Nerve conductions studies in experimental models of autoimmune neuritis: A meta-analysis and guideline

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List of abbreviations

CI = confidence interval

CMAP = compound muscle action potential

CV = conduction velocity

EAN = experimental autoimmune neuritis

MeSH = Medical Subject Headings

NCS = nerve conduction studies

NCV = nerve conduction velocity

SD = standard deviation

SE = standard error

SNAP = sensory nerve action potential

1. Introduction

Nerve conduction studies (NCS) are standard measures in experimental autoimmune neuritis (EAN), the most common experimental model of Guillain-Barré syndrome (GBS). There is consensus that NCS need standardization to produce high-quality results and to minimize inter- and intraobserver variability (Dillingham et al. , 2016, Dyck et al. , 2013, Litchy et al. , 2014). However, in contrast to human studies, experimental NCS in EAN lack standardization in terms of (i) normative reference values, (ii) technical performance, and (iii) criteria for reporting relevant information in scientific publications. These issues are at odds with the principles of replacement, reduction, and refinement (3R) in animal research. For instance, establishing individual NCS reference values for a specific experiment requires additional animals, which contradicts the principle of minimizing the number and exposure of test animals to this invasive and potentially painful procedure.

To provide a set of reference metrics and criteria for technical performance and reporting of NCS, we performed a systematic review and meta-analysis of NCS for the last 30 years of healthy Lewis rat breed, the most commonly used strain in autoimmune neuritis.

2. Methods

2.1 Literature search strategy

This meta-analysis follows the proposed Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline (Moher et al. , 2009) (**Additional file 1**). A systematic literature review of the database Medline (via pubmed) was performed on the 30th of November 2019. We used the MeSH- and search terms [experimental autoimmune neuritis], neurophysiology, [neural conduction], [electromyography], and neurophysio* and combined them with the term [rat, inbred Lew] (**Table 1**). We only included publications in English.

"[insert Table 1]"

We exported the studies to Endnote X9 (Clarivate Analytics, Philadelphia, Pennsylvania, USA) to find any possible duplications of studies due to indexing under more than one of the five search or MeSH-terms. We further defined a timeline of 30 years, between the 1st of January 1990 and the 30th of November 2019, to limit differences in technological advances (Dillingham, Chen, 2016).

2.2. Data collection

Exclusion criteria were papers written in other languages than English, number of examined animals <4 (to reduce bias), studies performed on dead rats, NCS after model induction, intraoperative electrodiagnostic studies (e.g., surgical exposure of the sciatic

nerve followed by NCS), NCS of non-immune mediated neuritis (e.g., reconstructive nerve lesions), and papers that reported only percentages and ratios but no numeric values. Inclusion criteria were papers written in English, NCS performed on healthy living rats (≥4 per study) before any intervention or any surgical procedure, graphically or numerically reported statistical values like mean, SD, or SE of NCS in immune-mediated neuritis models. Title, abstract, and whenever necessary, the entire publication of 468 studies were read and screened for exclusion criteria by two independent researchers (HL and FK). Rare discrepancies in study selection (overall five studies) were resolved by mutual discussion. For each study, we pooled all experimental groups, in which NCS was performed on fit rats and **before** an intervention.

We converted the means, standard deviations (SD), and standard errors (SE) in any paper with more than one group to a pooled mean, SD, and SE of the mean using the method described in the Cochrane Handbook for Systematic Reviews of Interventions (Higgins JPT, 2019). In 17 studies, results were only presented graphically. We used ImageJ software (ImageJ 1x- software, open-source) to measure and extract the data of interest (Schneider, 2012).

2.3 Statistical analysis

All analyses were performed using Stata 16 (StataCorp, College Station, Texas, USA). Descriptive statistics are reported as means and 95 % confidence intervals (CI). A meta-

