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A Systematic Review and Meta-Analysis of Anxiety- and Depressive-Like Behaviors in Rodent Models of Neuropathic Pain

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ABSTRACT

BACKGROUND: Epidemiological studies have frequently shown the concurrence of chronic pain with symptoms of anxiety and depression, particularly in women. Animal models are useful to understand the complex mechanisms underlying comorbidities, but the wide range of methods employed and the wealth of evidence sometimes impedes effective translation and reproducibility. In this systematic review and meta-analysis, we aimed to synthesize the evidence regarding the influence of variables such as sex and species on anxiety- and depressive-like behaviors in rodent models of neuropathic pain.

METHODS: Following PROSPERO registration, we searched EMBASE, Scopus, and the Web of Science from their inception to November 24, 2023, identifying 126 studies that met the inclusion criteria. The Hedges' g value for each experiment and study was calculated, and further subgroup and meta-regression analyses were performed.

RESULTS: Neuropathic pain significantly reduced the time that rats and mice spent in the open arms of the elevated plus and zero mazes ($g = -1.14$), time spent in the center of the open field ($g = -1.12$), sucrose consumption in the sucrose preference test ($g = -1.43$), and grooming time in the splash test ($g = -1.37$) while increasing latency to feed in the novelty-suppressed feeding test ($g = 1.59$) and immobility in the forced swimming ($g = 1.85$) and tail suspension ($g = 1.91$) tests. Sex differences were observed, with weaker effects in female than in male rodents for several behavioral paradigms, and funnel plots identified positive publication bias in the literature.

CONCLUSIONS: This meta-analysis emphasizes the effect of neuropathic pain on anxiety- and depressive-like behaviors in rodents, highlighting the importance of investigating sex differences in future experimental studies.

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Chronic pain affects a significant number of adults, approximately 20% to 30% of the population, and it is an important reason for clinical consultations (1–3). Chronic pain often coexists with psychiatric conditions, which may precede or follow the onset of pain and may in turn complicate effective treatment (1,4,5). Notably, approximately 69% of individuals with chronic pain meet the diagnostic criteria for major depressive disorder (6), while 20% to 30% experience anxiety-related disorders (4). Importantly, there is a higher prevalence of chronic pain (7–9) and stress-related disorders (8,10) in women, and similarly, the incidence of depression or anxiety disorders comorbid with chronic pain is higher among women than among men (11–13). Therefore, gaining a deeper understanding of the etiology underlying the comorbidity between pain and anxiety and depressive disorders may help to identify novel therapeutic targets that regulate pain-emotional circuits and establish relevant biomarkers for robust testing in clinical trials.

Preclinical models of pain are invaluable tools to unravel the driving forces behind pain-induced anxiety and depression (14,15). Among these models, considerable attention has been focused on neuropathic pain, i.e., pain that stems directly from disease or damage that affects the somatosensory system (16). Neuropathic pain is commonly a persistent syndrome with a predominantly peripheral origin that potentially arises from diverse conditions ranging from peripheral nerve injury to metabolic disorders such as diabetes. In laboratory settings, peripheral nerve injury is mainly simulated through total or partial compression, constriction, or ligation of the sciatic nerve, or its trifurcation, resulting in sensory hypersensitivity to various stimuli (mechanical, thermal, etc.). Behavioral tests such as the elevated plus maze (EPM) or its circular variant, the elevated zero maze (EZM), as well as the forced swimming test (FST) are frequently employed to evaluate anxiety- and depressive-like behaviors in rodents (14,17–19). Consequently, in several reports, anxiety- and depressive-like behavior has been evident in animal models of neuropathic pain (20–22), although the results of such studies have not always been conclusive (23–25).

The heterogeneity observed in the behavioral outcomes in neuropathic pain models may stem from experimental variations in sex, genetic factors, the material employed, and the

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duration of the lesions, all of which may profoundly influence the biological mechanisms underlying pain, as well as the regulation of emotions and their influence on behavioral outcomes. To address this issue and consolidate the experimental evidence, synthetic tools such as meta-analyses are becoming increasingly common to assess the methodological variance in preclinical studies (26–28). By identifying knowledge gaps, reducing replication, and highlighting influential factors, preclinical meta-analyses offer focused approaches to enhance the utility of animal models in research (29,30). Here, we carried out a meta-analysis to pinpoint specific variables that influence the behavioral changes in experimental traumatic models of neuropathic pain, summarizing the main effects observed. To this end, relevant literature was systematically collected and analyzed regarding the consequences of traumatic neuropathic pain in rodents compared with sham-operated control rodents. The data obtained emphasize the impact of neuropathic pain on anxiety- and depressive-like behaviors in rodents, highlighting the need to investigate sex differences in future studies.

METHODS AND MATERIALS

A detailed description is provided in the figure legends and in the Supplement. The data and code can be accessed on Open Science Framework (https://osf.io/4pezx/).

Protocol Preregistration, Study Search, and Screening

Methodologies were consistent with established guidelines for data analysis and reporting standards (Table S3) (31,32), as well as those specific to animal studies (26,30,33). The protocol for systematic review and meta-analysis underwent a prospective revision and was registered (PROSPERO CRD42022366275). The search strategies were formulated using a combination of standardized terms and keywords to effectively filter the results according to our inclusion and exclusion criteria (Table S1 and Figure 1A).

