Systematic review of rat models with temporomandibular osteoarthritis suitable for the study of emerging prolonged intra-articular drug delivery systems

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Abstract:

Purpose

Development of minimally invasive therapies for temporomandibular joint osteoarthritis (TMJOA) has focused on drug intra-articular injections to avoid the systemic adverse effects experienced when these substances are administered orally. Therefore, we performed a systematic review to answer the question "Which method of induction of a TMJOA-related-pain model in rats leads to prolonged painful symptoms, allowing the best assessment of a sustained drug delivery system?"

Materials and Methods

Following the PRISMA guidelines, we searched MEDLINE for papers published from 1994 to July 2020 on a TMJ arthritis model using rats. We identified the means of pain induction and of nociception assessment. We assessed protocol bias using an adaptation of the QUADAS-2 tool. Animal selection, the reference standard method of pain assessment, applicability of a statistical assessment, and flow and timing were assessed.

Results

Of the 59 full papers we reviewed, 41 performed no pain assessment after the first 7 days following induction of the TMJ-related pain model. We eventually identified 18 long-term TMJOA-related pain models. Pain was induced by injection of toxic substances, most commonly Freund's complete adjuvant (50 µg per 50 µl), formalin at various concentrations, or monosodium iodoacetate (50 mg per 50 µl), into the TMJ, or by physical methods. Few

studies reported data on pain after 21 days of follow-up. Heterogeneity of induction methods, pain assessment methods, and flow and timing biases precluded a meta-analysis.

Conclusion

Given that pain is 1 of the main symptoms of TMJOA, experimental study protocols should include long-term pain assessment.

Keywords: pain; osteoarthritis; temporomandibular joint disorders; models, animal; rats; drug liberation

Introduction:

Temporomandibular disorders (TMD) are a significant public health problem affecting approximately 5 to 12% of the general population.[1] This group of heterogeneous musculoskeletal disorders is characterized by either regional pain in the preauricular or facial area or by jaw movement limitation. Subtypes of TMD include pain-related disorders, such as myalgia, myofascial pain with or without pain referral, and arthralgia; and disorders associated with the temporomandibular joints (TMJ), such as internal derangements and degenerative joint disease. Either type results in pain and disability, impacting daily activities, psychosocial functioning, and altering the quality of life. DJD, also known as osteoarthrosis or osteoarthritis (TMJOA), is 1 of the most common taxonomic subtypes of TMD.[2] The prevalence of TMJOA varies greatly, clinical evidence of the disease being observed in 2 to 16% of the population, and structural involvement of the TMJ can be found in 35 to 94% of the patients with at least 1 symptom.[3] This entity is clinically associated with pain in the pre-auricular area with or without associated earache, pain during palpation, coarse crepitus with or without clicking, and limited mobility of the jaw.[2] Its diagnosis is mainly based on radiographic features, including pinching of the joint space, cortical bone resorption, subchondral cysts and geodes, subchondral bone sclerosis, and osteophyte formation.

TMJOA is characterized by progressive cartilage and bone destruction leading to joint inflammation. Therefore, pharmacologic approaches having paralleled those for symptomatic treatment of osteoarthritis have been developed, including NSAIDs[4] and intra-articular injections into the superior joint space (corticosteroids, hyaluronic acid or platelet-rich plasma from blood) [4–6]. However, use of these agents remains controversial in light of decades of mixed reports of intra-articular injections either accelerating TMJ destruction or triggering regeneration [4,6]. To date, no agents have allowed to reverse the underlying TMJ disease.

Consequently, current pain reduction techniques are effective in the early stages of the disease, but fail to alleviate chronic pain caused by severe degenerative joint disease.

There is a high need for sustained release agents, enabling to reduce pain for a long time without systemic adverse effects, which can be seen with current treatments such as NSAIDs [6]. In this light, the methods of intraarticular drug delivery to the TMJ (nano or microparticles), as well as emerging injectable controlled release systems with potential to improve TMJ drug delivery, were under development by numerous researchers to encourage further research in the development of sustained release systems for both long-term pain management and to enhance tissue engineering strategies for TMJ regeneration [7].

Animal models are a useful tool for understanding the pathophysiological mechanisms underlying TMJ disorders, and for evaluating the efficacy of intra-articular injections. A variety

of animal models have been used to evaluate various aspects of drug delivery to the TMJ, including adverse effects of existing intra-articular formulations and the efficacy of emerging treatments. Rodent models are commonly used in studies focusing on temporomandibular degenerative joint disease and TMJ pain, and at the first step in preclinical studies of TMJ drug delivery systems. Rat models of TMJ inflammation have been developed using a variety of methods ranging from repeated, manual, forced mouth opening (mechanical method), surgical procedures, to intra-articular injection of chemical agents. Various analytical methods, such as non-invasive meal pattern analysis, behavior monitoring, etc., have been published to assess as the results for painful symptoms.[8] However, 1 of the main difficulties consists in obtaining a model facilitating the induction of pain in a sufficiently prolonged manner to evaluate the analgesic effect of a long-term drug delivery system.

This review systematically discusses the rat models of TMJOA-related pain in order to identify the best option for assessing long-term controlled drug delivery systems.

Materials & Method:

This systematic review was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[9] This study followed the Declaration of Helsinki on medical protocol and ethics. Due to the bibliographic nature of this study, it was granted an exemption in writing by the University of Lille IRB.

Focused Question

The research question of this study was "Which method of induction of a TMJOA-related-pain model in rat leads to prolonged painful symptoms, particularly suitable for the assessment of a controlled long-term drug delivery system?"

Search Strategy

The search was performed in MEDLINE/PubMed databases, from 1994 to July 2020, using the following terms: "TMJ OR temporomandibular OR TMD", AND "nociception OR pain", AND "model", AND "rat".

Studies were eligible if a TMJ arthritis model using rats was described. Exclusion criteria were as follows: literature reviews or studies only describing the method of induction of the TMJ arthritis model, absence of pain assessment in the TMJ arthritis model, absence of control arm for pain assessment, language other than English, or unavailability of the full paper.

The process of searching and selecting the studies was conducted in duplicate by 2 authors (F.B. and R.N.) working independently. Studies were first screened based on an evaluation of the title and abstract, the potential articles were then carefully assessed according to the eligibility criteria of this review.

Data Extraction

First, listed the characteristics of the eligible experimental models were listed out. Sex, breed, and weight (in grams) of rats used in the selected studies were extracted. The method of induction of the arthritis model was described. For each of the chemical induction methods, type, volume, and concentration of the chemical agent for TMJ intra-articular injection were detailed. Similarly, for each of the mechanical induction methods, load, frequency and duration of application were extracted. The main methods of nociception assessment and the main categories of variables evaluated (clinical, biological, histological, radiological, and electrophysiological) were detailed. When a study used multiple assessment methods, we selected only the most reliable if one could be identified. Finally, the aim of each study was listed.

Further, to sort out the suitable methods for assessing controlled long-term drug delivery systems to treat TMD, the focus was set on all the studies presenting symptoms of TMD related pain that was lasting statistically more than 7 days longer than the control arm. The control group could be a group of rats injected with 0.9% NaCl (saline) or a therapeutic group of rats, in which an efficient therapeutic injection relieved the chemical-induced TMJOA-related pain. In addition to the data previously collected, the number of rats of the induction group and the control was extracted, and the longest duration (in days) of detectable TMJ pain caused by the arthritis induction method was compared to the control.