analysis of the parameters was performed using Stata's metan command. The metan command pools studies to produce an overall effect estimate using inverse variance-weighted meta-analysis. The meta-analysis was performed using a random-effects model (to account for the heterogeneity between studies), specifying each study-specific parameter as effect size and a 95 % CI, based on the reported SE. An overall summary and study-specific effect sizes and 95% CIs are reported in forest plots for parameters NCV and CMAP. We report heterogeneity via between-study variance (r^2), percentage of variability in the effect sizes, which is not caused by sampling error (I^2), and the total amount of variability of heterogeneity plus sampling variance (H^2). We used a restricted maximum likelihood (REML) function for unbiased estimates of variances. To address the risk of bias across the studies, we used the Systematic Review Center for Laboratory animal Experimentation (SYRCLE) tool for the risk of bias assessment (Hooijmans et al. , 2014). Further subgroup analyses for gender, animals' age, and animals' weight (in a range of 150-220g) were performed, as well as stratification for the anesthetics used and pulse amplitude. The sample sizes of studies reporting CMAP after stimulation at the ankle or hip were too small for further subgroup analysis.

3. Results

3.1 Literature search

The search led to 616 publications of interest, 58 of which were duplicates captured by more than one search term. Further, ninety studies had been published before 1990. Thus, 468 studies were eligible for further review, of which 412 were excluded after abstract and title screening. Fifty-six publications were accessed for full-text analysis, of which 28 were excluded because they met exclusion criteria (**Additional file 2**). Twenty-eight studies were left for qualitative assessment. Three studies (of which two exclusively) reported motor NCS of the tail nerve (Kafri et al. , 2002, Usuki et al. , 2010, Usuki et al. , 2006). Due to this low number of studies, no further analysis was performed concerning this nerve. Overall, 26 studies reporting NCV or/and CMAP of the sciatic nerve and its muscles, were included in the qualitative analysis (**Figure 1**).

"[insert Figure 1]"

Figure 1. Study flow-chart based on the PRISMA guidelines. PRISMA = Preferred Reporting Items for Systematic Reviews and Meta-Analyses, n= number of studies.

3.2 Data characteristics

Overall, the studies included a total of 735 Lewis-rats (mean 28.27 ± SD of 19.12 per study). Twenty-two of the 26 studies reported NCV, 13 the CMAP recorded from the dorsal foot muscles after stimulation at the sciatic notch, and 15 the CMAP recorded from the dorsal foot muscles after stimulation at the ankle or popliteal fossa. Six studies did not describe the animals' age, two studies did not report the gender distribution of

the animals included (**Table 2 and 3**). Reported methods differed widely regarding anesthesia, positioning, pulse duration, pulse amplitude, temperature monitoring, and technical devices (**Table 3**). The most frequently used anesthetics were xylazine and ketamine, followed by pentobarbital, halothane, fentanyl/fluanisone, and isoflurane. The drugs had been administered via intravenous, intraperitoneal, subcutaneous injection, or inhalation and differed remarkably in the used concentrations (**Table 3**). Two studies did not specify the used narcotics, and 12 studies did not report the used concentration. Also, the reported technical procedures were often incomplete and varied considerably. Only six studies reported pulse amplitude (between 1 and 30 mA), 23 studies a pulse duration (50 – 200 ms). Seven studies did not report temperature monitoring. The positioning of electrodes was described in 25 studies (**Table 3**).

“[insert Table 2]"

“[insert Table 3]"

3.3. Quality appraisal

The SYRCLE’s “risk of bias tool” assesses the bias of preclinical intervention studies (Hooijmans, Rovers, 2014). Some of these biases were not considered relevant for our purpose since we only included healthy rats in our analysis. We, therefore, decided to exclude whether allocation concealment was performed and whether animals were randomly assessed for outcome measurement. Random intervention group allocation

for, e.g., medication application should not bias baseline NCS, nor should allocation for outcome measurement (outcome NCS was not of interest in this analysis). Converting graphical data could introduce another bias, which we added to the assessment. The risk of bias assessment for the 26 studies, only regarding baseline NCS, is depicted in **Figure 2**.

"[insert Figure 2]"

Figure 2. Risk of bias assessment. Study quality is assessed by eight items using the SYRCLE risk of bias tool. Studies were allocated to 3 different categories: high risk for bias (orange), low risk for bias (grey), and unclear risk of bias (blue).

Poor reporting regarding experimental details led to an overall unclear risk of bias. Selection bias assessment showed that none of the studies described how group allocation had been performed. Fortunately, only 23% of the studies showed a high risk of bias regarding baseline characteristics. Performance bias was not further detailed in any of the studies as no references to random housing or caregiver/investigator blinding was made. Only 27% of the studies stated investigators' blindness, with the remaining studies not addressing possible detection bias. Attrition bias via incomplete outcome reporting was a concern in 37% of the studies, and selective outcome reporting was a low risk (12%). 65% of the included reports depicted their data graphically.