Data Extraction and Analysis

Data regarding the bibliographic, population, and methodological variables of the behavioral assays together with the statistical data were extracted for the 126 studies included in the meta-analysis (Table S4). The risk of bias was assessed using an adapted version of the SYRCLE tool (Table S2) (34). A primary meta-analysis was performed separately for 8 behavioral tests on both a "by experiment" and "by study" basis: EPM/EZM, open field (OF), light-dark box (LDB), novelty-suppressed feeding (NSF), FST, tail suspension test (TST), sucrose preference test (SPT), and splash test (SPH) (Figure 1B, C). The Hedges' g statistics were calculated together with the 95% CIs. A random-effects meta-analysis was carried out, followed by subgroup analysis and meta-regression.

RESULTS

Risk of Bias Analysis

Compliance with ethical standards of animal welfare and the appropriate approval of their protocols by an institutional committee were confirmed for the 126 studies identified for inclusion in the meta-analysis (Figure 2). A sample size calculation was referenced in only 9 of these studies: in 7 studies, a power analysis was performed, and in 2 studies, preexisting data were taken into consideration. Details regarding the randomization of the outcome assessments were not provided in 58% of the studies. A further 28 studies indicated that randomization had been implemented, but the method employed was not specified. The use of either automated analysis or blinded manual scoring was reported in 60% of the studies, although only 16% indicated that both methods were used simultaneously, and no information was specified in 21% of the studies. The evident nature of the neuropathic model often makes it impractical to perform studies with study participants blinded to experimental manipulation, although the data may be analyzed blinded to the manipulations. In 94% of the studies, no data exclusion or the absence of details regarding the data was reported, and in 71% of the studies, there was only a low risk of bias associated with selective outcome reporting, even in behavioral paradigms where a single outcome is typically reported. Of the studies that evaluated locomotor behavior, 4 included supplementary locomotor assessments such as wheel running, while outcome reporting was incomplete in 33 studies. In 86% of the studies, no private funding sources or affiliation were reported, while funding sources were not disclosed in 10 studies. Similarly, in 84% of the studies, authors declared no conflict of interest, while no information was provided in 16 studies. A corporate affiliation was disclosed in 4 reports.

Summary Effect Sizes for Behavioral Outcomes

The effect sizes and the measures of heterogeneity were assessed for all 8 behavioral outcomes in each type of experiment, as summarized below, and forest plots that organized the data by experiment and study are presented in the supplementary figures. The effect of neuropathic pain models was first explored in tests evaluating anxiety-like behaviors, such as the EPM/EZM, OF, and LDB, tests that generally induce a conflict between the innate exploratory behavior of the rodent and the fear generated by an open and/or bright area (17–19). Neuropathy significantly reduced the time spent in the open arms of the EPM/EZM ($g = -1.148$, $p < .0001$, $I^2 = 63\%$, $\tau^2 = 0.52$) (Figure 3 and Figure S1A), and the between-study heterogeneity for these tests was moderate, corresponding to 63% of the total variance observed. Less time was spent in the center of the OF in neuropathic pain animals ($g = -1.123$, $p < .0001$, $I^2 = 0.701\%$, $\tau^2 = 0.826$) (Figure 3 and Figure S2A), again with moderate between-study heterogeneity, although in this case, it only accounted for a small proportion of the total heterogeneity. The time spent in the light chamber in the LDB did not differ between sham and neuropathic pain animals ($g = -0.689$, $p = .3176$, $I^2 = 82\%$, $\tau^2 = 2.191$) (Figure 3 and Figure S3). The between-study heterogeneity was very high for this test, and it corresponded to 82% of the total variance observed.

The NSF test is a valuable tool to evaluate both anxiety- and depressive-like behavior because it creates a conflict by pitting the natural urge to consume a pellet against the apprehension of exploring the center of the testing environment. A prolonged