Assessment of Protocol Bias

Two authors (R.N. and F.B.) independently evaluated the methodological quality rating to verify the strength of scientific evidence on the selected methods of inducing TMJOA-related pain. Protocol bias assessment was performed using an adaptation of the QUADAS-2 tool. Animal selection, the reference standard method of pain assessment, applicability of a statistical assessment, and flow and timing were assessed.

Animal selection: given the role of sex in pain, we considered studies at high risk of bias when both male and female rats were included, at intermediate risk when only females were included, and at low risk when only males were included.

Reference standard method of pain assessment: we considered studies at high risk of bias when they measured the pain using the animals' meal pattern, at intermediate risk when standardized behavioral assessment such as head flinching or orofacial rubbing was performed, and at low risk when a measure of the threshold value using the head withdrawal test was included.

Applicability of a statistical assessment: we considered the studies at high risk of bias when the statistical analysis was performed on fewer than 5 rats in each group, at intermediate risk when between 5 and 7 rats were used, and at low risk when at least 8 rats were used.

Flow and timing: we evaluated if an appropriate time interval was considered between the induction of the model and the last checkpoint using the reference standard method of pain assessment for evaluating long-term TMJ-related pain. We considered studies at intermediate risk of bias when pain assessment was conducted for less than 21 days, and at low risk of bias when pain assessment was conducted for at least 21 days.

Results:

The initial search yielded a total of 174 results (Figure 1). Among them, 115 studies were nonrelevant or were not eligible, since they did not assess nociception, focused on wrong joint, concerned an injured joint, or used an animal other than the rat. Therefore, 59 full papers were reviewed. In order to sort out the suitable methods for assessing controlled long-term drug delivery systems, 41 studies were then excluded because there were no symptoms of TMD

related pain that was lasting statistically more than 7 days longer than the control arm. In the end, 18 papers were analyzed.

Studies Using a TMJ-related Pain Model

The results are shown in Table 1. Seven studies included only female rats,[10–16] and 4 included both female and male rats.[17–20] In all other studies, only male rats were included. Wistar rats or Sprague-Dawley rats were used in all but 3 studies, in which females Holtzman rats were used.[12–14]

The mechanical induction method was applied in 5 studies[12–14,17,21] and chemical 1 in the remaining 54. No study using surgical technique for inducing an osteoarthritis model was found.

All cases of the mechanically induced model consisted of applying a repeated daily mouth opening using a force of 2 N or 3.5 N. In all cases, the load was daily applied under general anesthesia for 60 minutes for 7 consecutive days. Interestingly, in 3 of the 4 studies using a Holtzman rat, the model induction method was mechanical.[12–14]

Several chemical agents have been used to induce TMJOA. Freund's adjuvant is an emulsified mineral oil solution of antigen with immunopotentiator characteristics. Its complete form, Freund's Complete Adjuvant (CFA), is composed of inactivated and dried mycobacteria (mainly *Mycobacterium tuberculosis*). Injection of CFA was used in 33 of 54 studies.[11,15,16,18,20,22–49] Among them, the standard dose used to induce the model was in most cases 50 µg of CFA in a 50 µl volume.[15,24,25,28–30,34–37,41,42,46,47] The volume and concentration of injected CFA is, however, quite variable in other studies.[16,18,20,22,23,26,31–33,38–40,43–45,48–50] Except the study of Ivanusic et al. [39], in which TMJ injection was made using 1 µg of CFA in a 2 µL volume, the CFA dose ranged from 15 µg to 250 µg with a volume varying from 15 to 100 µL. In 9 studies, osteoarthritis was induced by injecting formalin into the TMJ at a

concentration varying from 0.5 to 5%.[51–59] Monosodium iodoacetate (MIA) is an inhibitor of glycolysis, which disrupts chondrocyte metabolism and produces cartilage degradation.[10,11,19] Other chemical agents used were carrageenan,[60–63] mustard oil,[64,65] zymosan,[66,67] and methylated bovine serum albumin.[27,68]

Regarding the main method of nociception assessment, a threshold value was used in 34 of 59 studies.[10–15,17,19,21,23–26,28–37,39,41,42,44,46,50,60,63,66–68] With the exception of 1 study using the paw withdrawal test,[44] the head withdrawal test was used in all cases. The threshold value was obtained with a Von Frey[10,12–15,17,19,21,23–26,28–36,41,44,46,66–68] or Semmes-Weinstein[37,39] or unspecified digital aesthesiometer.[11,42,50,60,63] Behavioral evaluation was the main assessment method in 18 of 59 studies.[13,20,22,27,45,51–59,61,62,64,65] Behavioral assessment included head flinching,[27,51,53,54,57,59,61,62,64,65] orofacial rubbing,[20,22,27,45,51–59,61,62,64,65] or chewing.[51,64] In 1 case, a rat grimace scale was performed by scoring facial expression.[13] Meal pattern was used as the pain assessment method in some studies.[16,18,20,38,40,47,48,53] Sleep disturbance has also been used as an assessment method in 3 studies, all of which were from the same research team.[20,43,49]

Studies Evaluating Long-term TMJ-related Pain

The results concerning the long-term TMJOA-related pain model are shown in Table 2. Among the 18 studies analyzed, 3 used only female rats,[10–12] and 1 used both female and male rats.[19] In all other studies, only male rats have been used.[21,23,24,26,29–31,33,34,36,40,44,57,68] In all studies, they were Wistar rats[21,24,29,57,68] or Sprague-Dawley rats,[10,11,19,23,26,30,31,33,34,36,40,44] with the exception of 1 study using Holtzman rats.[12]

The TMJOA induction method was mechanical in 2 studies[12,21] and chemical in the remaining 16.[10,11,19,23,24,26,29–31,33,34,36,40,44,57,68]

Both cases of the mechanical model consisted of applying a repeated daily mouth opening

using a 2-N[21] or 3.5-N.[12] In all cases, the load was applied for 1 h daily for 7 consecutive days.

As mentioned before, 2 chemical agents (CFA and MIA) have been mainly used to induce the pain model. TMJ injection of CFA was used in 11 of 18 studies.[23,24,26,29–31,33,34,36,40,44] In most of the cases, animals were injected with a volume not exceeding 50 µL into the TMJ. Only 2 studies used a volume of 60 µL[33] or 100 µL of CFA solution[23]. The injected CFA concentration varied from 0.5 µg/µL[26,31,44] to 1 µg/µL.[23,24,29,30,33,34,36] Only 1 study used a concentration of 5 µg/µL.[40] TMJ injection of MIA was used in 3 studies.[10,11,19] In 2 of them, a solution containing 50 mg of MIA in a 50 µL volume was injected into the TMJ.[10,11] The third study compared 2 other doses (80 mg/mL, 16.6 mg/mL) of MIA.[19] Other chemical agents used were methylated bovine serum albumin (10 µg in a 10 µL volume)[68] and formalin (45 µL volume of formalin 1.5%).[57]

Regarding the method of nociception assessment, a threshold value was used in all the studies except 2, which used behavioral assessment[57] or meal pattern.[40] In most of the cases, the threshold value was obtained by using a head withdrawal test obtained with a von Frey aesthesiometer.