3.4 Study findings

We performed a meta-analysis of motor NCS data (NCV/CMAP) for the sciatic nerve based on the extracted data. The reported mean values for NCV (**Figure 3**) and CMAP after stimulation at the notch (**Figure 4**) or the ankle (**Figure 5**) were dispersed with considerable heterogeneity between the studies (reported $I^2 > 99\%$). This heterogeneity was accessed with further subgroup analyses of NCV stratified for gender and age, which did not reveal any significant impact of this parameter for NCV (**Figure 6 and Additional file 3**). To assess the impact of weight, we analyzed the NCV of studies that reported the animals' weight within a range of 150-220 g. Despite comparable mean weights, considerably different NCVs were found (**Additional file 4**). Methodical differences of the studies were further analyzed with subgroup analyses for used temperature monitoring, anesthetics, and pulse duration (**Additional files 5 to 7**). In all cases, considerably variable NCV were found. A meta-analysis for pooled mean NCV and CMAP was conducted, but the observed variability was too substantial and could not be explained by any meaningful factor. We reported the values as ranges in **Table 4**.

"[insert Figure 3]"

Figure 3. Meta-analysis with a random-effects model of the reported NCV. NCV values are in (m/s). Heterogeneity was considerable with a reported I^2 of 99.75%. The NCV with a 95% CI are reported with their percentual weight in generating the overall NCV (see Table 4).

“[insert Figure 4]"

Figure 4. Meta-analysis with a random-effects model of reported CMAP after simulation at the notch and recording in dorsal foot (plantar) muscles. Heterogeneity was considerable with $I^2 = 99.77\%$. The mean CMAP of each study with a 95% CI is reported and their percentual weight in generating the overall CMAP (see Table 4).

“[insert Figure 5]"

Figure 5. Meta-analysis with a random-effects model of reported pooled CMAP after stimulation at the ankle and recording in dorsal foot muscles. Heterogeneity was considerable with $I^2 = 100\%$. The mean CMAP of each study with a 95% CI is reported and their percentual weight in generating the overall CMAP (see Table 4).

“[insert Figure 6]"

Figure 6. Impact of gender on NCV. Meta-analysis with a random-effects model of reported NCV values stratified by gender (f = female, m = male, u = unknown). Heterogeneity is considerable for all conditions ($I^2 = 99.63\%$ for f, $I^2 = 98.90\%$ for m). The mean NCV in (m/s) of each study with a 95% CI is reported and their percentual weight in generating the overall NCV (overall NCV not shown due to heterogeneity).

4. Discussion

4.1 Summary of evidence

This is the first meta-analysis of NCS in EAN. All eligible studies reported NCS data from the sciatic nerve's motor fibers, except for two studies reporting NCS data only of the tail nerve. Three studies also reported data on sensory nerve conduction and sensory nerve action potential using the H-reflex of the sural nerve (Niknami et al. , 2013, Taylor and Pollard, 2001, 2003).

The preference for using the sciatic nerve for NCS in EAN can be explained by the fact that it is the largest peripheral nerve. It is relatively easy to access, and its anatomical hallmarks for stimulation are comparable to those of humans. The neglect of NCS of sensory fibers probably reflects the technical difficulties of accessing and recording sensory nerves. Sensory amplitudes are much smaller and require an experienced examiner, particularly when small rodents are used. NCS of sensory fibers are often contaminated by accidentally stimulated motor fibers and altered by artifacts, pressure, and positioning, even though some studies argue that sensory NCS offer a reliable assessment of nerve function in rats (Apfel et al. , 1992, Kurokawa et al. , 2004, Stanley, 1981).

The extracted data showed considerable heterogeneity in all our analyses, with I^2 values >99%. Potential explanations for this heterogeneity include the experience of the conducting researcher. Dyck et al. (Dyck, Albers, 2013) reported a high interrater variability even between experienced physicians for human nerve conduction studies, while intraobserver variability was not reported.

