46 A61P25/00 A61P29/00 ADD.		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) A61K A61P		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EPO-Internal, WPI Data, BIOSIS, CHEM ABS Data, Sequence Search, EMBASE		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DWORKIN ROBERT H ET AL: "Recommendations for the pharmacological management of neuropathic pain: an overview and literature update.", MAYO CLINIC PROCEEDINGS, vol. 85, no. 3 Suppl, March 2010 (2010-03), pages S3-S14, XP002684920, ISSN: 1942-5546 the whole document	1-4, 6-15,19, 20, 22-25, 29-35
X	----- US 7 026 118 B1 (WEI QUN [CN] ET AL) 11 April 2006 (2006-04-11) abstract; claims ----- -/-	1-4, 6-15,19, 20, 22-25, 29-35
☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 10 October 2012		Date of mailing of the international search report 02/11/2012
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Vandenbogaerde, Ann

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2012/042606

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SMITH HOWARD S: "Calcineurin as a nociceptor modulator.", PAIN PHYSICIAN 2009 JUL-AUG, vol. 12, no. 4, July 2009 (2009-07), pages E309-E318, XP002684921, ISSN: 2150-1149 cited in the application abstract -----	1-4, 6-15, 19, 20, 22-25, 29-35

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2012/042606

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		AU 5406099 A	21-03-2000
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