The number of rats in each group (model induction or control group) was less than 5 in 3 studies[11,33,34] and between 4 and 8 in 9 studies.[21,23,24,26,30,36,57,68] Only 6 studies compared groups each containing at least 8 rats.[10,12,19,29,40,44]

Finally, 6 studies described a TMJOA-related pain model with long-term pain lasting at least 3 weeks.[10,11,23,30,40,68] Three of them used CFA[23,30,40] for TMJ injection, 2 MIA,[10,11] and 1 methylated bovine serum albumin.[68]

Bias Assessment of the Selected Studies

The results are listed in Figure 2. Based on the QUADAS-2 tool, 2 studies had a low risk of

bias,[29,44] 11 studies had an unclear risk of bias,[10,12,21,23,24,26,30,31,36,57,68] and 5 showed a high risk of bias.[11,19,33,34,40]

Given the multiplicity of induction methods, the pain assessment method and the flow and timing biases, it was not possible to perform a meta-analysis.

Discussion:

Summary of Evidence

The experimental models of TMJ pain simulate either the symptoms or signs of TMJ pain mainly through the development of arthritis or osteoarthrosis, by using chemical and inflammatory agents, mechanical TMJ loading, or surgical procedures.[8] Although TMJ disorders have a complex taxonomy, all share common traits such as inflammation and pain.[1] Therefore, current therapeutic research axes focus on the development of pharmacological substances contributing to locally reducing inflammation and pain.[4] The specifications of good medication candidates must include the control of pain-related symptoms with low systemic adverse effects and long-term local efficacy. Considering these reasons, research has been directed toward the development of drug candidates combining with a sustained release system. Validation of such treatments requires the use of a specific experimental model that manifests pain-related symptoms long enough to allow an assessment of these long-term drug delivery systems. This systematic review thus focused on experimental models of TMJOA-related pain. Among the 18 studies selected, we identified some mechanical and chemical methods inducing a TMJOA-related pain model. Similarity in the profiles between these mechanical and chemical models suggests that they may induce similar molecular mediators and/or structural changes leading to painful symptoms.[4,8] We finally highlighted 6 studies that achieved an induction of joint pain for at least 3 weeks, but 4 of them had unclear risk of bias

and 2 had a high risk of bias. In all cases,[10,11,23,30,40,68] the induction method was chemical, mainly by the injection of CFA[23,30,40] or MIA.[10,11] The injection of these toxic substances into the TMJ has the advantage of being simple and reproducible. The recognition of specific landmarks previously described easily allows the operator to establish the location of the TMJ.[11,69] Moreover, unlike the surgical induction method, these chemical induction methods generally do not alter the joint anatomy.[70] On the other hand, it has been reported that injection of a toxin such as CFA into the TMJ causes morphological and molecular changes in the contralateral joint, suggesting that the unilateral injection of a toxic chemical agent is sufficient to induce a TMJOA-related pain model,[71] although the uni- or bilateral status of the TMJ injection is poorly documented in these studies. Thus, the injection of toxic substances into the TMJ seems to be able to establish a valuable model for subsequently performing the joint injections of medication candidates carried by a sustained drug delivery system. The main disadvantage of the chemical method could be that, a chemical substance is introduced into the TMJ, which could further interact with the therapeutic substances to be tested or local environment, either by the agent itself directly modifying these therapeutic substances due to its intrinsic properties, or by the solvent in the injection mixture interacting with the intra-articular environment.

Limitations

This systematic review assessed protocol bias using an adaptation of the QUADAS-2 tool. Evaluation of animal selection has shown that 4 of 18 long-term pain assessment studies included at least some female rats.[10–12,19] The role of gender in the occurrence of human TMD has been investigated for many years.[72] The intensity of painful symptoms appears to be greater in women for many anatomical locations, including the TMJ. In addition, sex differences in osteoarthritis prevalence and incidence have been shown, with females

generally at a higher risk for developing knee or hand osteoarthritis, particularly after menopause.[73] In a TMJ-osteoarthritis-related pain model, female rats demonstrated a similar spread of tactile hypersensitivity at the lower dose of MIA, whereas male rats did not develop ongoing pain or spread of tactile hypersensitivity outside the area of the ipsilateral TMJ.[19] It suggests that females have a higher susceptibility to develop ongoing pain and central sensitization compared with male rats, a susceptibility that is not due to differences in MIA-induced joint pathology. Therefore, studies evaluating TMJOA-related pain should include only rats of the same gender in order to avoid this selection bias. Given the likely hormonal character and the intra- and interindividual variability of this parameter, the choice should focus on male rats.

Several methods have been proposed in the literature to assess orofacial pain. We identified 4 types of assessment methods: meal patterns, behavioral assessment, sleep patterns, and the threshold value measurement by using the head withdrawal test. The 6 selected long-term TMJOA-related pain models used the paw or head withdrawal test, behavioral assessment, or meal patterns to objectify the pain. These 3 methods can be used with a quantitative dimension. Meal patterns differentiate the ingested meal quantity, frequency, and duration, and the animal stool.[40] Behavioral assessment can be measured by adding the sum of head flinching or orofacial rubbing during a defined lapse of time or use a standardized approach with a quantitative score such as the rat grimace scale.[13] The head withdrawal test classically uses an aesthesiometer, which is applied on the TMJ and leads to rat head withdrawal at a threshold value when the device pressure induces pain. However, the specificity of these 3 different methods seems different. While the withdrawal test appears to be quite specific for pain assessment, the behavioral assessment and the meal patterns could both be influenced by external factors such as stress or illness. Moreover, behavioral assessment is expected to lead to observer bias even if video-recording of rats is used to reduce it.[74] In addition, the

possibility of using an electronic aesthesiometer to perform the head withdrawal test considerably improves the reliability of the threshold value obtained. These are probably the reasons that most of the studies used the head withdrawal test as the main assessment method. Nevertheless, all studies fail to describe the daily rhythm of the measures performed, even though nociception exhibits a robust daily rhythmicity in rats: sensitivity to pain is highest late in the dark phase of the light-dark cycle and lowest at the light-dark transition.[75] It is likely that taking into account the circadian rhythm of pain would change the response to most of the pain assessment methods.

One of the major concerns in the evaluation of these models was the quality of the statistical method. A study design sequentially requires to decide the experimental setting, identify the most appropriate statistical tests, and calculate the sample size that guarantees identifying an expected outcome as statistically significant with appropriate power level. Power analysis must therefore be calculated to ascertain the number of animals per group. Usually, to calculate the number of animals required, one must know the effect size (the estimated difference between the 2 groups), the estimated standard deviation (for continuous variables), the desired power (usually 80%), and the significance level (usually 5%; $p < 0.05$). Neglecting to identify the appropriate sample size in the planning stage or having misestimated the variables necessary to calculate it may potentially compromise the results, since the sample size could turn out to be too small when testing the outcomes in the final statistical analysis.[76] It was quite surprising to see through this systematic review some studies carrying out statistical comparisons on 2 arms each containing 3 to 4 rats. Thereby, we selected a cut-off according to the minimum value necessary for the applicability of statistical tests to evaluate the statistical bias. In total, 12 of the 18 studies did not meet the sufficient conditions to perform relevant statistical tests.[11,21,23,24,26,30,31,33,34,36,57,68] In addition, no study has detailed the calculation of the statistical power required. Therefore, it seems that this point

remains 1 of the essential criticisms because even if the most relevant evaluation method was chosen in most studies, few have carried out statistical tests with numbers allowing relevant comparisons. On the other hand, we considered the flow and timing bias, pointing out studies with an inappropriate time interval between the induction of the model and the last checkpoint using the reference standard method of pain assessment to evaluate long-term TMJ-related pain. Consequently, the studies concerned might not have demonstrated pain for at least 3 weeks given their lack of long-term evaluation of this parameter.