229 Furthermore, significant variations in technical procedures were noted. These
230 differences comprised anesthesia, stimulating device, stimulation, positioning, and
231 temperature monitoring. It is conceivable that these inequalities also contribute to the
232 observed variability of reported NCS data.
233 For instance, Oh et al. (Oh et al. , 2010) observed a significant, dose-dependent
234 reduction of NCV following pentobarbital and ketamine/xylazine -induced anesthesia in
235 mice with fewer effects of isoflurane. In contrast, volatile agents as isoflurane or
236 halothane seem to suppress F-waves in a dose-dependent fashion, whereas
237 ketamine/xylazine did not affect F-waves (Nowicki et al. , 2014). Although only rarely
238 performed and therefore not included in our meta-analysis, F-waves may indicate
239 radiculopathy and can be useful as an outcome measure for EAN.
240 Temperature is also well known to bias NCS, as reduced temperatures may cause
241 slower NCV. NCS in humans are normally conducted within a temperature range of 32-
242 36°C measured by surface thermometers. Limbs should be warmed up to this range, if
243 necessary. In rodents, even the accurate measurement itself can be challenging. Most
244 studies that reported temperature control used heat lamps and thermostats for
245 maintaining the temperature between 34-37°C. According to Allen´s rule, smaller
246 animals have a higher surface/volume ratio leading to a faster cooldown of limbs during
247 NCS without heating lamps. Consequences on NCV and CMAP are immense: different
248 studies reported a CV reduction of 1.5-2 m/s per 1°C degree surface temperature

249 reduction for sensory and motor nerve velocity in humans (Halar et al. , 1981, Halar et
250 al. , 1983, Rutkove, 2001). Effects on CMAP are, however, conflicting with a reported
251 increase but also decrease of amplitude and duration (Rutkove, 2001).
252 Basic procedural steps of NCS are another pitfall. Anatomical knowledge of the
253 investigated nerve is essential for the correct placement of the needle electrodes.
254 Technical issues can also occur during stimulation of the nerve. For NCS, all axons
255 within the nerve must be depolarized sufficiently. Submaximal stimulation of the axons
256 can occur with suboptimal placement of the electrodes, or submaximal stimulation pulse
257 and duration.
258 The exact measurement of the distances between the electrodes is elementary to
259 calculate NCV. Variations of a few millimeters can increase or decrease NCV
260 significantly. Absolute changes in the distance in small rodents compared to human
261 NCS have a higher relative impact. For example, in humans, for distances less than 10
262 cm, the error in NCV was reported greater than 20 - 25% for a CV greater than 40 m/s
263 (Landau et al. , 2003, Maynard and Stolov, 1972). In human NCS, 3-4 cm between the
264 stimulating and recording electrodes is recommended as minimal distance (Landau,
265 Diaz, 2003). The hindlimb of a rat, depending on its age, has a length of 5-10 mm, so
266 that the stimulation electrode at the ankle has a distance to the dorsal foot muscles of 1
267 mm. Other variables that significantly influence nerve distances in rats is weight and
268 age. Therefore, these parameters should be assessed for each animal.

CMAP reflects the number and integrity of functioning axons of the nerve. During submaximal stimulation, the CMAP can be falsely low. Most of the studies used the sciatic notch and ankle as the two sites for the stimulation electrodes to evaluate conduction blocks and the proximal and distal integrity of the nerve. Pitarokoili and colleagues, on the other hand, reported insertion of the electrode at the popliteal fossa for distal stimulation (not included in the current meta-analysis due to exclusion criteria). The reports about the effect of the distances between stimulating and recording electrodes on CMAP amplitude are conflicting (Johnsen et al. , 2006, Li et al. , 2014, Mizuta et al. , 2008). Most human studies show an amplitude decay with proximal stimulation due to differences in CV and desynchronization (Johnsen, Fuglsang-Frederiksen, 2006, Olney et al. , 1987). These results were not confirmed in our meta-analysis. The mean reported CMAP amplitude of all 7 studies, which reported measurements after notch and ankle stimulation, were proximally higher than distally (**Figure 4 and 5**).