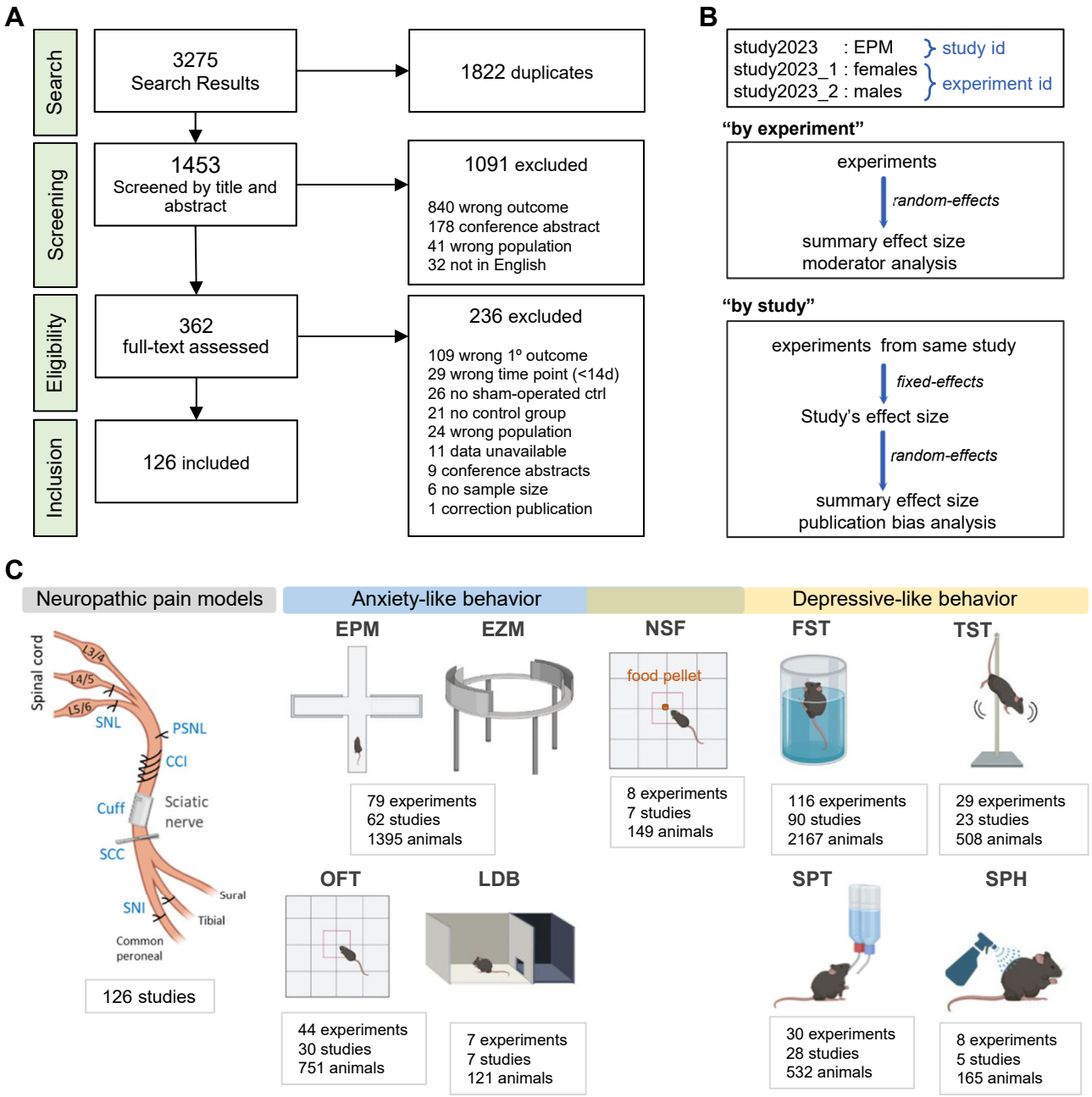

Figure 1. Flow diagram for the systematic review, analysis strategy, and contingency. **(A)** The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram for search retrieval, screening, and the inclusion and exclusion of studies (for further details, see the Supplement). **(B)** Preprocessing and analytic strategy. **(C)** Models of neuropathic pain and the behavioral tests and metadata included in the systematic review and the meta-analysis. CCI, chronic constriction injury; Cuff, sciatic nerve cuffing; EPM, elevated plus maze test; EZM, elevated zero maze test; FST, forced swimming test; LDB, light-dark box test; NSF, novelty suppressed feeding test; OFT, open field test; PSNL, partial sciatic nerve ligation; SCC, spinal cord crushing; SNI, sciatic nerve injury; SNL, sciatic nerve ligation; SPH, splash test; SPT, sucrose preference test; TST, tail suspension test.

delay in pellet consumption latency is indicative of anxiodepressive-like behaviors (17,35). In animal models of neuropathic pain, the latency to feed increased significantly compared with sham-operated control rodents (g = 1.594, p = .0086, I^2 = 75%, τ^2 = 1.134) (Figure 3 and Figure S4A), and the strong heterogeneity between studies corresponded to 75% of the total heterogeneity observed.

The FST and TST are common tests of behavioral despair used to model depressive-like behavior (36,37). Neuropathy significantly increased the time spent immobile in both the FST

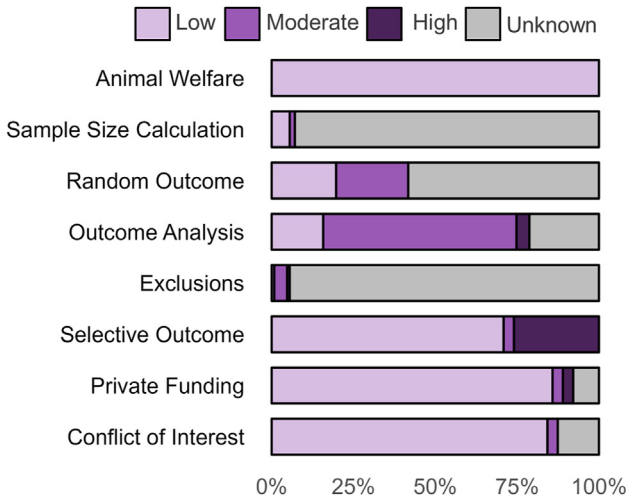

Figure 2. Publication bias analysis (SYRCLE). A stacked bar chart illustrates the quality assessments in terms of the risks of bias. The intensity of the purple shading corresponds to the level of bias identified, with the darkest purple representing a higher risk of bias, a medium tone of purple indicating some concerns, and light purple indicating a low risk of bias. The gray portion of the bars signifies the proportion of studies where no information or specification was available. This adaptation is derived from the rubric utilized by the Systematic Review Centre for Laboratory Animal Experimentation's Risk of Bias tool (for further details, see the Supplement).