In conclusion, the results of this systematic review showed that the chemical method is currently a valuable option to obtain a long-term TMJOA-related pain model. CFA (50 µg of CFA in 50 µL) and MIA (50 mg of MIA in 50 µL) are the 2 main chemical agents injected into the TMJ to induce this specific condition. The practical implication of this finding is that these methods seem both to be the best options for evaluating sustained drug delivery systems. Nevertheless, it appears that induction protocols for TMJOA focused mainly on long-term histopathologic assessment, and few clinical data are available after 21 days of follow-up. Given that pain is 1 of the main symptoms of TMJOA and the future direction of mini-invasive treatments, experimental protocols should include long-term pain assessment in order to allow the evaluation of sustained drug delivery systems.

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Study	Rat Sex	Rat Breed	Weight	Method of Model Induction	Main Method of Nociception Assessment	Parameters Evaluated	Aim of the study
Abdalla et al., 2020	Males	Wistar	200-250g	45µl Fomalin 1.5%	Behaviour: head flinching and orofacial rubbing	Clinical + Biological + Histological	To assess the viability, effectiveness and longevity of a PL-based micellar system containing 15d-PGJ2 (PL-15d-PGJ2) in a formalin-induced inflammatory pain model
Caminski et al., 2020	Males	Wistar	250-350g	50 µl CFA	Behaviour: orofacial rubbing	Clinical + Histological	To evaluate the antinociceptive effects of the CTK 01512-2 toxin with acute, inflammatory, chronic, and neuropathic orofacial pain models, as well as measuring the in vitro and in vivo glutamate levels

Table 1

Cornelison et al., 2020	Males + Females	Sprague-Dawley	350-500g (Males) 250-300g (Females)	Mouth openning: 2N or 3,5N, 1h every day, 7 days	Head withdrawal (Von Frey aesthesiometer)	Clinical + Biological	To test the hypothesis that exposure of animals to the identified TMD risk factors of neck muscle tension, prolonged jaw opening, and female gender would promote persistent sensitization of trigeminal neurons and enhanced nociception indicative of chronic TMD
Ribeiro et al., 2020	Males	Wistar	180-250g	50µl of Formalin 1.5%	Behaviour: head flinching and orofacial rubbing	Clinical + Biological	To evaluate the antinociceptive and antiinflammatory effects of Caulerpa racemosa
Sperry et al., 2020	Females	Holtzman	243-285g	Mouth openning: 2N or 3,5N, 1h every day, 7 days	Head withdrawal (Von Frey aesthesiometer)	Clinical + Biological + Histological	To investigate hypoxia-inducible factors and hypoxia after TMJ loading inducing sustained (3.5 N loading) or resolving (2 N loading) pain
de Sousa et al., 2019	Males	Wistar	200-250g	10 µg mBSA in 10 µl	Head withdrawal (Von Frey aesthesiometer)	Clinical + Biological	To investigate the morphological changes of the synovial membrane during the development of TMJ arthritis, as well as the participation of canonical Wnt and NF-κB pathways in the progression of this chronic disease.
Ferrara-Jr et al., 2019	Males	Sprague-Dawley	250-370g	100 µg CFA in 100 µl	Head withdrawal (Von Frey aesthesiometer)	Clinical + Biological	To evaluate the effects of Photobiomodulation, as well as the mechanisms involved
Garattini et al., 2019	Males	Wistar	160-220g	50 µg CFA in 50 µl	Head withdrawal (Von Frey aesthesiometer)	Clinical + Biological	To investigate whether the endogenous Hydrogen sulfide production pathway contributes to arousal and maintenance of orofacial inflammatory pain
Jin et al., 2019	Males	Sprague-Dawley	220-280g	50 µg CFA in 50 µl	Head withdrawal (Von Frey aesthesiometer)	Clinical + Histological	To examinate morphologic alterations of satellite glial cells in trigeminal ganglion following TMJ inflammation and changes in Connexin 43, glial fibrillary acidic protein and sodium channel 1.7 expression
Sannajust et al., 2019	Males + Females	Sprague-Dawley	225-275g Males / 175-200g Females	MIA 16.6mg/ml or 80mg/ml	Head withdrawal (Von Frey aesthesiometer)	Clinical + Biological + Histological	To characterize sex differences in development of ongoing pain and central sensitization
Scarabelot et al., 2019	Males	Sprague-Dawley	250-300g	25 µg CFA in 50 µl	Head withdrawal (Von Frey aesthesiometer)	Clinical + Biological + Histological	To investigate the effect of Transcranial Direct Current Stimulation, a non-pharmacological therapy, on local mechanical hyperalgesia, and remote thermal hyperalgesia
Zhang et al., 2019	Females	Sprague-Dawley	198-271g	0.5mg MIA in 50µl	Head withdrawal (Von Frey aesthesiometer)	Clinical + Histological	To evaluate the effects of weekly intra-articular injections of mesenchymal stem cells exosomes in model of TMJOA, and to investigate the molecular mechanism of exosome-mediated cellular processes and restoration of matrix homeostasis in TMJ repair and regeneration
Alves et al., 2018	Males	Wistar	180-240g	50µl Fomalin 1.5%	Behaviour: orofacial rubbing	Clinical + Biological + Histological	To investigate the unexplored anti-nociceptive and anti-inflammatory efficacy of Abelmoschus esculentus lectin in model of formalin-induced temporomandibular joint inflammatory hypernociception
Bonfante et al., 2018	Males	Wistar	150-250g	mBSA + CFA	Behaviour: head flinching and orofacial rubbing	Clinical + Biological	To investigate if a persistent model of albumin-induced arthritis hypernociception in the TMJ results in the release of pronociceptive factors by microglial cells located in the trigeminal subnucleus caudalis associated with sensitization of central nervous system
Ito et al., 2018	Males	Sprague-Dawley	200-300g	50 µg CFA in 50 µl	Head withdrawal (Von Frey aesthesiometer)	Clinical + Biological + Histological	To determine the involvement of TNF-α signaling in the trigeminal ganglion in the mechanical hypersensitivity of the masseter muscle during TMJ inflammation
Santos et al., 2018	Males	Wistar	250g	50 µg CFA in 50 µl	Head withdrawal (Von Frey aesthesiometer)	Clinical + Biological	To evaluate the hypothesis that TMJ inflammation-induced hyperalgesia and allodynia responses are mediated by endogenous hydrogen sulfide
Sperry et al., 2018	Females	Holtzman	268 +/- 21g	Mouth openning: 0N, 2N or 3,5N, 1h	Head withdrawal (Von Frey aesthesiometer) + Rat	Clinical + Biological +	To assess Rat Grimace Scale ability to detect TMJ pain induced using repeated TMJ loading that produces moderate osteoarthritic pathology in the joint