4.2.Limitations

Limitations of this review, as in many other reviews of animal studies, are the poorly reported methods and the variable methods applied by the researchers. The substantial heterogeneity of the studies could not be resolved by addressing methodical issues via subgroup analyses. Further bias assessment was hampered by incomplete method

289 reporting, and systematic errors cannot be excluded. As has been stated by other
290 reviews of animal studies, detailed and standardized reporting of animal study methods
291 is mandatory.
292 Further, potentially relevant studies could have been missed in our review, as our
293 literature search was restricted to one database. Finally, no study protocol was
294 published.
295
296
297 ## 5. Conclusion
298 Our meta-analysis emphasizes the need for standardization concerning conducting and
299 reporting NCS in EAN. We propose the use of a checklist of seven items, describing
300 essential information (**Table 5**)**.** Adhering to this protocol will help to improve the
301 quality of the studies and reduce intra- and interobserver variability. It will refine the
302 methodology and minimize the number of animals needed in adherence to the 3 R's
303 principles of (Russell and Burch, 1959).
304 **"[insert Table 4]"**
305 **"[insert Table 5]"**
306 We also calculated normative values for NCS in Lewis rats' sciatic nerve of (**Table 4**),
307 based on our meta-analytic data. These metrics may serve as a blueprint for the design
308 (e.g., enabling a power analysis to estimate the number of animals needed in

applications to institutional animal welfare committees) and interpretation of collected data studying EAN. Nevertheless, given the considerable heterogeneity of the data analyzed we still advise collecting a core set of normal values from a control group for each experiment.

Declarations

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Competing Interest

FK contributed to one study included in this review. The authors declared no further potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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476

Tables

Table 1. Study design.

Search or [MeSH] term	Number of papers
1. [neurophysiology]	6,585
2. [neural conduction]	31,727
3. [electromyography]	78,387
4. [Neuritis, Autoimmune, Experimental]	584
5. neurophysio*	115,977
6. [rats, inbred Lew]	22,832

Combination	Number of papers
1. AND 6.	0
2. AND 6.	113
3. AND 6.	79
4. AND 6.	349
5. AND 6.	75
6. AND 1. OR 2. OR 3. OR 4. OR 5.	616

The number of papers for each search and [MeSH]-Term are shown. The combination of the terms reduced the number and left 616 studies for further review.

482 **Table 2**. Characteristics of the used rats.

author	experimental groups	n total	gender	age (weeks)	body weight (g)
Ambrosius et al. , 2017	4	16	f	6-8	160-180
Calik et al. , 2012	1	18	m	a	200-240
Cavaletti et al. , 2000	3	54	u	u	180-200
Hartung et al. , 1990	3	36	f	8-12	180-220
Harvey and Pollard, 1992	2	48	m	u	250-320
Harvey et al. , 1994	3	18	f	11-12	175-205
Jung et al. , 1992	4	27	f	6-8	130-180
Jung et al. , 1993	4	26	f	6-8	160-190
Jung et al. , 1995	2	10	f	u	135-160
Kafri, Drory, 2002	1	5	f	8	175-210
Lawlor et al. , 2001	1	10	m	a	240-300
Lin et al. , 2007a	2	24	m	a	300-400
Lin et al. , 2007b	4	33	f	a	150-200
Moriguchi et al. , 2017	2	9	m	8-10	u
Motte et al. , 2018	2	23	f	6-8	160-180
Niknami, Wang, 2013	6	74	f	8-10	150-200
Pitarokoili et al. , 2014	4	11	f	6-8	160-180
Pitarokoili et al. , 2015	4	40	f	6-8	160-180

Pitarokoili et al. , 2017	8	80	f	6-8	160-180
Pitarokoili et al. , 2019	3	30	f	6-8	160-180
Sarkey et al. , 2007	3	12	m	a	300
Stevens et al. , 1990	3	24	f	u	160-200
Taylor and Pollard, 2001	2	40	b	u	190-400
Taylor and Pollard, 2003	3	43	m/f	u	190-400
Watson et al. , 1994	2	10	u	a	300-400
Yamawaki et al. , 1996	2	14	f	10-12	210-260

Numbers of rats in experimental group, total number (n) of rats used in the study, gender, age (in weeks) and body weight in (g) (m: male, f: female, u: unknown, a: adult).

