APPENDIX A Search strategies for the medical databases

PubMed

("Musculoskeletal Manipulations "[Mesh:noexp] OR "Manipulation, Chiropractic "[Mesh:noexp] OR "Manipulation, Osteopathic "[Mesh:noexp] OR "Manipulation, Spinal "[Mesh:noexp] OR "Manipulation, Orthopedic "[Mesh:noexp] OR musculoskeletal manipulation[tiab] OR musculoskeletal manipulations[tiab] OR osteopathic[tiab] OR chiropractic[tiab] OR manual therapy[tiab] OR manipulative therapy[tiab] OR manipulation therapy[tiab] OR manual treatment[tiab] OR manual treatments[tiab] OR manipulative treatment[tiab] OR manipulative treatments[tiab] OR manipulation treatment[tiab] OR manipulation treatments[tiab] OR spine manipulation[tiab] OR spine manipulations[tiab] OR spinal manipulation[tiab] OR spinal manipulations[tiab] OR spine mobilisation[tiab] OR spinal mobilisation[tiab] OR spine mobilisation[tiab] OR spinal mobilisation[tiab] OR spinal mobilisations[tiab] OR (thrust[tiab] AND manipulat*[tiab]) OR cervical manipulation[tiab] OR cervical manipulations[tiab] OR cervical mobilisation[tiab] OR cervical mobilisations[tiab] OR cervical mobilisation[tiab] OR neck manipulation[tiab] OR neck manipulations[tiab] OR neck mobilisation[tiab] OR thoracic manipulation[tiab] OR thoracic manipulations[tiab] OR thoracic mobilisation[tiab] OR lumbar manipulation[tiab] OR lumbar manipulations[tiab] OR lumbar mobilisation[tiab] OR lumbar mobilisations[tiab] OR lumbar mobilisation[tiab] OR joint manipulation[tiab] OR joint manipulations[tiab] OR joint mobilisation[tiab] OR joint mobilisations[tiab] OR joint mobilisation[tiab] OR joint mobilisations[tiab] OR ankle mobilisation[tiab] OR knee manipulation[tiab] OR knee manipulations[tiab] OR knee mobilisation[tiab] OR knee mobilisation[tiab] OR hip mobilisation[tiab] OR hip mobilisation[tiab] OR hip mobilisations[tiab] OR shoulder mobilisation[tiab] OR shoulder mobilisation[tiab] OR shoulder manipulation[tiab] OR shoulder manipulations[tiab] OR elbow mobilisation[tiab] OR hand manipulation[tiab] OR

hand manipulations[tiab] OR nerve mobilisation[tiab] OR nerve mobilisation[tiab] OR neural mobilisation[tiab] OR neural mobilisation[tiab]) AND ("Immune system"[Mesh] OR "Immune System Phenomena"[Mesh] OR "Intercellular Signaling Peptides and Proteins"[Mesh] OR "Endocrine cells"[Mesh] OR immune[tiab] OR immunity[tiab] OR immunological[tiab] OR immunoreactive[tiab] OR immuno-reactive[tiab] OR immunoregulatory[tiab] OR immuno-regulatory[tiab] OR inflammation[tiab] OR inflammasome[tiab] OR inflammasomes[tiab] OR inflammatory[tiab] OR proinflammatory[tiab] OR pro-inflammatory[tiab] OR antiinflammatory[tiab] OR anti-inflammatory[tiab] OR cytokine[tiab] OR cytokines[tiab] OR chemokine[tiab] OR chemokines[tiab] OR neurokine[tiab] OR neurokines[tiab] OR midkine[tiab] OR midkines[tiab] OR interferon[tiab] OR interferons[tiab] OR interleukin[tiab] OR interleukins[tiab] OR IL[tiab] OR IL6[tiab] OR IL1ra[tiab] OR IL10[tiab] OR lymphokin[tiab] OR lymphokine[tiab] OR lymphokines[tiab] OR tumor necrosis factor[tiab] OR TNF[tiab] OR phagocyte[tiab] OR phagocytes[tiab] OR phagocytic[tiab] OR phagocytosis[tiab] OR macrophage[tiab] OR macrophages[tiab] OR neutrophil[tiab] OR neutrophils[tiab] OR monocyte[tiab] OR monocytes[tiab] OR mast cell[tiab] OR mast cells[tiab] OR dendritic[tiab] OR lymphocyte[tiab] OR lymphocytes[tiab] OR leukocyte[tiab] OR leukocytes[tiab] OR glia[tiab] OR glial[tiab] OR microglia[tiab] OR non-neuronal[tiab] OR astrocyte[tiab] OR astrocytes[tiab] OR oligodendrocyte[tiab] OR oligodendrocytes[tiab] OR schwann[tiab] OR satellite cell[tiab] OR satellite cells[tiab] OR neuropeptide[tiab] OR neuropeptides[tiab] OR neuro-peptide[tiab] OR neuro-peptides[tiab] OR opioid[tiab] OR opioids[tiab] OR opiate[tiab] OR opiates[tiab] OR endorphin[tiab] OR endorphins[tiab] OR substance P[tiab] OR acetylcholine[tiab] OR acetylcholines[tiab] OR "neurokinin A"[tiab] OR NKA[tiab] OR dopamine[tiab] OR adrenaline[tiab] OR noradrenaline[tiab] OR nor-adrenaline[tiab] OR GABA[tiab] OR glutamate[tiab] OR glucocorticoid[tiab] OR

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WEB OF SCIENCE

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OR “phagocytosis” OR “macrophage” OR “macrophages” OR “neutrophil” OR “neutrophils” OR “monocyte” OR “monocytes” OR “mast cell” OR “mast cells” OR “dendritic” OR “lymphocyte” OR “lymphocytes” OR “leukocyte” OR “leukocytes” OR “glia” OR “glial” OR “microglia” OR “non-neuronal” OR “astrocyte” OR “astrocytes” OR “oligodendrocyte” OR “oligodendrocytes” OR “schwann” OR “satellite cell” OR “satellite cells” OR “neuropeptide” OR “neuropeptides” OR “neuro-peptide” OR “neuro-peptides” OR “opioid” OR “opioids” OR “opiate” OR “opiates” OR “endorphin” OR “endorphins” OR “substance P” OR “acetylcholine” OR “acetylcholines” OR "neurokinin A" OR “NKA” OR “dopamine” OR “adrenaline” OR “noradrenaline” OR “nor-adrenaline” OR “GABA” OR “glutamate” OR “glucocorticoid” OR “glucocorticoids” OR “endocannabinoid” OR “endocannabinoids” OR “endo-cannabinoid” OR “endo-cannabinoids” OR “peptide” OR “peptides” OR “anandamide” OR “nitric oxide” OR “growth factor” OR “growth factors” OR “endothelial” OR “endothelium” OR “epinephrine” OR “norepinephrine” OR “nor-epinephrine” OR “prostaglandin” OR “prostaglandins” OR “hormone” OR “hormones” OR “CRH” OR “Corticotropin releasing factor*” OR “ACTH” OR “catalase” OR “catecholamine” OR “catecholamines” OR “enzyme” OR “enzymes” OR “cortisol” OR “oedema” OR “edema” OR “CRP” OR “C-reactive protein” OR “serum” OR “plasma” OR “complement” OR “endocrine” OR “homeostasis” OR “neurotransmitter” OR “neurotransmitters” OR “neuro-transmitter” OR “neuro-transmitters” OR “Toll Like Receptor” OR “Toll Like Receptors” OR “TLR” OR “TLRs” OR “Danger signal*” OR “damage-associated molecular pattern” OR “damage-associated molecular patterns” OR “DAMP” OR “pathogen associated molecular pattern” OR “pathogen associated molecular patterns” OR “PAMP” OR “Microbe associated molecular pattern” OR “Microbe associated molecular patterns” OR “MAMP” OR “reactive oxygen species” OR “ROS” OR “nitrosylation”)

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MAMP.ti,ab,kw. OR reactive oxygen species.ti,ab,kw. OR ROS.ti,ab,kw. OR nitrosylation.ti,ab,kw.)