				every day, 7 days	Grimace Scale	Histological	
Alves et al., 2017	Males	Wistar	160-220g	Zymosan (2mg in 40µl)	Head withdrawal (Von Frey aesthesiometer)	Clinical + Biological	To investigate the unexplored anti-nociceptive and antiinflammatory effects of strontium ranelate on the zymosan-induced inflammatory hypernociception in the TMJ by evaluating the IL-1-β and TNF-α levels after strontium ranelate treatment
Koop et al., 2017	Males	Sprague-Dawley	350-500g	50 µg CFA in 50 µl	Head withdrawal (Von Frey aesthesiometer)	Clinical + Biological	To investigate the role of neuropeptide calcitonin gene-related peptide and protein kinase A in promoting cellular changes in the spinal trigeminal nucleus and trigeminal ganglion, and nociceptive response to mechanical stimulation
Kartha et al., 2016	Females	Holtzman	245 +/- 16.2g	Mouth openning: 2N or 3,5N, 1h every day, 7 days	Head withdrawal (Von Frey aesthesiometer)	Clinical + Biological + Histological	To adapt an existing model of mechanically induced TMJOA, to induce persistent orofacial pain by altering only the jaw-opening force, and to measure the expression of common proxies of TMJOA, including degradation and inflammatory proteins, in the joint
Lacković et al., 2016	Males	Wistar	300-330g	50 µl CFA	Head withdrawal (Von Frey aesthesiometer)	Clinical + Biological + Histological	To investigate the reactivity of cranial dura to trigeminal pain and the mechanism of botulinum toxin type A action on dural neurogenic inflammation
Scarabelot et al., 2016	Males	Sprague-Dawley	250-300g	25 µg CFA in 50 µl	Head withdrawal (Von Frey aesthesiometer)	Clinical	To evaluate the effect of acute melatonin administration in the nociceptive response and central biomarkers levels in a chronic inflammatory orofacial pain model
Magni et al., 2015	Males	Sprague-Dawley	200-250g	60 µl CFA in 60µl	Head withdrawal (Von Frey aesthesiometer)	Clinical + Biological + Histological	To understand the role of specific P2Y receptors in trigeminal ganglion-related pain
Cady et al., 2014	Males	Sprague-Dawley	300-400g	50 µg CFA in 50 µl	Head withdrawal (Von Frey aesthesiometer)	Clinical + Biological + Histological	To investigate the role of orexins in modulation of trigeminal nerve activation in response to acute and prolonged inflammation of the TMJ, which occurs in TMJ disorders
do Val et al., 2014	Males	Wistar	160-220g	Zymosan (2mg in 40µl)	Head withdrawal (Von Frey aesthesiometer)	Clinical + Histological	To investigate the unexplored antinociceptive and anti-inflammatory efficacy of T. toxicaria in the model of zymosan-induced TMJ inflammatory hypernociception
Cavalcante et al., 2013	Males	Wistar	180-200g	10 µl of Carrageenan 5%	Head withdrawal	Clinical + Biological + Histological	To highlight the role of NMDA receptors in the hypernociceptive process in the TMJ region
Hatch et al., 2013	Males	Sprague-Dawley	200-250g	50 µg CFA in 50 µl	Head withdrawal (Semmes-Weinstein aesthesiometer)	Clinical + Biological + Radiological	To investigate whether there was a change in the proportion or intensity of hyperpolarization-activated cyclic nucleotide-gated channel immunoreactivity in TMJ primary afferent neurons following inflammation
Li et al., 2013	Males	Sprague-Dawley	180-225g	50 µg CFA in 50 µl	Head withdrawal (Von Frey aesthesiometer)	Clinical + Biological	To further explore the mGluR5 involvement in inflammatory pain of the trigeminal system, particularly in the TMJ, to determine the expression of mGluR5 protein, and to investigate whether CFA-induced TMJ inflammation causes changes in the levels of mGluR5 protein expression in the trigeminal ganglion
Bi et al., 2012	Females	Sprague-Dawley	200-220g	50 µl CFA in 50 µl	Head withdrawal (Von Frey aesthesiometer)	Clinical + Biological + Histological	To examine whether TMJ inflammation could influence the expression of Nav1.7 in trigeminal ganglion and whether blocking Nav1.7 function in trigeminal ganglion could attenuate the hyperalgesia of TMJ
Garrett et al., 2012	Males	Sprague-Dawley	175-200g	50 µg CFA in 50 µl	Head withdrawal (Von Frey aesthesiometer)	Clinical	To test the reliability and validity of a novel rat-holding device designed to be used in conjunction with the plantar test apparatus for studying nocifensive behavioral responses in an established model of TMJ pathology
Mountziaris et a., 2012	Males	Sprague-Dawley	250-300g	15 µg CFA in 20 µl	Meal pattern	Clinical + Biological + Histological	To investigate the in vivo therapeutic efficacy of an intra-articular controlled release system consisting of biodegradable poly(dl-lactic-co-glycolic acid) (PLGA) microparticles encapsulating anti-inflammatory small interfering RNA (siRNA), together with branched poly(ethylenimine) (PEI) as a transfecting agent, in a model

