**[0024]** Figure 11: Quantification of the co-localization of Src and PSD95 in 14DIV hippocampal neurons with and without treatment with Tat-ND2 310-321 or Src 40-49-Tat.

**[0025]** Figures 12A-E. Immunoprecipitation experiments from rat brain lysates showing that antibodies against ND2, Src, PSD95, NR2B and NR1 can all immunoprecipitate a complex containing the other proteins.

**[0026]** Figures 13A-D. A. Immunoprecipitation using anti-NR2B antibody from 14DIV hippocampal neurons that have been treated with control, Tat-ND2 310-321 or Src 40-49-Tat at 1 uM for 1 hour. B. Same, treated with 3 uM for 2 hours. C. Repeat of A using the indicated antibody for IP. D. Similar to A, using anti-PSD95 as the immunoprecipitation antibody.

**[0027]** Figures 14A, B: ND2 310-321 inhibits PACAP-enhanced NMDA-evoked currents but Src 40-49 does not.

**[0028]** Figure 15: Co-immunoprecipitation using anti-NR2B antibodies from rat brains subjected to 3PVO or 3PVO and treatment with 3 uM Tat-ND2 310-321. C – contralateral brain extract; I – ipsilateral brain extract. Labels indicate the detection antibody used, and P-Tys indicates an antibody against phosphorylated tyrosine 100 of Src.

**[0029]** Figures 16A, B: Co-immunoprecipitations using either anti PSD95 or anti NR2B antibodies, demonstrating the status of proteins with the NR2B complex in the presence or absence of Src-Tat or Tat-ND2.

**[0030]** Figure 17: Treatment with Tat-ND2 310-321 reduces pain hypersensitivity in a model of CFA-induced pain.

**[0031]** Figure 18: Infarct sizes of rats subjected to 3PVO in the presence of Tat-NR2B9c, src40-49-Tat, or Tat-ND2 310-321.

**[0032]** Figure 19: Sequence of ND2, with predicted membrane topology. The location of 310-321 is highlighted and predicted to be intracellular.

**[0033]** Figure 20: Effect of Tat-ND2, myristoylated ND2 and NA-1(also known as Tat-NR2B9c) on blood pressure when injected intravenously into rats at high concentrations.

**[0034]** Figure 21: Graph depicting the minimum blood pressure (maximum blood pressure drop) observed following intravenous injection of NA-1, Tat-ND2 or myr2-ND2.

**[0035]** Figure 22: Graph demonstrating the effect of Tat-ND2, myr-ND2 and myr2-ND2 in protecting the rat brain against stroke as induced in the 3PVO model when given intravenously 1 hour after the onset of the stroke.

**[00186] Measurement of Paw Withdrawal Threshold**: Based on the procedure described by Pitcher et al. (Journal of Neuroscience Methods, 1999), animals were placed on a platform containing holes 1.5 mm in diameter in a transparent plexiglass observation chamber (30x30x30 cm). An ascending series of calibrated von Frey filaments (Stoelting, USA) (.008g – 15g) was applied to the plantar surface of a hind paw to determine the minimum stimulus needed to elicit paw withdrawal. Each filament was applied perpendicularly for 2 seconds or until withdrawal occurred. Each successive filament was applied 5 times at 5-second intervals. The threshold was determined by the filament that elicited 3 positive responses out of 5 applications. Filaments greater than 15g were not used to avoid tissue damage. Paw withdrawal threshold was measured prior to CFA injection, 8 hours after CFA injection, 60 minutes following peptide injection and 120 minutes following peptide injection.

## Example #6 : ND2 INHIBITORS ARE EFFECTIVE AT REDUCING BRAIN DAMAGE FOLLOWING STROKE

**[00187]** The efficacy of ND2 inhibitors in the treatment of stroke was examined using the three pial vessel occlusion (3PVO) model of stroke. This is a permanent model of stroke that creates small but consistent infarcts at 24 hours.

**[00188]** Male Sprague Dawley rats (n=10 in each group) were surgically subjected to permanent occlusion of the pial vessels. One hour post surgery, animals were given an intravenous injection of Saline, Tat-NR2B9c (Aarts et al, Science, 2002), Src 40-49-Tat or Tat-ND2 310-321 and allowed to further recover in their cages. 24 hours after surgery, brains were harvested, sliced and incubated with TTC to visualize the areas of infarction. The infarct volume of each animal was determined, as well as the average volumes for each group of ten. Figure 18 shows the results of one such experiment. Tat-ND2 310-321 and Tat-NR2B9c both show an approximately 40% decline in infarct volume, whereas Src 40-49-Tat was not statistically effective in reducing infarct volumes in this model. This suggests that ND2 inhibitors of the Src-ND2 interaction, and Tat-ND2 310-321 in particular, can be effective drugs for the treatment of stroke.