Table B.1.: SYRCLE's tool for assessing risk of bias

Type of bias	**Domain**	**Description of domain**	**Review authors judgment**
Selection bias	Sequence generation	The methods used to generate the allocation sequence is reported in sufficient detail to allow an assessment whether it could produce comparable groups	Was the allocation sequence adequately generated and applied?
Selection bias	Baseline characteristics	Describe all the animal characteristics that are compared in order to judge whether or not the intervention and control group groups were similar at the start of the experiment	Were the groups similar at baseline or were they adjusted for confounders in the analysis?
Selection bias	Allocation concealment	The method used to conceal the allocation sequence is reported in sufficient detail to determine whether the intervention allocations could have been foreseen before or during enrolment	Was the allocation adequately concealed?
Performance bias	Random housing	All measures used to house the animals randomly within the animal room	Were the animals randomly housed during the experiment?
Performance bias	Blinding	All measures used to blind trial caregivers and researchers from	Were the caregivers and/or investigators blinded from

		knowing which intervention each animal received. Any information provided relating to whether the intended blinding was effective	knowledge which intervention each animal received during the experiment?
Detection bias	Random outcome assessment	Whether or not animals were selected at random for outcome assessment, and which methods was used to select the animals	Were animals selected at random for outcome assessment?
Detection bias	Blinding	All measures used to blind outcome assessors from knowing which intervention each animal received. Any information provided relating to whether the intended blinding was effective	Was the outcome assessor blinded?
Attrition bias	Incomplete outcome data	The completeness of data for each main outcome, including attrition and exclusion from the analysis. State whether attrition and exclusion were reported, the numbers in each intervention group, reasons for attrition or exclusions, and any re-inclusions in analysis for review	Were incomplete outcome data adequately addressed?

Reporting bias	Selective outcome reporting	State how selective outcome reporting was examined and what was found	Are reports of the study free of selective outcome reporting?
Other	Other sources of bias	State any important concerns about bias not covered by other domains in the tool	Was the study apparently free of other problems that could result in high risk of bias?

Table C.1. GRADE approach for animal studies

Outcome	Neuroimmune response under study
Studies	Number of studies
Design	Randomized or non-randomized controlled trial
Limitations risk of bias	The risk of bias was rated as high, unclear or low [31].A summary assessment of the risk of bias was based on the likelihood to seriously alter the results [31].
Inconsistency	Inconsistency was based on the overlap between confidence intervals, the magnitude and direction of effect of the individual studies, the P-value of the test for heterogeneity and I^2. Single studies are always considered to be inconsistent and imprecise.
Imprecision	Imprecision was based on interpreting the results of preclinical animal studies, the direction of the effect is often perceived to be more important than the exact magnitude of effect. Judgment of imprecision was based on whether the confidence interval includes no effect or not. Single studies are always considered to be inconsistent and imprecise.
Publication bias	Publication bias was based on symmetry on the funnel plot. If a funnel plot could not be made, publication bias could not be ruled out and was rated as unclear.
Indirectness layer 1	Indirectness from preclinical animal studies to the preclinical animal PICO.
Indirectness layer 2	Indirectness of preclinical evidence to clinical PICO (translatability)

Other considerations	The factors for upgrading include large magnitude of effect, presence of dose response relationship and opposing direction of plausible residual confounding.

Table D.1.: Overview of the experimental models to induce neuromusculoskeletal conditions

Mimicked neuromusculoskeletal disorder	**Induction**	**Anesthesia used**	**Antibiotic**	**Euthanasia**
Intervertebral foramen inflammation	In vivo delivery of inflammatory mediators directly into the lumbar intervertebral foramen at L5. Thirty µl containing bradykinin, 5-HT, histamine, and prostaglandin was injected at a pH of 7.45.	Sodium pentobarbital 40 mg/kg i.p.	Augmentin, for 7 days after surgery 7.52g per 500 ml in the drinking water.	-
Sciatic Crush Injury	Sciatic nerve is crushed 1 cm above its trifurcation for 30 seconds with a 2-mm wide forceps.	80 mg/kg i.p. ketamine 55mg/kg i.p xylazine	On the skin 10% povidone iodine	Deeply anesthetized with 15% chloral hydrate (0.5 g/kg, i.p.) and perfused transcardially with cold phosphate-buffered saline (0.1 M; pH 7.4; 300 ml) followed by cold buffered paraformaldehyde (4%; pH 7.4; 500 mL)
Median Nerve compression	Chromic catgut 4.0 suture line in 4 points, with an approximate distance of 1 mm, on the medial nerve, in the proximal region of the right elbow.	Ketamine (50mg/kg) and xylazine 10mg/kg solution	-	-
Inflammatory Ankle Injury	0.05 ml of 3% carrageenan in 0.9% saline (pH 7.4) was injected into the right ankle.	-	-	Rats were euthanized by carbon dioxide inhalation and cervical dislocation
CCI	Four ligatures proximal to the sciatic trifurcation, tied loosely around the nerve at a spacing of approximately 1 mm spacing.	Halothane	-	Decapitation under light halothane anesthesia

Compression-decompression of the dorsal root ganglion	Surgically implantation of a stainless steel rod unilateral into the intervertebral foramen at L4 and L5. Decompression was mimicked by removal of the previous inserted rod.	Sodium pentobarbital 40 mg/kg i.p.	Augmentin, for 7 days after surgery 7.52g per 500 ml in the drinking water.	-
Knee joint immobilisation	The right knee joint was shaved, and 2 plastic glass splints were placed on the medial and lateral sides of the knee joint and then gently wrapped with adhesive plaster around the limb to restrain the movement of the joint. The right knee joint was immobilized in the extended position (150°). A plastic glass belt was attached surrounding the abdomen area to ensure rats would keep their hind paw apparatus.	90 mg/kg of ketamine and 10 mg/kg of xylazine	-	Decapitation
Chronic post-ischemia pain	An elastic O-ring with 1.2-mm internal diameter was placed around the right hindlimb just proximal to the ankle joint, producing ischemia. O-rings were left on the limb for 3 hours.	A bolus (7%, 0.6 mL/kg, i.p.) of chloral hydrate and 20% of the initial volume at the end of the first and second hour.	-	-
Knee joint immobilisation	The right knee was shaved, and 2 plastic glass splints were placed on the medial and lateral sides of the knee joint and then gently wrapped with edhesive plaster around the limb to restrain the movement of the joint.	90mg/kg of ketamine and 10 mg/kg of xylazine	-	Decapitation
NGF-induced trunk mechanical hyperalgesia	Injections of NGF were made into the left multifidus muscle at the vertebral level of L5 (3 mm lateral to the spinous process) on Days 0 and 5.	brief isoflurane anesthesia	-	-