							of painful TMJ inflammation
Wang et al., 2012	Males	Sprague-Dawley	200-250g	25 µg CFA in 50 µl	Head withdrawal	Clinical + Histological	To test the hypothesis that glial activation would regulate the expression of the NMDAR subunit 1 in the trigeminal subnucleus caudalis (Sp5C) induced by TMJ inflammation
Wang et al., 2012	Females	Sprague-Dawley	180-200g	0.5mg MIA in 50µl	Head withdrawal	Clinical + Histological	To evaluate whether MIA injection into the upper compartment of the TMJ can be used to create a comprehensive TMJOA model
Ivanusic et al., 2011	Males	Sprague-Dawley	100-300g	1 µg CFA in 2 µl	Head withdrawal (Semmes-Weinstein aesthesiometer)	Clinical + Biological	To determine whether peripheral NMDA receptors are involved in inflammation-induced mechanical hypersensitivity of the TMJ
Burgos et al., 2010	Males	Wistar	200-250g	50µl of Formalin 2.5%	Behaviour: orofacial rubbing	Clinical + Biological	To evaluate the systemic effect of the cannabinoid agonist WIN 55,212-2 (WIN) and two antagonists (SR141716A and SR144528) on 2 different models of inflammatory orofacial pain ; To compare the effect of WIN on orofacial inflammatory pain with its effect in a model of spinal inflammatory pain (the paw formalin test) ; To compare the antinociceptive effectiveness of WIN with other well-known analgesic drugs such as morphine, indomethacin, and ketamine
Kramer et al., 2010	Males	Sprague-Dawley	250g	250 µg CFA in 50 µl	Meal pattern	Clinical + Biological	To show that a meal pattern can measure a persistent increase in TMJ nociception
Nicoll et al., 2010	Males	Wistar	397+/-93g	Mouth openning: 2N, 1h every day, 7 days	Head withdrawal (Von Frey aesthesiometer)	Clinical + Histological	To develop a model of TMJ pain and to characterize in it the development and temporal response of behavioral hypersensitivity as well as to evaluate if and to what extent a loading protocol is associated with histological changes in the TMJ consistent with osteoarthritic pathology
Villa et al., 2010	Males	Sprague-Dawley	200-250g	50 µg CFA in 50 µl	Head withdrawal (Von Frey aesthesiometer)	Clinical + Biological + Histological	To characterize the reaction of peripheral nervous system and central nervous system glial cells to the injection of CFA into the TMJ
Bonjardim et al., 2009	Males	Wistar	200-300g	Mustard oil 1.5, 2.5 or 4.5%	Behaviour: head flinching and orofacial rubbing	Clinical	To improve the previously reported Mustard oil-induced TMJ nociception model by reducing the concentration of the Mustard oil injected and to investigate the potential analgesic activity of systemic dipyrone and tramadol on the nociceptive behavioral responses induced by TMJ application of the Mustard oil
Denadai-Souza et al., 2009	Males	Wistar	250-300g	10 µl of Carrageenan 5%	Head withdrawal	Clinical	To determine the time course of some vascular and cellular events (such as vascular permeability, leukocyte influx, TNF-α and IL-1-β production and pain) secondary to carrageenan-induced TMJ arthritis, and the putative involvement of the tachykinin receptor NK1 in the mediation of these events
Schütz et al., 2009	Males + Females	Wistar	NA	100 µl CFA	Behaviour: orofacial rubbing + Sleep pattern + Meal pattern	Clinical + Biological	To investigate the effect of orofacial pain upon the behavioral and sleep patterns of both sexes and of females in different phases of the estrous cycle
Wang et al., 2009	Males	Sprague-Dawley	200-300g	50 µg CFA in 50 µl	Head withdrawal	Clinical + Biological + Histological	To examine the hypothesis that the upregulation of NR1 in Sp5c following inflammation of the unilateral TMJ region would be regulated by IL-6 and NF-κB
Lee et al., 2008	Males	Sprague-Dawley	220-280g	50µl of Formalin 5%	Beahviour: orofacial rubbing	Clinical + Biological	To evaluate the hypothesis that central cannabinoid might modulate the antinociceptive roles of mGluRs in formalin-induced TMJ nociception
Schütz et al., 2007	Males	Wistar	NA	100 µl CFA	Sleep pattern	Clinical	To examine the role of cyclo-oxygenase-2 in that painful condition, while trying to establish the extent to which this enzyme influences the sleep patterns of animals, both when subjected to this experimental model and when merely manipulated
Okamoto et al.,	Males	Sprague-	150-250g	25 µg CFA in 50 µl	Paw withdrawal (Von Frey	Clinical +	To investigate whether persistent TMJ inflammation affects nocifensive behavioral responses evoked by

2006		Dawley			aesthesiometer)	Biological	formalin injection into the hindpaw or withdrawal thresholds of mechanical stimulation to the hindpaw
Rodrigues et al. 2006	Males	Wistar	200-300g	Carrageenan (100 µg in 15 µl)	Behaviour: head flinching and orofacial rubbing	Clinical + Biological	To show that administration of indomethacin before the initiation of inflammation would diminish the TMJ hyperalgesia
Ahn et al. 2005	Males	Sprague-Dawley	220-280g	50µl of Formalin 5%	Behaviour: orofacial rubbing	Clinical + Biological	To investigate the effects of intraarticular or intracisternal injection of IL-1-**β** on the formalin induced behavioral responses in the TMJ of freely moving rats
Gameiro et al., 2005	Males	Wistar	200-230	Stress exposure + Formalin	Behaviour: head flinching and orofacial rubbing	Clinical + Biological	To evaluate the effects of acute and chronic restraint stress on the nociceptive responses induced by TMJ formalin test
Guan e tal., 2005	Females	Sprague-Dawley	200-225g	10 µg CFA in 50 µl	Meal pattern	Clinical + Biological + Histological	To test the effect of estrogen on TMJ swelling and monocytic cell number
Kerins et al., 2005	Males	Sprague-Dawley	175g	50 µg CFA in 50 µl	Meal pattern	Clinical	To test the efficacy of COX-2-I anti-inflammatory drug Rofecoxib on TMJ inflammation
Okamoto et al., 2005	Males	Sprague-Dawley	150-200g	25 µg CFA in 50 µl	Behaviour: orofacial rubbing	Clinical	To evaluate the effect of local administration of the 5HT2AR antagonist, ketanserin, or the 5HT1AR antagonist, propranolol, on the orofacial nocifensive behavior evoked by the injection of formalin
Oliveira et al., 2005	Males	Wistar	150-250g	Carrageenan (100 µg in 25 µl)	Behaviour: head flinching and orofacial rubbing	Clinical	To investigate whether activation of P2X receptors located within the TMJ region induces nociception
Takeda et al., 2005	Males	Wistar	280 - 320g	50 µg CFA in 50 µl	Head withdrawal (Von Frey aesthesiometer)	Clinical + Biological + Electrophysiological	To investigate whether the local release of substance P from the trigeminal root ganglion neurons innervating TMJ modulates the excitability of β-trigeminal root ganglion neurons innervating the facial skin via the paracrine mechanism
Kerins et al., 2004	Males	Sprague-Dawley	NA	15 µg CFA in 50 µl	Meal pattern	Clinical	To confirm previous findings and extend them by using Rofecoxib, a selective cyclooxygenase-2 inhibitor/
Gameiro et al., 2003	Males	Wistar	200-300g	50µl of Formalin 1.5%	Behaviour: head flinching and orofacial rubbing	Clinical	To evaluate the effect of acute and chronic administration of ethanol and ethanol withdrawal on the pain
Hartwig et al., 2003	Males	Sprague-Dawley	300-400g	Mustard oil 1, 10 or 20%	Behaviour: chewing + head flinching and orofacial rubbing	Clinical + Biological	To describe and quantify spontaneous noxious stimulus-evoked behaviors in awake rats induced by articular injection of mustard oil
Kerins et al., 2003	Males + Females	Sprague-Dawley	190g (Males) 230g (Females)	10 µg CFA in 50 µl	Meal pattern	Clinical + Biological	To further validate our animal model by determining whether aspects of CFA-induced TMJ inflammation/pain are reversed with Ibuprofen treatment
Schütz et al., 2003	Males	Sprague-Dawley	NA	100 µl CFA	Sleep pattern	Clinical + Biological	To assess an experimental behavioral model of orofacial pain induced by Freund's adjuvant applied into the TMJ while evaluating the sleep pattern and the effect of indomethacin
Roveroni et al., 2001	Males	Wistar	150-250g	50µl of Formalin 0.5, 1.5, 2.5 or 5%	Behaviour: chewing + head flinching and orofacial rubbing	Clinical	To apply concentrations of formalin into the TMJ region to develop an experimental behavioral model of TMJ pain and verify if the model proposed is sensitive to morphine and to the hydrophilic lidocaine derivative, QX-314 (2%).