**[00189]** In a second experiment, the efficacy of myristoylated versions of two ND2 peptides were assessed relative to Tat-ND2. The sequences are provided following the examples. Both peptides were myristoylated via an amide bond to the alpha-amino group of the N-terminal amino acid of the peptide. Myr2-ND2 provided equivalent or superior protection against damage from the stroke in this model, and myr2-ND2 was less effective

(Figure 22). Thus, both Tat-ND2 and myr2-ND2 are effective drugs for the treatment of stroke when administered after a stroke. We have also found these inhibitors effective when administered prior to a stroke.

**Three pial vessel occlusion model of ischemia**

**[00190]** Male Sprague Dawley rats (n=10 in each group) weighing between 230 and 290 g were used for this study. Experiments were performed on fasted rats (Free overnight access to water but not food). For permanent three pial vessel occlusion (3PVO) was performed as described previously (Forder J *et al.*, 2005, supra). In brief, rats were anesthetized with a 0.5 ml/kg intramuscular injection of ketamine (100 mg/kg), acepromazine (2 mg/kg), and xylazine (5 mg/kg), supplemented with one-third of the initial dose as required. An anal temperature probe was inserted, and the animal was placed on a heating pad maintained at 37°C. The skull was exposed via a midline incision and scraped free of tissue. Using a dissecting microscope and a pneumatic dental drill, a 6- to 8-mm cranial window was made over the right somatosensory cortex (2 mm caudal and 5 mm lateral to bregma) by drilling a rectangle through the skull and lifting off the piece of skull while keeping the dura intact. The 3 pial arteriolar middle cerebral artery branches around the barrel cortex were selected and electrically cauterized through the dura. After the cauterizations, the scalp was sutured. Each rat was returned to its individual cage under a heating lamp to maintain body temperature until the rat fully recovered. One hour after 3PVO ischemia, rats were injected with indicated drug (3nmol/g) or saline by intravenous tail vein. Food and water was supplied. Twenty-four hours after surgery, the brain was quickly harvested. Coronal slices (2mm) were taken through the brain and incubated in 2% triphenyltetrazoliumchloride (TTC) (Sigma) for 15 min at 37°C. Images were scanned (Canon 4200F).

## Example #7 : ND2 INHIBITORS REDUCE PAIN HYPERSENSITIVITY IN RODENT MODELS OF NEUROPATHIC PAIN

**[00191]** We next tested the effect of Tat-ND2 310-321 versus the control scrambled ND2 negative control (sTat-ND2 310-321) on neuropathic and inflammatory pain behaviors using a model of peripheral nerve injury (PNI) in rats and the Complete Freund's Adjuvant model (CFA), both characterized by a reduction of mechanical paw withdrawal threshold. The effect of Tat-ND2 310-321 on mechanical withdrawal threshold was assessed 8–15 d after PNI. We found that Tat-ND2 310-321, but not sTat-ND2 310-321, caused a significant increase in paw withdrawal threshold ipsilateral to the nerve injury when administered intravenously. Similar

and vehicle into the lumbar spinal cord and tested PWT 45min, 90min, 135min and 180min post injection. Both concentrations of myr-ND2 tested demonstrated decreased sensitivity to pain across all time points tested (Figure 23). Thus, ND2 peptides, including myr-ND2, are effective inhibitors of pain.

## Example #9 : MYRISTOYLATION MAINTAINS EFFICACY IN PAIN AND STROKE MODELS WITHOUT REDUCING BLOOD PRESSURE AT HIGH DOSAGE LEVELS

**[00197]** ND2 peptide have been shown herein to be effective at reducing both pain and damage following stroke. Tat peptides have previously been shown to cause a reduction in blood pressure when administered rapidly in high doses. We tested the effective of applying a rapid bolus of high concentration myr2-ND2 peptide relative to Tat-ND2 and NA-1 (Tat-NR2B9c). Rats with surgically implanted devices to measure arterial pressure were dosed with ~25 mg/kg of the respective peptides as a bolus injection into the tail vein at 10 minutes post monitoring. Both of the peptides containing Tat sequences resulted in a drop of mean arterial blood pressure to levels about one half of normal. These pressure drops resolved themselves within about 10-15 minutes without intervention. The myr2-ND2 peptide, however, showed no decreases in blood pressure at similar concentrations in any of the animals tested (Figure 20). Figure 21 shows the lowest points in blood pressure, further demonstrating that myr2-ND2 had no effect on blood pressure in this model. Thus, myr2 -ND2 can be administered at a higher dose level than Tat-ND2 without or with a lower chance of observing blood pressure side effects in humans.