Table E.1.: Applied nerve and joint mobilisation and or manipulation

Intervention	Intervention settings	Animal position	Anesthesia used	Start time	Time until obduction
Neural Mobilisation in CCI group	The right knee joint was positioned in full extension (0-degrees) The right hip joint was bent between 70 and 80 degrees until a small amount of resistance was feld. The ankle joint was angled between 30 -45 degrees. Thereafter oscillatory movements were initiated. The right ankle was maintained in dorsiflexion (30-45 degrees) at approximally 20 oscillations per minute for 2 minutes followed by a 25 second rest. The treatment required 10 minutes, and in the last minute the cervical spine was fully flexed.	Animals were positioned in the left lateral position to mobilize the right side (ipsilateral to the CCI)	Halothane with continuous flow of medicinal oxygen (5ml/l)	NM was initiated 14 days after injury NM sessions occurred every other day for a total of 10 sessions	Unclear
Median nerve mobilisation	Repeated oscillations of wrist flexion-extensions were made for 1 minute. or 3 minutes. The control groups receives on the same days of treatment anaesthesia and were then placed back into their boxes.	The animal was placed in dorsal recumbent position, with lateral cervical flexion to the left, depression of the shoulder girdle and slight abduction, external rotation and supination, with extension at the elbow and wrist until	-	Six sessions were performed on alternate days between the third and 13th postoperative days.	Unclear

		resistance to movement was observed.			
Knee mobilisation	Knee mobilisation/ translation was applied by flexing and extending the ipsilateral knee joint to its end range of extension while the tibia was simultaneously translated in an anterior-to posterior direction using grade III and IV mobilisation forces. Flexion and extension were performed at a frequency of 20 times per minutes. 3 repetitions of 3 minutes with a 1- to 2-minute rest interval between repetitions	-	Isoflurane (2%-5%)	Knee mobilisation was performed 24 hours after carrageenan injection	30 minutes after anaesthesia
Motorised spinal mobilisation	10 min using a computer controlled feedback motor to deliver forces equivalent to 0.9N at 1.2 Hz.	-	isoflurane anaesthesia (1–2%).	The day following the first NGF and every other 12 days.	-
Ankle mobilisation[51]	Dorsal flexion and plantar flexion were realized until the end range of motion. Three treatment sessions of 3 minutes were performed with rest intervals of 30 seconds. Ankle joint mobilisation was performed every other day, with 48 hours of rest between each session. Treatment comprised 15 sessions.	The rats were placed in the lateral recumbent position	Anaesthesia was induced using 2% isoflurane in 100% oxygen; anaesthesia was subsequently maintained using 1% isoflurane.	-	35 days after surgery. Unknown time after mobilisation
Ankle joint mobilisation[73]	The experimenter's hand stabilized the knee joint while the ankle joint was flexed and extended to full amplitude, rhythmically with a movement frequency of approximately 40 cycles per minute. Movement frequency was performed	-	Lightly anesthetised with 1%–2% isoflurane	Animals received daily treatments of 9-minute MT between the 2nd to 11th day after the disease induction.	All animals were euthanised 30 min after the last treatment

	with assistance of a metronome. Treated animals received a total of 9 minutes of MT divided in 3 series of 3 minutes each with a 30 second interval between series.				
ASMT[86]	The activator adjusting instrument delivers short duration (<0.1 ms) mechanical force, manually assisted spinal manipulative thrusts. The force was applied to spinous process of L5 and L6. The ASMT was applied at a rostral direction at an angle of 40° to 50° to the vertebral horizontal line. Two different force setting were used ASMT-1 and ASMT-2.	-	-	A series of 10 ASMT was initiated 2 days after DRG decompression on the 10th day after CCD surgery and subsequently applied daily for consecutive 5 days (12-16 days) and every other day for another 5 ASMT, the last ASMT was on the 26th day after surgery, that is 14 days after compression-decompression of the dorsal root ganglion.	-
ASMT[85]	The activator adjusting instrument delivers short duration (<0.1 ms) mechanical force, manually assisted spinal manipulative thrusts. The force was applied to spinous process of L5 and L6. The ASMT was applied at a rostral direction at an angle of 40° to 50° to the vertebral horizontal line.	-	-	A series of 10 ASMT was initiated 24 hours after surgery and subsequently applied daily for 7 consecutive days and every other day during the second week.	-
ASMT[25]	Activator adjusting instrument delivered at spinous process of L4 and L5.	-	-	Three weeks, three times a week.	-

ASMT-sham[25]	Sham group had the activator adjusting instrument lightly touching the L4-L5 spinous processes	-	-	Three weeks, three times a week.	-
Lumbar Spinal manipulation	Single high velocity, low amplitude adjustive manipulation at the L4/L5-S1 region.	n.a.	n.a.	-	n.a.
Cervical Spinal Manipulation	Single high velocity, mid-range, left rotational force to C5-C6 or to the site of pain and/or restriction, with right side bending and left rotation.	n.a.	n.a.	n.a.	n.a.
Cervical Spinal Mobilisation	Grade III posterior-anterior joint oscillatory mobilisation technique applied to the articular pillar of C5/6 on the subjects symptomatic side. The treatment involved three, 1-min applications with a 1-min interval in-between.	n.a.	n.a.	n.a.	n.a.
Thoracic spine manipulation and mobilisation	The study group received a three-week duration of mobilisation and manipulation application, consisting of nine sessions in a frequency of three per week. Passive traction mobilisation applied on a seated and supine position at the level of the disc joints, and bilateral manipulation performed in the supine position at thoracic vertebrae level of T1-T8 facet joints.	n.a.	n.a.	n.a.	n.a.

Table F1: GRADE results for: "Pooled data for joint and nerve mobilisation compared to no intervention or sham in animals suffering from induced neuromusculoskeletal conditions for neuroimmune responses"

Pooled data for joint and nerve mobilisation compared to no intervention or sham in animals suffering from induced neuromusculoekeletal conditions for neuroimmune responses												
Population: Animals with induced neuromusculoskeletal conditions **Intervention:** Joint and nerve mobilisation **Comparison:** No-intervention or sham intervention												
Certainty Assessment										**Summary of findings**		
										No of patients		**Effect**
Outcomes	**Studies**	**design**	**Limitations RoB**	**Inconsistency**	**Imprecision**	**Publication bias**	**Indirectness**		**Other considerations**	**Experimental**	**No intervention**	**Standardized mean difference (95% CI)**
							Layer 1	Layer 2				
Red blood cell												
Glutathione Peroxidase	1[25]	NCT	Unclear[1]	Serious limitation[2a]	Serious limitation[2a]	Unclear[4]	No serious limitation[5]	Unclear	None[7]	6	12	-0.86 [-2.10, 0.38] P = 0.17
Lipid Hydroperoxide	1[25]	NCT	Unclear[1]	Serious limitation[2a]	Serious limitation[2a]	Unclear[4]	No serious limitation[5]	Unclear	None[7]	6	12	-1.08 [-2.19, 0.03] P = 0.06
Catalase	1[25]	NCT	Unclear[1]	Serious limitation[2a]	Serious limitation[2a]	Unclear[4]	No serious limitation[5]	Unclear	None[7]	6	12	-1.34 [-2.50, -0.18] P = 0.02]
Serum												
IL-10	1[86]	NCT	Unclear[1]	Serious limitation[2a]	Serious limitation[2a]	Unclear[4]	No serious limitation[5]	Serious limitation[6]	None[7]	12	6	0 36 [-0.63, 1.36] P = 0.47
IL-1β	1[86]	NCT	Unclear[1]	Serious limitation[2a]	Serious limitation[2a]	Unclear[4]	No serious limitation[5]	Serious limitation[6]	None[7]	12	6	0.24 [-0.75, 1.23]