(g = 1.851, $p < .0001$, I^2 = 79%, τ^2 = 1.313) (Figure 3 and Figure S5A) and TST (g = 1.910, $p < .0001$, I^2 = 74%, τ^2 = 1.547) (Figure 3 and Figure S6A), and in both cases, the strong heterogeneity between studies accounted for approximately 75% of the total heterogeneity. Sucrose consumption in the SPT is considered to be a proxy for anhedonia (38), and it was significantly reduced in neuropathic pain animals (g = −1.433, p = .0001, I^2 = 75%, τ^2 = 1.446) (Figure 3 and Figure S7A). The heterogeneity between studies was high and accounted for 75% of the total variance. Grooming time in the SPH is used as a measure of motivational behavior and self-care (21), and it was reduced significantly in neuropathic pain animals (g = −1.373, p = .0003, I^2 = 22%, τ^2 = 0.049) (Figure 3 and Figure S8A). The between-study heterogeneity was very low, and it only accounted for 22% of the total variance.

Moderator Analysis

Moderator analyses, such as subgroup analyses and meta-regression, were undertaken to define potential sources of heterogeneity among the distinct experimental outcomes (Figures 4 and 5). This was of particular interest for the data obtained from the EPM/EZM, OF, NSF, FST, TST, and SPT given their widespread use in behavioral assessment and the volume of data gathered through these tests. Categorical variables that were assessed included species, rat and mouse strain, model, sex, whether the animals were naïve for other tests (including behavioral despair tests), housing, the type of material used for nerve injury, and the type or shape of the maze in the case of the EPM/EZM and OF tests. The continuous variables such as number of days from nerve injury or the width of the material used to induce nerve injury were of particular interest in these settings. These analyses were contingent on having a sufficiently robust sample of experiments to ensure adequate statistical power, and more details are provided in the Supplement (Figures S9–S14).

The effect of neuropathy on the heterogeneity of the outcomes on the EPM/EZM test was significantly explained by the rat ($Q_{4,51}$ = 39.858; $p < .0001$) and mouse ($Q_{2,26}$ = 9.283; p = .009) strain, with F344 rats and BALB/c mice remaining longest in the open arms (Figure 4A and Figure S9A). Sex also explained a significant proportion of the heterogeneity between groups ($Q_{1,78}$ = 4.402, p = .035), with female animals spending more time in the open zones than male animals, although it is important to note that only 5% of the experiments studied female animals. No differences were found for the species, the neuropathic model, or in the experiments that employed an EPM or EZM. A subgroup analysis of the OF experiments showed a significant effect of species ($Q_{1,43}$ = 8.295, p = .003) (Figure 4B and Figure S10A), with mice showing less anxiety-like behavior than rats. No significant differences were found for strain, sex, or the neuropathic model or between experiments that used a square or round arena. The latency to feed in the NSF differed significantly

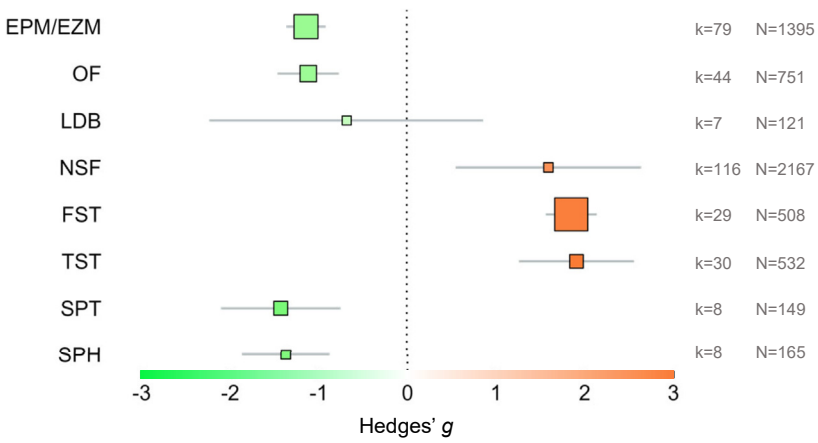

Figure 3. Summary forest plot of the 8 behavioral tests by experiment. The Hedges' g, with 95% CIs, are plotted for each behavioral test, and the size of the square is correlated with the total N of the experiments included in each meta-analysis. The x-axis color scale symmetrically extends from bright green at g = −3 to dark orange at g = 3, with white at the midpoint. The null effect is represented by a dotted line. Individual forest plots by experiment, by study, and repeated measures are available in the Supplement. k indicates number of experiments; N indicates total animal sample. EPM, elevated plus maze test; EZM, elevated zero maze test; FST, forced swimming test; LDB, light-dark box test; NSF, novelty suppressed feeding test; OF, open field test; SPH, splash test; SPT, sucrose preference test; TST, tail suspension test.