Table 2

Study	Rat Sex	Rat Breed	Weight	Method of Model Induction	Main Method of	Number of	Painful symptoms

					Nociception Assessment	rats	duration
Abdalla et al., 2020	Males	Wistar	200-250g	45µl Fomalin 1.5%	Behaviour: head flinching and orofacial rubbing	6/group	14 days
de Sousa et al., 2019	Males	Wistar	200-250g	10 µg mBSA in 10 µl	Head withdrawal (Von Frey aesthesiometer)	6/group	35 days
Ferrara-Jr et al., 2019	Males	Sprague-Dawley	250-370g	100 µg CFA in 100 µl	Head withdrawal (Von Frey aesthesiometer)	6-11/group	21 days
Garattini et al., 2019	Males	Wistar	160-220g	50 µg CFA in 50 µl	Head withdrawal (Von Frey aesthesiometer)	6/group	14 days
Sannajust et al., 2019	Males + Females	Sprague-Dawley	225-275g (Males) 175-200g (Females)	MIA 16.6mg/ml or 80mg/ml	Head withdrawal (Von Frey aesthesiometer)	9-12/group	14 days
Scarabelot et al., 2019	Males	Sprague-Dawley	250-300g	25 µg CFA in 50 µl	Head withdrawal (Von Frey aesthesiometer)	? (N=52 for 6 groups)	15 days
Zhang et al., 2019	Females	Sprague-Dawley	198-271g	0.5mg MIA in 50µl	Head withdrawal (Von Frey aesthesiometer)	8/group	56 days
Santos et al., 2018	Males	Wistar	250g	50 µg CFA in 50 µl	Head withdrawal (Von Frey aesthesiometer)	8/group	10 days
Koop et al., 2017	Males	Sprague-Dawley	350-500g	50 µg CFA in 50 µl	Head withdrawal (Von Frey aesthesiometer)	5-7/group	21 days
Kartha et al., 2016	Females	Holtzman	245 +/- 16.2g	3,5N mouth openning (1h every day during 7 days)	Head withdrawal (Von Frey aesthesiometer)	10-12/group	14 days
Scarabelot et al., 2016	Males	Sprague-Dawley	250-300g	25 µg CFA in 50 µl	Head withdrawal (Von Frey aesthesiometer)	? (N=35 for 6 groups)	14 days
Magni et al., 2015	Males	Sprague-Dawley	200-250g	60 µg CFA in 60µl	Head withdrawal (Von Frey aesthesiometer)	4/group	10 days
Cady et al., 2014	Males	Sprague-Dawley	300-400g	50 µg CFA in 50 µl	Head withdrawal (Von Frey aesthesiometer)	4/group	14 days
Garrett et al., 2012	Males	Sprague-Dawley	175-200g	50 µg CFA in 50 µl	Head withdrawal (Von Frey aesthesiometer)	6/group	14 days

Wang et al., 2012	Females	Sprague-Dawley	180-200g	0.5mg MIA in 50µl	Head withdrawal	3/group	21 days
Kramer et al., 2010	Males	Sprague-Dawley	250g	250 µg CFA in 50 µl	Meal pattern	13-14/group	19 days to 42 days
Nicoll et al., 2010	Males	Wistar	397+/-93g	2N mouth openning (1h every day during 7 days)	Head withdrawal (Von Frey aesthesiometer)	4-8/group	15 days
Okamoto et al., 2006	Males	Sprague-Dawley	150-250g	25 µg CFA in 50 µl	Paw withdrawal (Von Frey aesthesiometer)	12/group	14 days

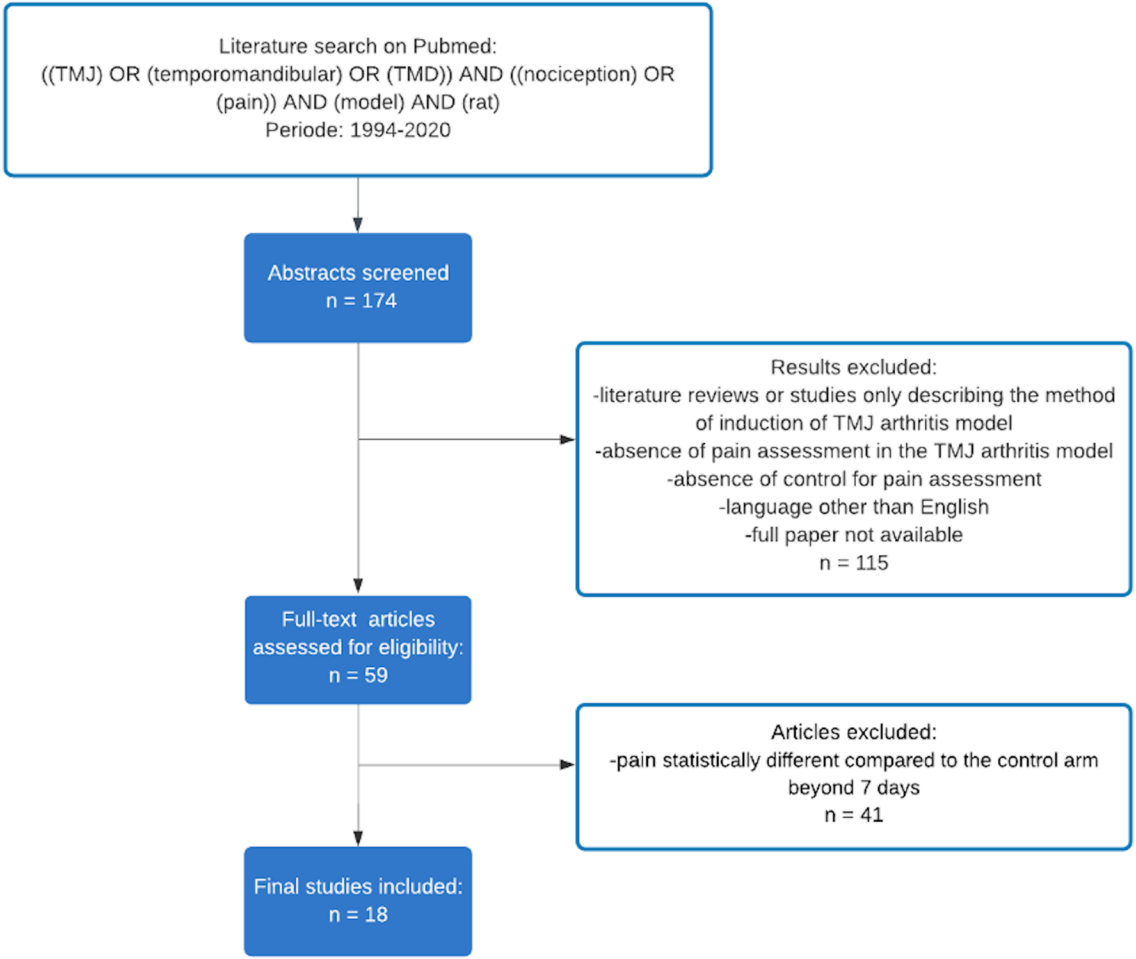
Tables and Figures:

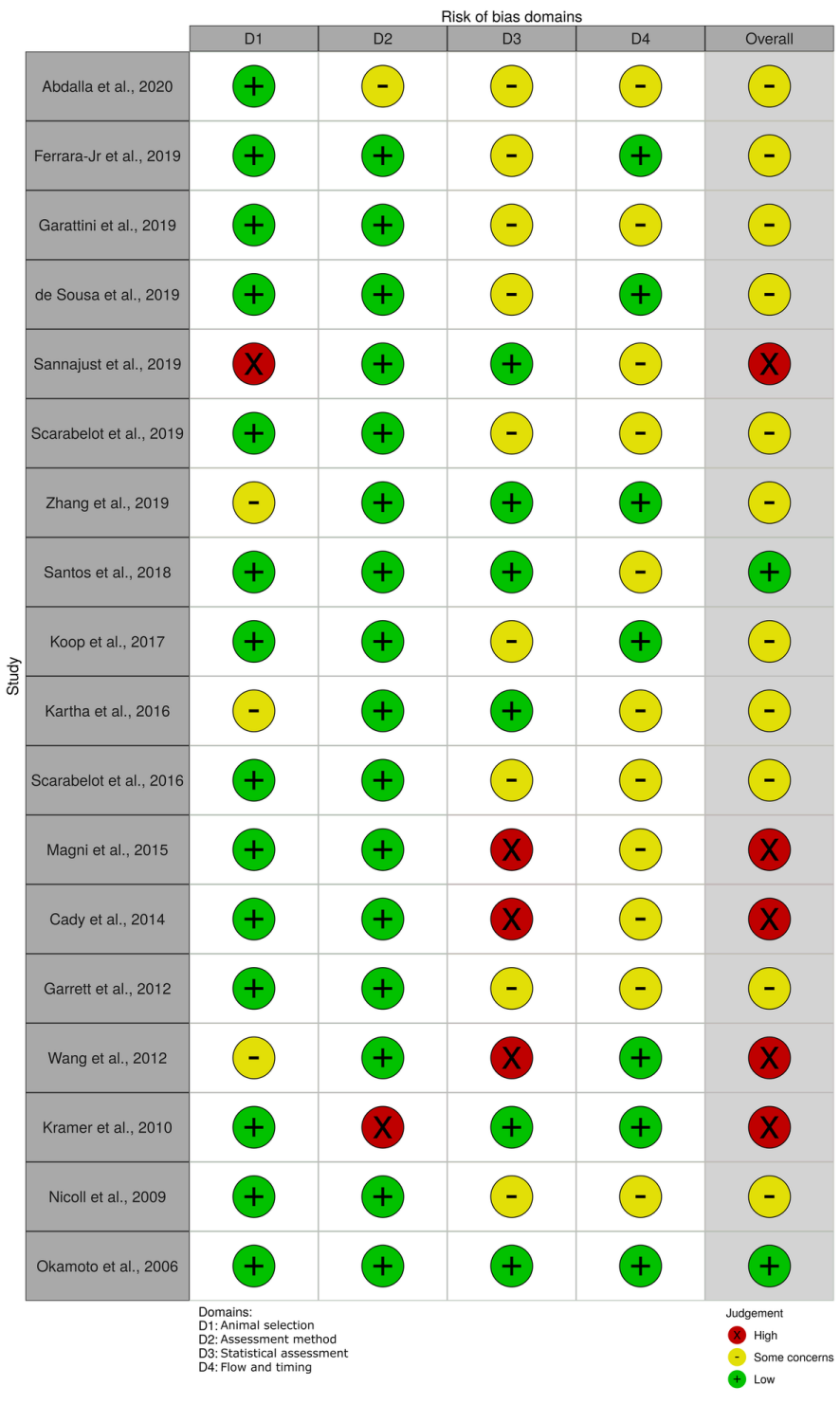
Table 1: Characteristics of eligible studies including a temporomandibular joint osteoarthritis-related pain model.

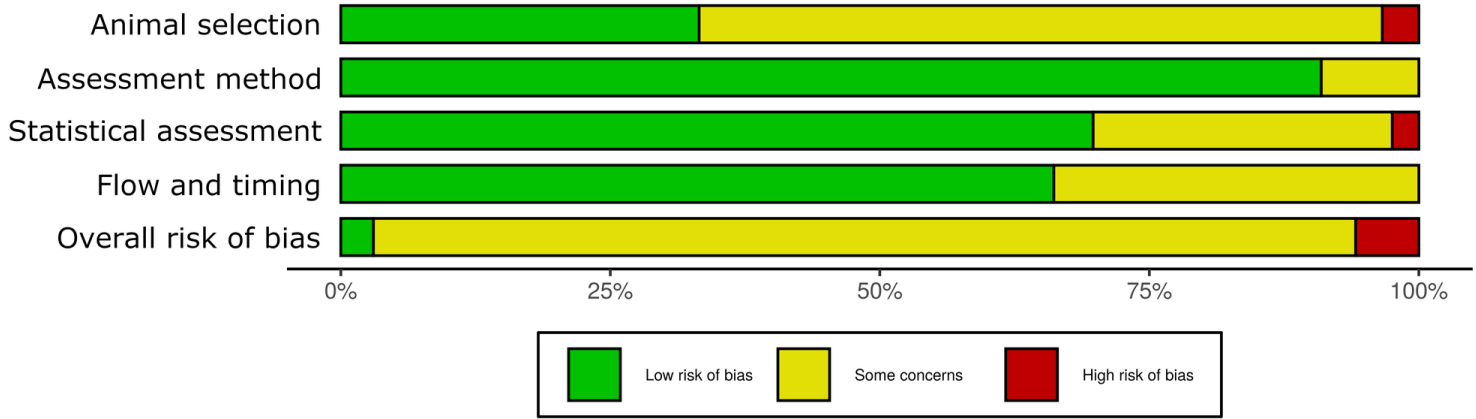
Table 2: Characteristics of studies including a long-term temporomandibular joint osteoarthritis-related pain model.

Figure 1: Flowchart following PRISMA statement.

Figure 2: Quality assessments of included studies using QUADAS-2 tool. A - Risk of bias summary through a tabular representation resuming the authors' judgments about the risk of each bias item for each included study. B – Risk of bias graph in which items presented as percentages across all included studies.


Literature search on Pubmed:
((TMJ) OR (temporomandibular) OR (TMD)) AND ((nociception) OR (pain)) AND (model) AND (rat)
Periode: 1994-2020
Abstracts screened
n = 174
Results excluded:
-literature reviews or studies only describing the method of induction of TMJ arthritis model
-absence of pain assessment in the TMJ arthritis model
-absence of control for pain assessment
-language other than English
-full paper not available
n = 115
Full-text articles assessed for eligibility:
n = 59
Articles excluded:
-pain statistically different compared to the control arm beyond 7 days
n = 41
Final studies included:
n = 18


Risk of bias domains
D1
D2
D3
D4
Overall
Study
Abdalla et al., 2020
Ferrara-Jr et al., 2019
Garattini et al., 2019
de Sousa et al., 2019
Sannajust et al., 2019
Scarabelot et al., 2019
Zhang et al., 2019
Santos et al., 2018
Koop et al., 2017
Kartha et al., 2016
Scarabelot et al., 2016
Magni et al., 2015
Cady et al., 2014
Garrett et al., 2012
Wang et al., 2012
Kramer et al., 2010
Nicoll et al., 2009
Okamoto et al., 2006
Domains:
D1: Animal selection
D2: Assessment method
D3: Statistical assessment
D4: Flow and timing
Judgement
High
Some concerns
Low


Animal selection
Assessment method
Statistical assessment
Flow and timing
Overall risk of bias
0%
25%
50%
75%
100%
Low risk of bias
Some concerns
High risk of bias