												P = 0.63
TNF-α	1[86]	NCT	Unclear[1]	Serious limitation[2a]	Serious limitation[2a]	Unclear[4]	No serious limitation[5]	Serious limitation[6]	None[7]	12	6	0.09 [-0.89, 1.07] P = 0.85
NO metabolites	1[25]	NCT	Unclear[1]	Serious limitation[2a]	Serious limitation[2a]	Unclear[4]	No serious limitation[5]	Unclear	None[7]	6	12	-0.25 [-1.24, 0.73] P = 0.61
Superoxide Dismutase	1[25]	NCT	Unclear[1]	Serious limitation[2a]	Serious limitation[2a]	Unclear[4]	No serious limitation[5]	Unclear	None[7]	6	12	-0.24 [-1.23, 0.75] P = 0.63
DRG												
Il-10	1[86]	NCT	Unclear[1]	Serious limitation[2a]	Serious limitation[2a]	Unclear[4]	No serious limitation[5]	Serious limitation[6]	None[7]	12	6	-0.02 [1.01, 0.98] P = 0.97
Il-1β	1[86]	NCT	Unclear[1]	Serious limitation[2a]	Serious limitation[2a]	Unclear[4]	No serious limitation[5]	Serious limitation[6]	None[7]	12	6	-5.52 [-8.12, -2.92] P < 0.0001
TNF-α	1[86]	NCT	Unclear[1]	Serious limitation[2a]	Serious limitation[2a]	Unclear[4]	No serious limitation[5]	Serious limitation[6]	None[7]	12	6	-0.29 [-1.28, 0.70] P = 0.57
Number of non-neuronal cells	2[85,86]	NCT	Unclear[1]	No serious limitation[2]	No serious limitation[3]	Unclear[4]	No serious limitation[5]	Serious limitation[6]	None[7]	7	7	-7.02 [11.11, -2.93] P = 0.0008
Spinal Cord												
Il-10	1[86]	NCT	Unclear[1]	Serious limitation[2a]	Serious limitation[2a]	Unclear[4]	No serious limitation[5]	Serious limitation[6]	None[7]	12	6	6.47 [3.46, 9.48] P < 0.0001
Il-1β	1[86]	NCT	Unclear[1]	Serious limitation[2a]	Serious limitation[2a]	Unclear[4]	No serious limitation[5]	Serious limitation[6]	None[7]	12	6	-0.01 [-0.99, 0.97] P = 0.98

TNF-α	1[86]	NCT	Unclear[1]	Serious limitation[2a]	Serious limitation[2a]	Unclear[4]	No serious limitation[5]	Serious limitation[6]	None[7]	12	6	-0.4 [-0.6, 1.40] P = 0.43
GFAP	2[51,77]	NCT	Unclear[1]	No serious limitation[2]	No serious limitation[3]	Unclear[4]	No serious limitation[5]	Serious limitation[6]	None[7]	11	11	-3.55 [04.84, -1.86] P < 0.0001

[1]Risk of bias was unclear for most items (e.g. no information about randomization and blinding) except for blinding of investigators (high risk of bias).[2] Considering the overlap between confidence intervals, the magnitude and direction of effect of the individual studies, the P-value of the test for heterogeneity and I^2. [2a]Single studies are always considered to be inconsistent and imprecise. [3]Interpreting the results of preclinical animal studies, the direction of the effect is often perceived to be more important than the exact magnitude of effect. Judgment of imprecision was based on whether the confidence interval includes no effect or not.[4]Funnel plot could not be made, publication bias could not be ruled out.[5]Preclinical study fits the preclinical PICO. However the clinical relevance change is unknown.[6]Used animal model is more severe (axonal damage) compared to human compression neuropathies. [7]Consistency across species could not be determined.

Abbreviations: *NCT*: Non-randomized trial; *GFAP*: Glial Fibrillary Acidic Protein; *RoB*: Risk of Bias; *CI*: Confidence Interval; *IL-10*: Interleukin-10; *TNF-α*: Tumor Necrosis Factor-α; *IL-1β*: Interleukin-1β

Table F2: GRADE results for: “Neural mobilisation technique compared to no intervention in Wistar rats suffering from chronic constriction injury for neuroimmune responses”

Neural mobilisation technique compared to no intervention in Wistar rats suffering from chronic constriction injury for neuroimmune responses												
Population: Wistar rats with CCI **Intervention:** Neural mobilisation **Comparison:** No intervention												
Certainty Assessment										**Summary of findings**		
										No of patients		**Effect**
Outcomes	**Studies**	**design**	**Limitations RoB**	**Inconsistency**	**Imprecision**	**Publication bias**	**Indirectness**		**Other considerations**	**Neural mobilisation**	**No intervention**	**Standardized mean difference (95% CI)**
							Layer 1	Layer 2				
DRG												
TRPV1	1[78]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[2]	Unclear[3]	No serious limitation[4]	Serious limitation[5]	None[6]	6	6	-6.17 [-9.39,-2.95] P=0.002
Substance-P	1[78]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[2]	Unclear[3]	No serious limitation[4]	Serious limitation[5]	None[6]	6	6	-3.27 [-5.22,-1.31] P = 0.001
NGF	1[78]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[2]	Unclear[3]	No serious limitation[4]	Serious limitation[5]	None[6]	6	6	-2.55 [-4.24,-0.87] P = 0.003
DOR	1[78]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[2]	Unclear[3]	No serious limitation[4]	Serious limitation[5]	None[6]	6	6	N.D.
MOR	1[78]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[2]	Unclear[3]	No serious limitation[4]	Serious limitation[5]	None[6]	6	6	18.6 [9.45, 27.74] P < 0.081

KOR	1[78]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[2]	Unclear[3]	No serious limitation[4]	Serious limitation[5]	None[6]	6	6	N.D.
GFAP	1[78]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[2]	Unclear[3]	No serious limitation[4]	Serious limitation[5]	None[6]	6	6	-2.99 [-1.14,-4.84] P = 0.002
Nerve												
NGF	1[23]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[2]	Unclear[3]	No serious limitation[4]	Serious limitation[5]	None[6]	6	6	6.26 [3.00, 9.51] P = 0.0002
Spinal Cord												
GFAP	1[77]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[2]	Unclear[3]	No serious limitation[4]	Serious limitation[5]	None[6]	6	6	-3.39 [-1.39, -5.40] P = 0.0009
NGF	1[77]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[8]	Unclear[3]	No serious limitation[4]	Serious limitation[5]	None[6]	6	6	-0.45 [-1.60, 0.70] P = 0.44
Midbrain												
BDNF	1[26]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[2]	Unclear[3]	No serious limitation[4]	Serious limitation[5]	None[6]	5	5	-2.66 [-4.38, -0.94] P = 0.002
MOR	1[23]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[2]	Unclear[3]	No serious limitation[4]	Serious limitation[5]	None[6]	6	6	-1.27 [-2.56, 0.02] P = 0.05
DOR	1[76]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[2]	Unclear[3]	No serious limitation[4]	Serious limitation[5]	None[6]	6	6	16.12 [8.17, 24.06] P < 0.0001
KOR	1[76]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[2]	Unclear[3]	No serious limitation[4]	Serious limitation[5]	None[6]	6	6	5.07 [2.35, 7.79] P = 0.003

OX-42	1[26]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[2]	Unclear[3]	No serious limitation[4]	Serious limitation[5]	None[6]	5	5	-6.47 [-10.32, -2.62] P = 0.0010
GFAP	1[26]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[2]	Unclear[3]	No serious limitation[4]	Serious limitation[5]	None[6]	5	5	-3.65 [-6.05, -1.25] P = 0.003
Thalamus												
BDNF	1[26]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[2]	Unclear[3]	No serious limitation[4]	Serious limitation[5]	None[6]	5	5	-1.86 [-3.32, -0.41] P = 0.01
OX-42	1[26]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[2]	Unclear[3]	No serious limitation[4]	Serious limitation[5]	None[6]	5	5	-3.69 [-1.27, -6.10] P = 0.003
GFAP	1[26]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[2]	Unclear[3]	No serious limitation[4]	Serious limitation[5]	None[6]	5	5	-2.64 [-0.71, -4.58] P = 0.007

[1]Risk of bias was unclear for most items (e.g. no information about randomization and blinding) except for blinding of investigators (high risk of bias). [2]Single studies are always considered to be inconsistent and imprecise. [3]Funnel plot could not be made, publication bias could not be ruled out. [4]Preclinical study fits the preclinical PICO. However the clinical relevance change is unknown. [5]Used animal model is more severe (axonal damage) compared to human compression neuropathies. [6]Consistency across species could not be determined.