References:


1. Liu, X.J., *et al.*, *Treatment of inflammatory and neuropathic pain by uncoupling Src from the NMDA receptor complex.* Nat.Med., 2008. **14**(12): p. 1325-1332.
2. Gingrich, J.R., *et al.*, *Unique domain anchoring of Src to synaptic NMDA receptors via the mitochondrial protein NADH dehydrogenase subunit 2.* Proc.Natl.Acad.Sci.U.S.A, 2004. **101**(16): p. 6237-6242.
3. Liu, X.J., *et al.*, *Treatment of inflammatory and neuropathic pain by uncoupling Src from the NMDA receptor complex.* Nature Medicine, 2008. **14**(12): p. 1325-1332.
4. Husi, H., *et al.*, *Proteomic analysis of NMDA receptor-adhesion protein signaling complexes.* Nat.Neurosci., 2000. **3**(7): p. 661-669.

**WHAT IS CLAIMED IS:**

1. An ND2 peptide having 4-40 residues identical to residues of SEQ ID NO:60 of which at least 4 of the residues are contiguous residues between amino acids 289-321 of SEQ ID NO:60.

2. An ND2 peptide having an amino acid sequence consisting of amino acids 310 to 321 of SEQ ID NO:60 provided that up to six amino acids can be deleted, inserted or conservatively substituted.

3. The ND2 peptide of claim 1 or 2, wherein the ND2 peptide has an amino acid sequence consisting of 4-12 contiguous residues between amino acids 310—321 of SEQ ID NO:60.

4. The ND2 peptide of claim 1 or 2, wherein the ND2 peptide has an amino acid sequence consisting of 4-10 contiguous residues between amino acids 310-321 of SEQ ID NO:60.

5. The ND2 peptide of claim 1 or 2, wherein the ND2 peptide consists of amino acids 310-321.

6. The ND2 peptide of any preceding claim, wherein the ND2 peptide is lipidated.

7. The ND2 peptide of claim 6, wherein the ND2 peptide is lipidated by being linked to a fatty acid.

8. The ND2 peptide of claim 7, wherein the ND2 peptide is myristoylated.

9. The ND2 peptide of claim 8, wherein the ND2 peptide is myristoylated at its N-terminus.

10. The ND2 peptide of any of claims 1-5, wherein the ND2 peptide is linked to an internalization peptide.

11. The ND2 peptide of claim 10, wherein the internalization peptide is linked to the N-terminus of ND2 peptide.

12. The ND2 peptide of claim 10, wherein the internalization peptide is linked to the C-terminus of the ND2 peptide.

13. The ND2 peptide of claim 10, 11 or 12, wherein the internalization peptide and ND2 peptide are linked as a fusion peptide.

14. The ND2 peptide of any of claims 10-13, wherein the internalization peptide includes at least 5 arginine or lysine residues and has a total length of up to 15 amino acids.

15. The ND2 peptide of any of claim 10-13, wherein the internalization peptide is a Tat peptide.

16. A chimeric peptide up to 50 amino acids in length, comprising an ND2 peptide comprising at least 3 contiguous amino acids located between amino acids 289 and 321 of SEQ ID NO:60, and an internalization peptide linked to the ND2 peptide.

17. The chimeric peptide of claim 16 that is up to 25 amino acids in length.

18. The chimeric peptide of claim 16, wherein the ND2 peptide has an amino acid sequence consisting of 4-20 contiguous residues between amino acids 289 and 321 of SEQ ID NO:60.

19. The chimeric peptide of claim 16, wherein the ND2 peptide has an amino acid sequence consisting of 4-12 contiguous residues between amino acids 310—321 of SEQ ID NO:60.

20. The chimeric peptide of claim 16, wherein the ND2 peptide has an amino acid sequence consisting of 4-10 contiguous residues between amino acids 310-321 of SEQ ID NO:60.

21. The chimeric peptide of claim 16, wherein the ND2 peptide consisting of amino acids 310-321 of SEQ ID NO:60 provided that up to six amino acids can be deleted, inserted or substituted.

22. The chimeric peptide of claim 16, wherein the ND2 peptide has an amino acid sequence consisting of amino acids 310 to 321 of SEQ ID NO:60.

23. The chimeric peptide of claim 16, wherein the internalization peptide is linked to the N-terminus of ND2 peptide.

24. The chimeric peptide of claim 16, wherein the internalization peptide is linked to the C-terminus of the ND2 peptide.

25. The chimeric peptide of claim 22 or 23, wherein the internalization peptide and ND2 peptide are linked as a fusion peptide.