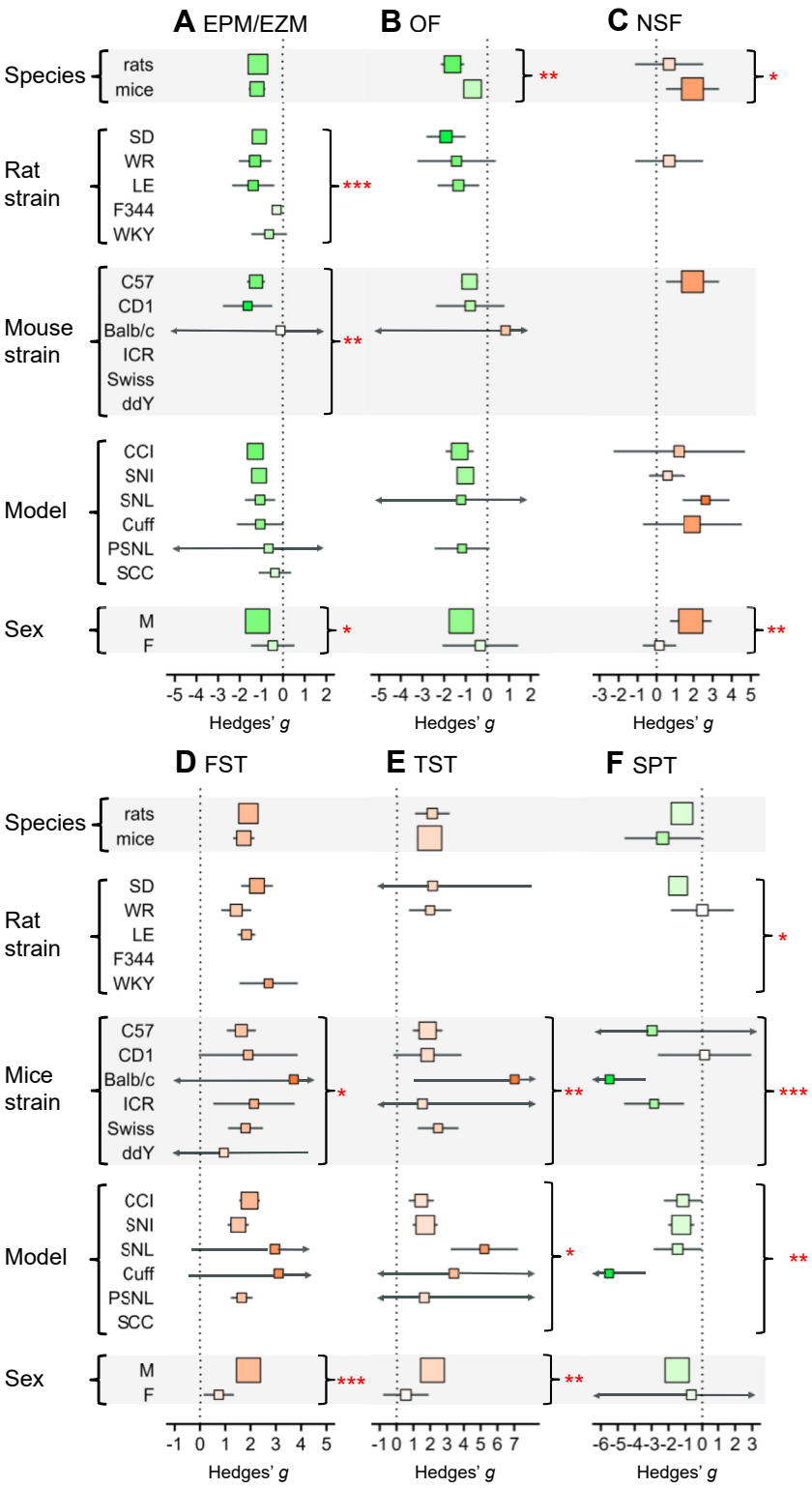

Figure 4. Forest plots for the subgroup analysis of the behavioral outcomes. The between-experiment heterogeneity was analyzed for the **(A)** EPM/EZM, **(B)** OF, **(C)** NSF, **(D)** FST, **(E)** TST, and **(F)** SPT. The null effect is represented by a dotted line, the arrows indicate that the 95% CI extends beyond the limit of the x-axis, and the asterisks indicate the level of statistical significance: $^{*}p \leq .05$, $^{**}p \leq .01$, $^{***}p \leq .001$. CCI, chronic constriction injury; EPM, elevated plus maze test; EZM, elevated zero maze test; F, female; FST, forced swimming test; LE, Long-Evans; M, male; NSF, novelty suppressed feeding test; OF, open field test; PSNL, partial sciatic nerve ligation; SCC, spinal cord crushing; SD, Sprague-Dawley; SNI, spared nerve injury; SNL, sciatic nerve ligation; SPT, sucrose preference test; TST, tail suspension test; WR, Wistar rat.