487 **Table 3:** Technical characteristics of the studies.

author	device	anesthesia	concentration	temperature	pulse amplitude (mA)	pulse duration (ms)	positioning
Ambrosius et al. , 2017	Keypoint, Dantec	Xylazine/ketamine	10/50 mg/kg i.p.	yes	u	50	yes
Calik et al. , 2012	Teca Synergy, Care-Fusion	Xylazine/ketamine	u	yes	25	50	yes
Cavaletti et al. , 2000	u	u	u	yes	u	u	yes
Hartung et al. , 1990	Medelec MS 91B	Fentanyl/fluanisone	1 ml/kg s.c.	yes	30-40	50	yes
Harvey and Pollard, 1992	Devices Mk IV	Halothane/O2	u	no	u	50	yes
Harvey et al. , 1994	Medelec MS 91	Fentanyl/fluanisone	0.5ml/kg s.c.	yes	u	50	yes
Jung et al. , 1992	Medelec MS 91A	Fentanyl/fluanisone	u	yes	30-40	50	yes
Jung et al. , 1993	Medelec MS 91A	Fentanyl/fluanisone	u	yes	30-40	50	yes
Jung et al. , 1995	Medelec MS 91	Fentanyl/fluanisone	u	yes	u	u	u
Kafri, Drory, 2002	Teca Modell Mno	Pentobarbital	30 (mg/kg) i.p.	no	u	200	yes
Lawlor et al. , 2001	Clarc davis Medical System	Xylazine/ketamine	u	7-30	50	yes	u
Lin et al. , 2007a	Neuromax	Pentobarbital	60 mg/kg i.p.	u	u	100	yes
Lin et al. , 2007b	Neuromax	Pentobarbital	60 mg/kg i.p.	u	u	100	yes

Moriguchi et al. , 2017	Neuropack y	Isoflurane	u	no	u	u	yes
Motte et al. , 2018	Keypoint, Dantec	Xylazine/ketamine	10/50 mg/kg i.p.	yes	u	50	yes
Niknami, Wang, 2013	Neuromax	Halothane/O2	u	yes	u	100	yes
Pitarokoili et al. , 2014	Keypoint, Dantec	Xylazine/ketamine	10/50 mg/kg i.p.	yes	u	50	yes
Pitarokoili et al. , 2015	Keypoint, Dantec	Xylazine/ketamine	10/50 mg/kg i.p.	yes	u	50	yes
Pitarokoili et al. , 2017	Keypoint, Dantec	Xylazine/ketamine	10/50 mg/kg i.p.	yes	u	50	yes
Pitarokoili et al. , 2019	Keypoint, Dantec	Xylazine/ketamine	10/50 mg/kg i.p.	yes	u	50	yes
Sarkey et al. , 2007	u	Xylazine/ketamine	u	yes	25	50	yes
Stevens et al. , 1990	Toennies DA-IIR	Xylazine/ketamine	3/63 mg/kg i.p	no	u	50	yes
Taylor and Pollard, 2001	Medelec MS92b	Pentobarbital	30/60 mg/kg f/m i.p	no	u	50	yes
Taylor and Pollard, 2003	Medelec MS92b	Pentobarbital	30/60 mg/kg f/m i.p.	no	u	50	yes
Watson et al. , 1994	Devices Mark IV	u	u	yes	u	50	yes
Yamawaki et al. , 1996	u	Pentobarbital	u	yes	u	50	yes

488 Technical device, anesthesia, concentration, temperature, pulse amplitude, pulse
489 duration, and positioning of the rat are shown. U stands for unknown, f for female, m

for male. Anesthesia concentration is given in (mg/kg) or (ml/kg) and application route is described if possible (s.c.: subcutaneous, i.p.: intraperitoneal).

Table 4. Proposed normative values of motor NCS of the sciatic nerve in Lewis rats.

NCS	**Mean values with 95% CI**
NCV	47.24 m/s [43.65 – 50.84]
CMAP sciatic notch	12.16 mV [9.72 – 14.60]
CMAP ankle	17.41 mV [11.87 – 22.95]

As results of the meta-analyses, the mean values of NCV, CMAP of the sciatic notch and the ankle with a 95% confidence interval are shown.