26. The chimeric peptide of claim 16, wherein the internalization peptide includes at least 5 arginine or lysine residues and has a total length of up to 15 amino acids.

27. The chimeric peptide of claim 16, wherein the internalization peptide is a Tat peptide.

28. A peptidomimetic of a chimeric peptide or the ND2 peptide of any preceding claim.

29. The peptidomimetic of claim 28, which is a retro-inverso peptidomimetic.

30. A method of treating or effecting prophylaxis of a neurological disease or disorder, comprising administering an effective regime of a chimeric peptide, ND2 peptide or peptidomimetic of any preceding claim to a patient having or at risk of developing a neurological disorder.

31. The method of claim 30, wherein the neurological disease or disorder is stroke, traumatic injury to the CNS, epilepsy, anxiety, or a neurodegenerative disease.

32. A method of treating or effecting prophylaxis of pain, comprising administering an effective regime of a chimeric peptide, an ND2 peptide, or peptidomimetic of any of claim 1-29 to a patient having or at risk of developing pain.

33. The method of claim 32, wherein the pain is neuropathic, physiological or inflammatory pain.

34. A method of treating or effecting prophylaxis of cancer, comprising administering an effective regime of chimeric peptide, ND2 peptide or peptidomimetic of any of claims 1-29 to a patient having or at risk of developing cancer.

35. A method of identifying an agent that inhibits ND2-Src interaction, comprising

contacting a Src peptide and ND2 peptide with an agent; and

determining binding between the Src peptide and ND2 peptide, wherein reduced binding in the presence of the agent relative to a control assay lacking the agent indicates the agent is an inhibitor of Src-ND2 interaction; wherein the agent is a chimeric or ND2 peptide or a peptidomimetic thereof as defined in any of claims 1-27.

36. A method of identifying an agent that inhibits Src-ND2 interaction, comprising

contacting a Src peptide and an ND2 peptide as defined in any of claims 15-19 with an agent; and determining binding between the Src peptide and ND2 peptide, wherein reduced binding in the presence of the agent relative to a control assay lacking the agent indicates the agent is an inhibitor of Src-ND2 interaction.

37. The method of claim 35 or 36 further comprising testing the agent for pharmacological activity against a neurological disease, pain or cancer in an animal model of one of neurological disease, pain or cancer.

**WHAT IS CLAIMED IS:**

1. An ND2 peptide, the amino acid sequence of which consists of up to 20 residues of SEQ ID NO:60 including residues 310-321, the peptide inhibiting ND2 interaction with Src, wherein the peptide is lipidated or linked to an internalization peptide.

2. The ND2 peptide of claim 1, wherein the ND2 peptide is linked to an internalization peptide.

3. The ND2 peptide of claim 2, wherein the internalization peptide is linked to the N-terminus of ND2 peptide.

4. The ND2 peptide of claim 2, wherein the internalization peptide is linked to the C-terminus of the ND2 peptide.

5. The ND2 peptide of claim 2, wherein the internalization peptide and ND2 peptide are linked as a fusion peptide.

6. The ND2 peptide of claim 2, wherein the internalization peptide includes at least 5 arginine or lysine residues and has a total length of up to 15 amino acids.

7. The ND2 peptide of claim 2, wherein the internalization peptide is a Tat peptide.

8. The ND2 peptide of claim 1, wherein the ND2 peptide consists of amino acids 307-321, of SEQ ID NO:60.

9. The ND2 peptide of claim 1, wherein the ND2 peptide is lipidated.

10. The ND2 peptide of claim 9, wherein the ND2 peptide is lipidated by being linked to a fatty acid.

11. The ND2 peptide of claim 10, wherein the ND2 peptide is myristoylated.

12. The ND2 peptide of claim 11, wherein the ND2 peptide is myristoylated at its N-terminus.

13. A chimeric peptide up to 25 amino acids in length, comprising an ND2 peptide as defined in claim 1, and an internalization peptide linked to the ND2 peptide.

14. The chimeric peptide of claim 13, wherein the internalization peptide is linked to the N-terminus of ND2 peptide.

15. The chimeric peptide of claim 13, wherein the internalization peptide is linked to the C-terminus of the ND2 peptide.

16. The chimeric peptide of claim 13, wherein the internalization peptide includes at least 5 arginine or lysine residues and has a total length of up to 15 amino acids.

17. The chimeric peptide of claim 13, wherein the internalization peptide is a Tat peptide.

18. The chimeric peptide of claim 13, wherein the ND2 peptide has an amino acid sequence consisting of amino acids 307-321 or 310 to 321 of SEQ ID NO:60.

19. The chimeric peptide of claim 18, wherein the internalization peptide and ND2 peptide are linked as a fusion peptide.