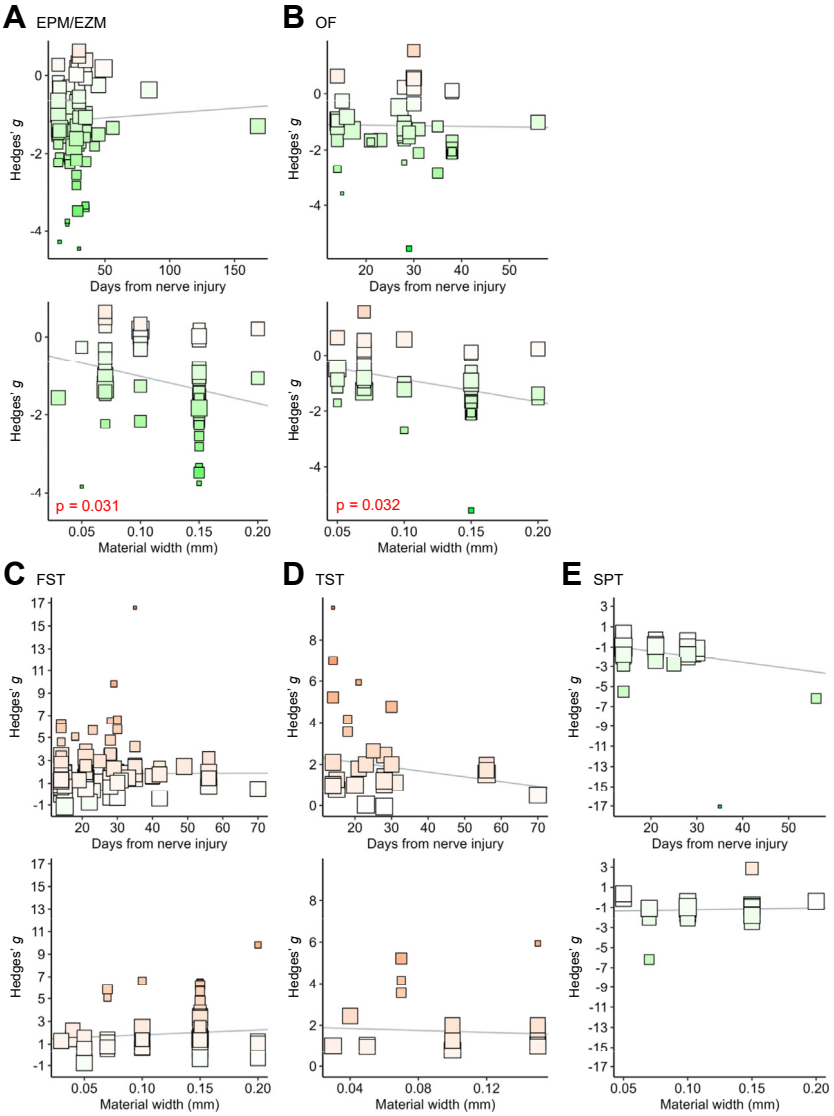

Figure 5. Meta-regression plots to analyze continuous variables. The number of days from nerve injury and the suture width were assessed as continuous moderators in the **(A)** EPM/EZM, **(B)** OF, **(C)** FST, **(D)** TST, and **(E)** SPT. The continuous variable is represented on the x-axis against the effect size of each study on the y-axis. EPM, elevated plus maze test; EZM, elevated zero maze test; FST, forced swimming test; OF, open field test; SPT, sucrose preference test; TST, tail suspension test.

between species ($Q_{1,7}$ = 4.807, p = .0283) and sexes ($Q_{1,7}$ = 6.762, p = .0093) (Figure 4C and Figure S11), with no effect of nerve damage on female animals. Meta-regression showed a significant moderator effect of the width of the material that was used to damage the nerve in the EPM/EZM (β = −6.881, $F_{1,58}$ = 4.879, p = .031) (Figure 5A and Figure S9C) and OF experiments (β = −7.936, $F_{1,37}$ = 4.949, p = .032) (Figure 5B and Figure S10C), although no effect was evident in any subgroup analysis when the time passed since nerve injury was considered (Figures S9B and S10B). Heatmaps for within-experiment time points are provided for visual inspection (Figure S16A). No meta-regressions were performed for the NSF test due to insufficient power.

The effect of neuropathic pain was significantly modified by the mouse strain in the FST ($Q_{5,39}$ = 11.115, p = .049), TST ($Q_{4,25}$ = 16.057, p = .002), and SPT ($Q_{3,6}$ = 39.389, p < .0001) (Figure 4D–F and Figures S12A–S14A), with BALB/c mice showing the longest immobility times. Rat strain was significant for the SPT ($Q_{1,22}$ = 4.163, p = .041). Sex also significantly accounted for some of the between-study heterogeneity in the FST ($Q_{1,113}$ = 32.644, p < .0001) (Figure 4D and Figure S12A) and TST ($Q_{1,28}$ = 11.66, p = .0006) (Figure 4E and Figure S13A), with females spending less time immobile than males. In the TST ($Q_{4,28}$ = 12.73, p = .0126) and SPT ($Q_{3,29}$ = 15.27, p = .0001), the type of neuropathic pain model also accounted for a significant proportion of the heterogeneity observed (Figure 4E, F and Figures S13A–S14A), where cuffed and spinal nerve ligation models produced the strongest effects in the TST, like the cuff model in the SPT. Interestingly, none of the following variables proved to have a significant influence: the species, whether animals were naïve for other tests or behavioral

despair tests, the housing conditions, the type of material used for nerve injury, or the hind paw injured (left/right). No continuous moderators explained the heterogeneity observed in the FST, TST, and SPT (Figures S12B, S12C, S13B, S13C, S14B, and S14C). Heatmaps for within-experiment time points are provided for visual inspection (Figure S16B). Moderator analyses were omitted for the LDB and SPH due to insufficient power and the limited representation of various subgroups.