497 **Table 5.** Checklist for performance and reporting NCS in rats

Number	Item	Recommendation for reporting
1	Animals	Species, strain, gender, mean age + range, mean weight + range
2	Anesthesia	Used drug, route of administration, concentration per (kg) bodyweight
3	Temperature	Should be controlled with heat lamp or pad, Monitoring with infrared skin thermometer or rectal probe
4	Electrode Type	Needle electrodes for sciatic nerve Needle or ring electrodes for tail nerve
5	Placement / Positioning	Standardized distances in (mm), rather than anatomical landmarks, for sciatic nerve: stimulation at sciatic notch and ankle, recording at dorsal foot (plantar) muscles
6	Pulse amplitude / duration	Supramaximal amplitude 7-30 (mA), duration 50 (ms)
7	Data reporting	Mean values, Standard Error of the Mean or Standard Deviation

498 The proposed seven items should be addressed when NCS are conducted in rats.

Additional files

Additional file 1. PRISMA-Checklist.

Section/topic		Checklist item	Reported on page
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2,3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	4
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	16
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5,6
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	5
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	5,6

Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	5,6
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	5,6
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	6,7
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	6,7
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	6,7
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	7
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	6,7
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	7,8
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	8,9
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	6,7
Results of individual	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each	8-12

studies		intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	8,9
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	9,10
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	11,12
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	12-16
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	16
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	17
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	19,20

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Additional file 2. Table of excluded studies (n = 28) with corresponding exclusion criteria.

author	reason for exclusion
Aronovich 2012 et al.	NCS after EAN induction
Bechtold 2005 et al.	intraoperative, terminal NCS
Doppler 2019 et al.	display of ratio only, for SNAP only mean without SD/SE
Gabriel 1997 et al.	terminal NCS
Gabriel 1998 et al.	terminal NCS
Hadden 2001 et al.	NCS after EAN induction
Han (1) 2016 et al.	NCS after EAN induction
Han (2) 2016 et al.	NCS after EAN induction
Harvey 1992 et al.	display of ratio only
Kafri 2005 et al.	NCS after EAN induction
Kajii 2014 et al.	NCS after EAN induction
Kim 1994 et al.	NCS after experimental injections
Kremer 2019 et al.	NCS after EAN induction
Lawlor 2002 et al.	display of ratio only
Lonigro 2009 et al.	intraoperative, terminal NCS
Mix 1992 et al.	terminal NCS
Pollard 1995 et al.	display of ratio only

Redford (1) 1997 et al.	NCS after EAN induction
Redford (2) 1997 et al.	intraoperative, terminal NCS
Schmidt 2003 et al.	data not shown
Spies 1995 et al.	data not shown
Stanley 1992 et al.	intraoperative NCS
Taylor 2017 et al.	display of ratio only
Wiethölter 1992 et al.	NCS after EAN induction
Yan 2000 et al.	display of ratio only
Yan 2014 et al.	display of ratio only
Zhang 2014 et al.	NCS after EAN induction
Zielasek 1993 et al.	data not shown

Additional file 3. pdf. Stratification of NCV for age in weeks. A stands for adult rats, u for unknow (age not documented in the studies). Heterogeneity was considerable throughout all groups. Most studies (n= 8) reported rats with an age of 6-8 weeks (Heterogeneity of $I^2 = 99.69\%$). Mean NCV with a 95% CI of each study is displayed with the percentual weight in the overall analysis.

Additional file 4.pdf. Impact of weight on NCV. This meta-analysis with a random-effects model of reported NCV values only included studies that reported animals' weight within a range of 150-220 g. Heterogeneity was considerable with $I^2 = 99.84\%$. The mean NCS in (m/s) of each study with a 95% confidence interval and their percentual in generating the overall NCS is reported (overall NCV not shown due to heterogeneity).

Additional file 5. pdf. Subgroup analysis of studies reporting temperature monitoring for NCV. Heterogeneity was considerable with $I^2= 99.71\%$. Mean NCV with a 95% CI of each study is displayed with the percentual weight in the overall analysis.

Additional file 6.pdf. Subgroup analysis of NCV after stratification for anesthetics used. Heterogeneity was considerable throughout all groups. Mean NCV with a 95% CI of each study is displayed with the percentual weight in the overall analysis.

Additional file 7.pdf. Subgroup analysis of reported NCV after stratification for pulse duration. Pulse duration is displayed in (ms). Heterogeneity was considerable throughout all groups. Mean NCV with a 95% CI of each study is displayed with the percentual weight in the overall analysis.