Abbreviations: *NCT*: Non-randomized controlled trial; *DRG*: Dorsal Root Ganglion; *TRPV1*: Transient Receptor Potential Vanilloid-1; *NGF*: Nerve Growth Factor; *DOR*: δ-opioid receptor; *MOR*: μ-opioid receptor; *KOR*: κ-opioid receptor; *GFAP*: Glial Fibrillary Acidic Protein*; OX-42*: a microglia marker; *BDNF*: Brain Derived Neurotrophic Factor; *RoB*: Risk of Bias; *CI*: Confidence Interval

Table F3: GRADE results for: “Activator assisted manipulative therapy compared to no intervention in Sprague-Dawley rats suffering from neuropathy for neuroimmune responses”

Activator assisted manipulative therapy compared to no intervention in Sprague-Dawley rats suffering from neuropathy for neuroimmune responses												
Population: Sprague-Dawley rats with decompression neuropathy **Intervention:** ASMT **Comparison:** No-intervention												
Certainty Assessment										**Summary of findings**		
										No of patients		**Effect**
Outcomes	**Studies**	**design**	**Limitations RoB**	**Inconsistency**	**Imprecision**	**Publication bias**	**Indirectness**		**Other considerations**	**ASMT**	**No intervention**	**Standardized mean difference (95% CI)**
							Layer 1	Layer 2				
Serum												
IL-10	1[86]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[3]	Unclear[4]	No serious limitation[5]	Serious limitation[6]	None[7]	12	6	0 36 [-0.63, 1.36] P = 0.47
IL-1β	1[86]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[3]	Unclear[4]	No serious limitation[5]	Serious limitation[6]	None[7]	12	6	0.24 [-0.75, 1.23] P = 0.63
TNF-α	1[86]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[3]	Unclear[4]	No serious limitation[5]	Serious limitation[6]	None[7]	12	6	0.09 [-0.89, 1.07] P = 0.85
DRG												
IL-10	1[86]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[3]	Unclear[4]	No serious limitation[5]	Serious limitation[6]	None[7]	12	6	-0.02 [1.01, 0.98] P = 0.97

IL-1β	1[86]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[3]	Unclear[4]	No serious limitation[5]	Serious limitation[6]	None[7]	12	6	-5.52 [-8.12, -2.92] P < 0.0001
TNF-α	1[86]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[3]	Unclear[4]	No serious limitation[5]	Serious limitation[6]	None[7]	12	6	-0.29 [-1.28, 0.70] P = 0.57
Spinal cord												
IL-10	1[86]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[3]	Unclear[4]	No serious limitation[5]	Serious limitation[6]	None[7]	12	6	6.47 [3.46, 9.48] P < 0.0001
IL-1β	1[86]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[3]	Unclear[4]	No serious limitation[5]	Serious limitation[6]	None[7]	12	6	-0.01 [-0.99, 0.97] P = 0.98
TNF-α	1[86]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[3]	Unclear[4]	No serious limitation[5]	Serious limitation[6]	None[7]	12	6	-0.4 [-0.6, 1.40] P = 0.43
Number of non-neuronal cells	1[85]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[3]	Unclear[4]	No serious limitation[5]	Serious limitation[6]	None[7]	4	4	-7.61[-13.03, -2.20] P = 0.006
Number of non-neuronal cells	1[86]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[3]	Unclear[4]	No serious limitation[5]	Serious limitation[6]	None[7]	3	3	-6.24[-12.48, -0.01] P = 0.05

[1]Risk of bias was unclear for most items (e.g. no information about randomization and blinding) except for blinding of investigators (high risk of bias). [2]Single studies are always considered to be inconsistent. [3]Single studies are always considered to be imprecise. [4]Funnel plot could not be made, publication bias could not be ruled out. [5]Preclinical study fits the preclinical PICO. However the clinical relevance change is unknown. [6]Used animal model is more severe (axonal damage) compared to human compression neuropathies. [7]Consistency across species could not be determined. Abbreviations: *ASMT*: Activator Assisted Manipulative Therapy; *NCT*: Non-randomized controlled trial; *IL-10*: Interleukin-10; *TNF-α*: Tumor Necrosis Factor-α; *IL-1β*: Interleukin-1β; *CI*: Confidence Interval;

RoB: Risk of Bias; *CI*: Confidence Interval

Table F4: GRADE results for: "Ankle joint mobilisation compared to no intervention in Wistar rats suffering from sciatic nerve crush injury for neuroimmune responses"

Ankle joint mobilisation compared to no intervention in Wistar rats suffering from sciatic nerve crush injury for neuroimmune responses												
Population: Wistar rats suffering from a sciatic nerve crush injury **Intervention:** Ankle joint mobilisation **Comparison:** No-intervention												
Certainty Assessment										**Summary of findings**		
										No of patients		**Effect**
Outcomes	**Studies**	**design**	**Limitations RoB**	**Inconsistency**	**Imprecision**	**Publication bias**	**Indirectness**		**Other considerations**	**Ankle joint mobilisation**	**No intervention**	**Standardized mean difference (95% CI)**
							Layer 1	Layer 2				
Spinal cord GFAP	1[51]c	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[2]	Unclear[3]	No serious limitation[4]	Serious limitation[5]	None[6]	5	5	-3.29 [-1.06, -5.52] P = 0.004
Spinal cord CD11b/c	1[51]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[2]	Unclear[3]	No serious limitation[4]	Serious limitation[5]	None[6]	5	5	-1.68 [-0.12, -3.23] P = 0.04
[1]Risk of bias was unclear for most items (e.g. no information about randomization and blinding) except for blinding of investigators (high risk of bias). [2]Single studies are always considered to be inconsistent and imprecise. [3]Funnel plot could not be made, publication bias could not be ruled out. [4]Preclinical study fits the preclinical PICO. However the clinical relevance change is unknown. [5]Used animal model is more severe (axonal damage) compared to human compression neuropathies. [6]Consistency across species could not be determined. Abbreviations: *NCT*: Non-randomized trial; *GFAP*: Glial Fibrillary Acidic Protein; *CD11b/c*: a microglia marker; *RoB*: Risk of Bias; *CI*: Confidence Interval												

Table F5: GRADE results for: “Median nerve mobilisation compared to no intervention in Wistar rats suffering from a median nerve compression injury for neuroimmune responses”