Publication Bias and Sensitivity Analysis

All the studies were evaluated as a singular unit to assess publication bias and perform a sensitivity analysis. The experiments within these studies were nested using a fixed-effects model that accounted for their execution within a consistent environment, the use of identical resources, and whether they were overseen by the same researchers. Subsequently, the individual studies were depicted in funnel plots, subjected to asymmetry testing with an Egger regression and scrutinized utilizing a trim-and-fill analysis (Figure S15). The leave-one-out analysis assessed sensitivity through a series of successive meta-analyses, systematically excluding one study at a time. This method allowed the impact of excluding each study on the summary effect to be examined, thus providing insight into the robustness of the findings. These approaches demonstrated optimal reliability when applied to datasets from more than 10 studies, a criterion met only by the EPM/EZM test, OF test, SPT, FST, and TST. For the LDB, NSF, and SPH, the funnel plots were presented for visual examination (Figure S15C, D, and H), although we deliberately refrained from employing methods sensitive to statistical power in these cases.

When we analyzed by study, we still observed a significant effect of neuropathic pain models in the EPM/EZM (Figure S1B). A similar analysis of the OF test also still reflected a significant effect (Figure S2B). The Egger regression indicated funnel plot asymmetry in both tests (Figure S15A, B), and the trim-and-fill analysis led to mild attenuation of the effect without changes in the significance. The LDB experiments included in our meta-analysis were all from different studies. Neuropathic pain models still had a significant effect on the latency to feed in the NSF when analyzed by study (Figure S4B).

Regarding the FST and TST, a significant effect of neuropathic pain models was still evident when analyzed by study (Figures S5B and S6B). Egger regression indicated funnel plot asymmetry (Figure S15E, F), and the trim-and-fill analysis attenuated the effect of neuropathy on immobility, but it remained significant. A significant effect of neuropathic pain models was seen when sucrose consumption was assessed in the SPT studies (Figure S7B), and the Egger regression indicated significant asymmetry of the funnel plot (Figure S15G). The inclusion of 6 studies in the trim-and-fill analysis mitigated the effect of neuropathic pain models on sucrose consumption, but the significance persisted. Neuropathy also decreased the grooming time in the SPH when analyzed by study (Figure S8B). In the leave-one-out sensitivity analysis, the overall effect size remained consistent across all tests except for the LDB, where omitting one study, Boccella *et al.* (39), reduced heterogeneity and had a significant effect ($g = -1.159$, $p = .0332$, $I^2 = 68\%$).

DISCUSSION

In this systematic review and meta-analysis of over a hundred studies involving thousands of animals, light was shed on the impact of traumatic models of neuropathic pain in diverse affective behavioral paradigms. Our findings emphasize the prevalence of anxiety-like behaviors among animals experiencing neuropathic pain compared with their sham-operated controls, as reflected in a variety of tests including the EPM/EZM, OF, and NSF. In addition, significant depressive-like behaviors were observed in the FST, TST, SPT, and SPH paradigms. Notably, the LDB anxiety test did not reveal discernible differences, although it was performed in a limited number of experiments and with a relatively small number of animals. Among the anxiety-related behavioral paradigms, the EPM/EZM and OF demonstrated moderate effect sizes, whereas depressive-like and anxiodepressive behaviors in the SPT, SPH, and NSF tests manifested slightly higher effect sizes. The most marked effects of neuropathy were witnessed in stress coping paradigms such as the FST and TST. While the predictive validity of unconditioned anxiety and depression tests in relation to chronic neuropathic pain remains unclear, in rodent models of traumatic neuropathic pain, an anxiodepressive phenotype is clearly induced, as is evident in most tests commonly used to assess this domain.

A moderator analysis revealed significant variation in the impact of neuropathic pain contingent on factors such as species, strain, and the specific model employed. Notably, a sex disparity emerged wherein the effects of neuropathy were lower in female than male animals. Conversely, no discernible distinctions were evident for variables such as prior exposure to other or behavioral despair tests, housing conditions, surgical materials, or the site of injury. Intriguingly, when a wider surgical suture was used in the models, the effect size in anxiety-related behavioral assessments was amplified. Conversely, continuous moderators such as the time since surgery failed to significantly affect behavior. However, some caution is warranted in drawing conclusions from these analyses due to the inadequate size of some subgroups, which perhaps compromised statistical power. While the meta-analysis took 79 experiments into consideration for the EPM/EZM paradigms and 116 FST experiments, only 4 and 3 of these were performed on female rodents. Similarly, where strain variation proved to be significant, the underrepresentation of the Balb/c, CD1, or ICR strains led to wider confidence intervals and a greater risk of type I errors. Inevitably, certain comparisons suffered from inadequate sample sizes owing to the diverse experimental settings. Nonetheless, power and sensitivity analyses (40), as well as recent methods to address the hierarchical nature and interdependent experimental settings of animal datasets (41,42), offer means to mitigate these issues.

Certain results surfaced consistently across a variety of the behavioral paradigms analyzed, suggesting some degree of robustness in the effects observed. Notably, discrepancies between species (rats/mice) were apparent on the OF and NSF tests. In addition, specific strains such as neuropathic BALB/c

mice exhibited pronounced depressive-like behavior but not signs of anxiety. Consequently, distinct comorbidities may arise in different species and strains. Interestingly, anatomical and functional variation has been highlighted in areas implicated in pain processing and emotional regulation (43–47), underlining the complexity of interpreting behavioral outcomes across different models. Moreover, the nature of the traumatic neuropathic pain model emerged as a significant factor in the TST and SPT meta-analyses. Specifically, cuffed and spinal nerve ligation models exhibited the most prominent effects, emphasizing the importance of considering the specific methods used to induce neuropathic pain.