Median nerve mobilisation compared to no intervention in Wistar rats suffering from a median nerve compression injury for neuroimmune responses												
Population: Wistar rats suffering from median nerve compression **Intervention:** Neural mobilisation **Comparison:** No-intervention												
Certainty Assessment										**Summary of findings**		
										No of patients		**Effect**
Outcomes	**Studies**	**design**	**Limitations RoB**	**Inconsistency**	**Imprecision**	**Publication bias**	**Indirectness**		**Other considerations**	**Median nerve mobilisation**	**No intervention**	**Standardized mean difference (95% CI)**
							Layer 1	Layer 2				
Nerve BDNA mRNA	1[50]	NCT	Unclears[1]	Serious limitation[2]	Serious limitation[2]	Unclear[3]	No serious limitation[4]	Unclear[5]	None[6]	12[7]	6	N.D.
Nerve NGF mRNA	1[50]	NCT	Unclears[1]	Serious limitation[2]	Serious limitation[2]	Unclear[3]	No serious limitation[4]	Unclear[5]	None[6]	12[7]	6	N.D.
[1]Risk of bias was unclear for most items (e.g. no information about randomization and blinding) except for blinding of investigators (high risk of bias). [2]Single studies are always considered to be inconsistent and imprecise. [3]Funnel plot could not be made, publication bias could not be ruled out. [4]Preclinical study fits the preclinical PICO. However the clinical relevance change is unknown. [5]Unclear of this animal model can be translated to human neuropathies. [6]Consistency across species could not be determined.[7]One and three minutes neural mobilisation combined. Abbreviations: *BDNF*, Brain Derived Neurothrophic Factor; *mRNA*, messenger ribonucleic acid; *NGF*, Nerve Growth Factor; *NCT*, Non-randomized trial; *RoB*, Risk of Bias; *CI*, Confidence Interval												

Table F6: GRADE results for: “Knee mobilisation compared to no intervention in Sprague- Dawley rats suffering from inflammatory ankle injury for neuroimmune responses”

Knee mobilisation compared to no intervention in Sprague- Dawley rats suffering from inflammatory ankle injury for neuroimmune responses												
Population: Sprague-Dawley rats **Intervention:** Knee mobilisation **Comparison:** No intervention												
Certainty Assessment										**Summary of findings**		
										No of patients		**Effect**
Outcomes	**Studies**	**design**	**Limitations RoB**	**Inconsistency**	**Imprecision**	**Publication Bias**	**Indirectness**		**Other Considerations**	**Knee mobilisation**	**No intervention**	**Standardized mean difference (95% CI)**
							Layer 1	**Layer 2**				
Whole genome expression spinal cord	1[69]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[2]	Unclear[3]	No serious limitation[4]	Unclear[5]	None[6]	3	3	N.D.

[1]Risk of bias was unclear for most items (e.g. no information about randomization and blinding) except for blinding of investigators (high risk of bias). [2]Single studies are always considered to be inconsistent and imprecise. [3]Funnel plot could not be made, publication bias could not be ruled out. [4]Preclinical study fits the preclinical PICO. However the clinical relevance change is unknown. [5]Unclear of this animal model can be translated to human conditions. [6]Consistency across species could not be determined.

Abbreviations: *NCT*: Non-randomized trial; *RoB*: Risk of Bias; *CI*: Confidence Interval

Table F7: GRADE results for: "Ankle joint mobilisation compared to no intervention in Swiss mice suffering from chronic post-ischemia pain for neuroimmune responses"

Ankle joint mobilisation compared to no intervention in Swiss mice suffering from chronic pot-ischemia pain for neuroimmune responses												
Population: Male swiss mice **Intervention:** Ankle mobilisation **Comparison:** No intervention												
Certainty Assessment										**Summary of findings**		
										No of patients		**Effect**
Outcomes	**Studies**	**design**	**Limitations RoB**	**Inconsistency**	**Imprecision**	**Publication Bias**	**Indirectness**		**Other Considerations**	**Knee mobilisation**	**No intervention**	**Standardized mean difference (95% CI)**
							Layer 1	**Layer 2**				
Malondialdehyde	1[73]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[2]	Unclear[3]	No serious limitation[4]	Unclear[5]	None[6]	8	8	-1.23 [-2.32, -0.13]
Carbodnyl protein	1[73]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[2]	Unclear[3]	No serious limitation[4]	Unclear[5]	None[6]	8	8	-1.44 [-2.58, -0.30]
Superoxide dismutase	1[73]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[2]	Unclear[3]	No serious limitation[4]	Unclear[5]	None[6]	8	8	0.99 [-0.07, 2.05]
Catalase	1[73]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[2]	Unclear[3]	No serious limitation[4]	Unclear[5]	None[6]	8	8	1.58 [0.41, 2.74]
[1]Risk of bias was unclear for most items (e.g. no information about randomization and blinding) except for blinding of investigators (high risk of bias). [2]Single studies are always considered to be inconsistent and imprecise. [3]Funnel plot could not be made, publication bias could not be ruled out. [4]Preclinical study fits the preclinical PICO. However the clinical relevance change is unknown. [5]Unclear of this animal model can be translated to human conditions. [6]Consistency across species could not be determined. Abbreviations: *NCT*: Non-randomized trial; *RoB*: Risk of Bias; *CI*: Confidence Interval												

Table F8: GRADE results for: “Spinal manipulation compared to no intervention in male Wistar rats suffering from knee joint immobilisation for neuroimmune responses”

Spinal manipulation compared to no intervention in male Wistar rats suffering from knee joint immobilisation for neuroimmune responses												
Population: Wistar rats rats **Intervention:** Spinal manipulation **Comparison:** No intervention												
Certainty Assessment										**Summary of findings**		
										No of patients		**Effect**
Outcomes	Studies	**design**	**Limitations RoB**	**Inconsistency**	**Imprecision**	**Publication Bias**	**Indirectness**		**Other Considerations**	**Knee mobilisation**	**No intervention**	**Standardized mean difference (95% CI)**
							Layer 1	**Layer 2**				
Superoxide dismutase[7]	1[25]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[2]	Unclear[3]	No serious limitation[4]	Unclear[5]	None[6]	6	6	-0.48 [-1.64, 0.67]
Superoxide dismutase[8]	1[25]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[2]	Unclear[3]	No serious limitation[4]	Unclear[5]	None[6]	6	6	-0.02 [-1.15, 1.11]
Catalase[7]	1[25]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[2]	Unclear[3]	No serious limitation[4]	Unclear[5]	None[6]	6	6	-1.88 [-3.33, -0.42]
Catalase[8]	1[25]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[2]	Unclear[3]	No serious limitation[4]	Unclear[5]	None[6]	6	6	-0.96 [-2.19, 0.26]
Lipid hydroperoxide[7]	1[25]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[2]	Unclear[3]	No serious limitation[4]	Unclear[5]	None[6]	6	6	-0.76 [-1.95, 0.43]

Lipid hydroperoxide[8]	1[25]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[2]	Unclear[3]	No serious limitation[4]	Unclear[5]	None[6]	6	6	-1.76 [-3.18, -0.34]
Nitric Oxide metabolites[7]	1[25]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[2]	Unclear[3]	No serious limitation[4]	Unclear[5]	None[6]	6	6	-0.27 [-1.41, 0.87]
Nitric Oxide metabolites[8]	1[25]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[2]	Unclear[3]	No serious limitation[4]	Unclear[5]	None[6]	6	6	-0.28 [-1.42, 0.86]
Glutathione peroxidase[7]	1[25]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[2]	Unclear[3]	No serious limitation[4]	Unclear[5]	None[6]	6	6	-1.56 [-2.93, -0.20]
Glutathione peroxidase[8]	1[25]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[2]	Unclear[3]	No serious limitation[4]	Unclear[5]	None[6]	6	6	-0.31 [-1.45, 0.83]

[1]Risk of bias was unclear for most items (e.g. no information about randomization and blinding) except for blinding of investigators (high risk of bias). [2]Single studies are always considered to be inconsistent and imprecise. [3]Funnel plot could not be made, publication bias could not be ruled out. [4]Preclinical study fits the preclinical PICO. However the clinical relevance change is unknown. [5]Unclear of this animal model can be translated to human conditions. [6]Consistency across species could not be determined. [7]Compared to sham. [8]Compared to no-intervention.

Abbreviations: *NCT*: Non-randomized trial; *RoB*: Risk of Bias; *CI*: Confidence Interval

Table F9: GRADE results for: “Spinal mobilisation compared to no intervention in female Sprague- Dawley rats suffering from NGF-induced low back pain for neuroimmune responses”

Spinal mobilisation compared to no intervention in female Sprague- Dawley rats suffering from NGF-induced low back pain for neuroimmune responses												
Population: Sprague-Dawley rats **Intervention:** Spinal mobilisation **Comparison:** No intervention												
Certainty Assessment										**Summary of findings**		
										No of patients		**Effect**
Outcomes	Studies	**design**	**Limitations RoB**	**Inconsistency**	**Imprecision**	**Publication Bias**	**Indirectness**		**Other Considerations**	**Knee mobilisation**	**No intervention**	**Standardized mean difference (95% CI)**
							Layer 1	**Layer 2**				
CGRP L1	1[66]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[2]	Unclear[3]	No serious limitation[4]	Unclear[5]	None[6]	3	3	-2.30 [-5.04, 0.44]
CGRP L2	1[66]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[2]	Unclear[3]	No serious limitation[4]	Unclear[5]	None[6]	3	3	-2.94 [-6.21, 0.32]
CGRP L3	1[66]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[2]	Unclear[3]	No serious limitation[4]	Unclear[5]	None[6]	3	3	-0.23 [-1.84, 1.39]
CGRP L4	1[66]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[2]	Unclear[3]	No serious limitation[4]	Unclear[5]	None[6]	3	3	-0.00 [-1.60, 1.60]
CGRP L5	1[66]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[2]	Unclear[3]	No serious limitation[4]	Unclear[5]	None[6]	3	3	-0.52 [-2.20, 1.16]
CGRP L6	1[66]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[2]	Unclear[3]	No serious limitation[4]	Unclear[5]	None[6]	3	3	-0.06 [-1.66, 1.54]

CGRP R1	1[66]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[2]	Unclear[3]	No serious limitation[4]	Unclear[5]	None[6]	3	3	-1.71 [-4.00, 0.59]
CGRP R2	1[66]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[2]	Unclear[3]	No serious limitation[4]	Unclear[5]	None[6]	3	3	-0.79 [-2.56, 0.98]
CGRP R3	1[66]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[2]	Unclear[3]	No serious limitation[4]	Unclear[5]	None[6]	3	3	-0.72 [-2.47, 1.02]
CGRP R4	1[66]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[2]	Unclear[3]	No serious limitation[4]	Unclear[5]	None[6]	3	3	0.50 [-1.18, 2.17]
CGRP R5	1[66]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[2]	Unclear[3]	No serious limitation[4]	Unclear[5]	None[6]	3	3	-0.45 [-2.11, 1.21]
CGRP R6	1[66]	NCT	Unclear[1]	Serious limitation[2]	Serious limitation[2]	Unclear[3]	No serious limitation[4]	Unclear[5]	None[6]	3	3	0.19 [-1.42, 1.81]

[1]Risk of bias was unclear for most items (e.g. no information about randomization and blinding) except for blinding of investigators (high risk of bias). [2]Single studies are always considered to be inconsistent and imprecise. [3]Funnel plot could not be made, publication bias could not be ruled out. [4]Preclinical study fits the preclinical PICO. However the clinical relevance change is unknown.[5]Unclear of this animal model can be translated to human conditions. [6]Consistency across species could not be determined. Abbreviations: *NCT*: Non-randomized trial; *RoB*: Risk of Bias; *CI*: Confidence Interval; *R*: Right; *L*: Left

Table F10: GRADE results for: “Manual therapy compared to no intervention in humans suffering from spinal pain for neuroimmune responses”

Manual therapy compared to no intervention in humans suffering from spinal pain for neuroimmune responses													
Population: Humans with spinal pain **Intervention:** spinal manipulation or mobilisation **Comparison:** No intervention or Sham													
Certainty Assessment										**Summary of findings**			
										No of patients		**Effect**	
Outcomes	**Studies**	**design**	**Limitations RoB**	**Inconsistency**	**Heterogeneity**	**Indirectness**	**Imprecision**	**Publication bias**	**Other considerations**	**Manual therapy**	**No intervention**	**Standardized mean difference (95% CI)**	**Certainty in the evidence**
Cortisol or β-endorphin													
Serum Cortisol	1[62]	RCT	No serious limitation[1]	Serious limitation[2]	Unclear	Unclear	Serious limitation[2]	Unclear	None	15	15	N.D.	⊕OOO VERY LOW
Serum Cortisol	1[46]	RCT	Unclear[1b]	Serious limitation[2]	Unclear	Unclear	Serious limitation[2]	Unclear	None	13	15	0.00 [-0.74, 0.74] P = 1.0	⊕OOO VERY LOW
Salivary Cortisol	1[91]	RCT	No serious limitations[1]	Serious limitation[2]	Unclear	Unclear	Serious limitation[2]	Unclear	Upgrade due to large effect size	28[3]	26	9.36 [7.45, 11.27] P < 0.00001	⊕⊕OO LOW
Salivary Cortisol	1[91]	RCT	No serious limitations[1]	Serious limitation[2]	Unclear	Unclear	Serious limitation[2]	Unclear	Upgrade due to large effect size	28[4]	26	14.86 [11.9, 17.82] P < 0.00001	⊕⊕OO LOW
Plasma	1[75]	RCT	No serious limitations[1]	Serious limitation [2]	Unclear	Unclear	Serious limitation[2]	Unclear	None	6	6	N.D.	⊕OOO

β-endorphin													VERY LOW
Serum interleukin-1β[5]	1[97]	RCT	No serious limitations[1]	Serious limitation [2]	Unclear	Unclear	Serious limitation[2]	Unclear	Unclear	11	11	-0.36 [-1.20, 0.49]	⊕OOO VERY LOW
Serum interleukin-1β[6]	1[97]	RCT	No serious limitations[1]	Serious limitation [2]	Unclear	Unclear	Serious limitation[2]	Unclear	Unclear	11	11	-0.80 [-1.68, 0.07]	⊕OOO VERY LOW

GRADE Working Group grades of evidence
High quality: Further research is very unlikely to change the confidence in the estimate of effect.
Moderate quality: Further research is likely to have an important impact on the confidence in the estimate of effect and may change the estimate.
Low quality: Further research is very likely to have an important impact on the confidence in the estimate of effect and is likely to change the estimate.
Very low quality: We are very uncertain about the estimate

[1]Median score was low risk of bias. [1b]Median score was unclear risk of bias. [2] Single study. Therefore considered to be inconsistent and imprecise. [3]Cervical spinal mobilisation versus sham manipulation. [4]Cervical spinal manipulation versus sham manipulation. [5] 20 minutes after one treatment sessions. [6]After 3 weeks treatment. Abbreviations: *RCT*: Randomized Controlled Trial; *RoB*: Risk of Bias; *CI*: Confidence Interval; N.D.: could not be determined