As anticipated, sex emerged as a consistent moderator across tests such as the EPM/EZM, NSF, FST, and TST. Surprisingly, however, we found that the effects of neuropathic pain in female rodents were weaker than in male rodents, with female rodents spending less time in the open arms of the EPM, for example. Nevertheless, the appropriateness of certain paradigms to assess sex differences must be considered given that they might have been initially developed and validated predominantly using male animals (48,49). It is also relevant to consider that some studies indicated sex differences in behavior among naïve animals (48,50–52). Female rodents demonstrated higher levels of anxiety than male rodents without differences in locomotor activity (53), potentially reaching the test's limits of detection. Our results are particularly intriguing given the higher prevalence of pain, anxiety, and depression disorders among women compared with men (10,12,54), which raises questions about the validity of these rodent pain models in replicating human conditions. Overall, this highlights the importance of incorporating female animals in experiments because sex may significantly influence not only the quantitative but also the qualitative aspects of pain-related phenomena and their biological underpinnings (55). This highlights the need for efforts to implement policies that promote sex parity in animal research (56) without necessarily increasing sample size or the costs of research (57). Recent studies in pain research have increasingly been including female animals, reflecting a growing awareness of the importance of sex as a biological variable in experimental models (58).

Regarding the external validity and reliability of the summary effect sizes and heterogeneity observed in our meta-analysis, our findings generally replicated the direction and magnitude of previous research synthesis related to anxiety and depressive-like behavior. Inflammatory pain and lipopolysaccharide depression rodent models achieved similar effect sizes on the EPM and FST compared with neuropathic pain models (28,59). Moreover, a chronic restraint stress model yielded similar effect sizes for the SPT in mice (60), but bigger effect sizes in rats (61,62). However, other stress models, such as maternal separation or sleep deprivation, led to lower effect sizes for the EPM (63), SPT, TST, and FST (64) than neuropathic pain models. There is a lack of research synthesis on the effects of neuropathic pain models on hypersensitivity, as well as the impact of polyneuropathic models, which are highly prevalent in humans, on anxiety- and depressive-like behavioral outcomes as assessed in this meta-analysis.

Previous evidence suggested that the nature of the material used and the paw side affected could influence hypersensitivity responses (65) and anxiety-like behavior (66), respectively. However, no disparities were observed between experiments that utilized silk, chromic, or synthetic sutures or when the left or right paws were affected, consistent with data from a prior meta-analysis on inflammatory pain induced by Complete Freund's adjuvant injection (28). Surprisingly, the width of the suture did yield significant differences in the meta-regression analysis of the EPM/EZM and OF. To the best of our knowledge, this is the first instance of such an effect being reported.

A limitation of this study shared with many other preclinical meta-analyses lies in its reliance on accurate and detailed methodological reporting (67). Variables such as housing conditions (68), the presence of environmental enrichment, habituation protocols (69), and the specific details of behavioral testing (e.g., lighting conditions, maze dimensions, or water depth) are often underreported factors that could significantly influence the outcomes observed (70). Variables that have been explored less often, such as the gender of the experimenters, have recently been associated with variations in pharmacological responses during assessments of anxiety-related behavior (71). The lack of transparency in reporting methodology not only compromises the reproducibility of individual studies but also undermines the synthesis of research findings (72,73). This is exacerbated by factors such as low statistical power due to small sample sizes and selective reporting, both of which may inflate type I and type II errors (74,75). This issue was evident in our funnel plot analyses, with all meta-analyses revealing publication bias toward significant results. Although this bias did not affect the significance of our summary effect size in our study, this does ultimately raise fundamental questions about the adequacy of animal research as a cornerstone in biomedicine (76,77).

There has been an important evolution in standards over recent decades to further standardize procedures in research, experimentation, and reporting. This progress is characterized by the emergence of guidelines, preregistration protocols, and data auditing at various levels (78–80). These developments hold considerable promise to enhance the overall reproducibility of preclinical research in the future. However, concerns remain regarding the gap between these prescribed standards and the practical realities of experimental settings. Protocols are often designed and adjusted to meet specific requirements, leading to methodological diversity. In response to this challenge, innovative, unbiased, and automated approaches are emerging as alternatives. Tools such as DeepLabCut (81) or Bonsai (82) have already been proven to be valuable in assessing pain behavior (83,84). In parallel, future psychiatric behavioral research may benefit from integrating considerations of environmental complexity and temporality into the evaluation of behavioral outcomes (85) together with factorial and multidimensional analytical tools (86–88). These approaches may improve the comprehensiveness of preclinical studies in behavioral science, thereby aiding the systematization of research methods.

Conclusions

While earlier studies provided thorough narrative reviews, this study distinguishes itself by quantifying the effects of experimental traumatic neuropathic pain models across 8 commonly

used behavioral paradigms. Moreover, it stratified these effects based on various variables of interest, including sex, species, and strain. This systematic approach offers a framework to refine and optimize the selection of behavioral paradigms, study populations, and sample sizes, thereby deepening our understanding of the influence of different neuropathic pain models on affective behavior.

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