Table G.1.: Method and results primary and secondary outcome

Author	Groups[a]	Method primary outcome[b]	Effect primary outcome[c]	Method secondary outcome[d]	Effect secondary outcome[e]
Ruhlen 2014	E: KJM C: NI	Microarray hybridization	No difference in gene expression between E and C	Voluntary wheel running[1]	No difference in voluntary wheel running between E and C
Salgado 2019	E: AJM C: NI	Spectrophotometry Method of Lowry	MDA E < C Carbonyls protein E < C SOD E = C CAT E > C Complex I, II, IV activity E = C	Mechanical hyperalgesia - Von Frey test	Frequency response after the 2th session E < C
Duarte 2019	E: ASMT C1: ASMT-sham C2: NI	Spectrophometry	Lipid hydroperoxides E = C1 Lipid hydroperoxides E 56% < C2 Nitric oxide metabolites E = C1 and E = C2 Superoxide dismutase E = C1 and E = C2 Glutathione peroxidase E 50% < C1 Glutathione peroxidase E = C1	Mechanical threshold - Von Frey Sciatic Functional Index (SFI) Tibial Functional Index (TFI) Peroneal Functional Index (PFI)	No difference in mechanical threshold E = C1 = C2 after 3 weeks SFI at three weeks E > C1; E > C2 TFI at three weeks E > C1; E > C2 PFI at three weeks E > C1; E > C2

			Catalase E < C1 and E < C2		
Reed 2020	E: MSM C: NI	Immunohistochemistry	CGRP Left L1 and L2 segmental E < C CGRP L3, L4, L5, L6, R1, R2, R3, R4, R5, R6 E = C.	Mechanical threshold - von Frey Thermal threshold - hotplate assay Functional field test Rat Grime Scale (RGC)	Trunk mechanical threshold E > C hindpaw Mechanical threshold E > C Ipsilateral hind paw thermal allodynia E = C Functional field test E = C RGS E < C on day 5 RGS E = C day 8 and 10.
Giardini 2018	E: NM C: NI	Western blot Immunohistochemistry Western blot Immunohistochemistry Western blot Immunohistochemistry	Thalamus GFAP E 70% < C Midbrain GFAP E 64% < C VPL PAG Thalamus OX-42 E 47% < C Midbrain OX-42 E 46% < C VPL PAG Thalamus BDNF E 36% < C Midbrain BDNF E 41% < C VPL PAG	No^2	
Santos 2014	E: NM C: NI	Western blot Western blot	PAG DOR E 50% > C PAG KOR E 40% > C	Sciatic functional index (SFI) Tibiales anterior muscle strength (maximal tetanic force (MTF))	SFI after the 7^{th} session E > C MTF E 172% > C

		Western blot	No difference PAG MOR E = C		
Santos 2012	E: NM C: NI	Western blot Immunohistochemistry Western blot Immunohistochemistry	DRG NGF E 110% < C No difference S.C. NGF E = C S.C. NGF-IR E < C DRG GFAP E 68% < C S.C. GFAP E 108% < C S.C. GRAP-IR E < C	Mechanical threshold - Test of Randall & Selitto - Von Frey test Thermal threshold - Hargreaves test	Mechanical threshold after the 2th session E > C Thermal threshold after the 4th session E > C
Da Silva 2015	E: NM C: NI	Western blot	Sciatic nerve NGF E 48% > C	Transmission electron microscopy (ultrastructural morphology of sciatic nerve) - Nerve fiber diameter (NFD) - Axon diameter (AD) - Myelin sheath thickness (MST) - G-ratios Myelin protein zero in sciatic nerve (MPZ)	- NFD E 2.1 > C - AD E 1.1 > C - MST E 0.38 > C - G-ratio E 0.1 > C -Sciatic nerve MPZ E 42% > C
Santos 2018	E: NM C: NI	Western blot Western blot Western blot Western blot	DRG Substance-P E 65% < C DRG TRPV1 E 110% < C No difference DRG DOR E = C No difference DRG KOR E = C DRG MOR E 43% > C	No[2]	
Martins 2011	E: AJM C: NI	Immunohistochemistry Immunohistochemistry	Dorsal S.C. GFAP-IR E < C Dorsal S.C. CD11b/c E < C	Mechanical hyperalgesia Von Frey Cold hyperalgesia Acetone drop	Mechanical Threshold E > C[4] Cold threshold E > C[4] SFI E > C[4]

				Walking track analysis Sciatic Functional Index (SFI) Static Sciatic Index (SSI) Morphology analysis - Degeneration debris (DD) - Area connective tissue (ACT) - Area myelinated fibers (AMF) - Density myelinated fibers (DMF) - Myelin sheath thickness (MST)	SSI E > C[4] - No difference DD E = C - No difference ACT E = C - No difference AMF E = C - No difference DMF E = C > MST Exp. = Co.
Marcioli 2018	E: NM C: NI	RT-PCR RT-PCR	Median nerve NGF mRNA Non-detectable Median nerve BDNF mRNA Non-detectable	Histomorphometric analysis - Axon count - Nerve fiber diameter[3] - Axon diameter[3] - Myelin sheath thickness[3] - Myelin/axon[3] - Quotient G[3] Mechanical hyperalgesia[3] - Von Frey Grip strength[3]	- No difference E = C - No difference E = C - No difference E = C - No difference E = C - No difference E = C - No difference E = C - No difference E = C - No difference E = C
Song 2016	E: ASMT C: NI	Light dissection microscope ELISA ELISA ELISA	Non-neuronal DRG cells E < C No difference Serum TNF-α E = C No difference Serum IL-1β E = C No difference Serum IL-10 E = C No difference DRG TNF-α E = C DRG IL-1β E < C No difference DRG IL-10 E = C	Mechanical allodynia Electro von Frey Thermal hyperalgesia Heat stimulation Whole cell current clamp recording (small ≤30 µm DRG neurons) Action potential current threshold Number of spikes[5] % Neurons discharging[5] c-Fos immunoreactivity in L4-L5 laminae I-IV PKCγ immunofluorescence in L4-L5 Laminae I-IV	Mechanical threshold E > C Thermal threshold E > C Threshold E > C Spikes E < C Discharging E < C S.C. c-Fos E < C S.C. PKCγ E < C

			No difference S.C. TNF-α E = C No difference S.C. IL-1β E = C S.C. IL-10 E > C		
Song 2006	E: ASMT C: NI	Light dissection microscope	Non-neuronal DRG cells E < S.C.	Mechanical allodynia - von Frey filaments Thermal hyperalgesia - Heat stimulation Whole cell current clamp recording (small ≤30 µm DRG neurons) - Resting membrane potential (Vm) - Action potential current threshold (ACPT) - % Neurons discharging[5] Whole cell current clamp recording (medium ≤31-49 µm DRG neurons) - Resting membrane potential (Vm) - Action potential current threshold (ACPT) - % Neurons discharging[5] Whole cell current clamp recording (large >49 µm DRG neurons) - Resting membrane potential (Vm) - Action potential current threshold (ACPT) - % Neurons discharging[5]	Mechanical threshold E > C Thermal threshold E > C Vm E > C Threshold E > C Spikes E > C Vm E > C Threshold E >C Spikes E < C Vm E > C (Threshold E > C Spikes E < C
Sanders 1990	E: LSM L4/L5/S1 C: NI	Radio-immuno assay	No difference serum β-endorphin E = C	VAS	Slight reduction E < C
Padayachy 2010	E: LSM C: NI	N.D.	Serum cortisol E > C	No	

Lohman 2018	E: CSM C: Sham	Milliplex map magnetic bead panel immunoassay	No difference serum cortisol E = C	No	-
Valera-Calero 2019	E1: CSM E2: CM C: Sham	ELISA	Salivary cortisol E1 > C Salivary cortisol E2 > C	VAS PPT	VAS E1 = C VAS E2 = C PPT E1=E2=C
Zemadanis 2019	E: TSM C: Sham	ELISA	Serum IL-1β E = C direct and after three weeks	NDI NPRS	NDI E < C NPRS E < C