

## Supplemental Digital Content (SDC)

### Methods

#### *Search Strategies*

Database(s): **Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily 1946 to Present**

Search Strategy:

#	Searches	Results
1	exp animal experimentation/ or exp models, animal/ or animals/ or mammals/ or vertebrates/ or exp fishes/ or exp amphibia/ or exp reptiles/ or exp birds/ or exp hyraxes/ or exp marsupialia/ or exp monotremata/ or exp scandentia/ or exp chiroptera/ or exp carnivora/ or exp cetacea/ or exp Xenarthra/ or exp elephants/ or exp insectivora/ or exp lagomorpha/ or exp rodentia/ or exp sirenia/ or exp Perissodactyla/ or primates/ or exp strepsirhini/ or haplorhini/ or exp tarsii/ or exp platyrrhini/ or catarrhini/ or exp cercopithecidae/ or gorilla gorilla/ or pan paniscus/ or pan troglodytes/ or exp pongo/ or exp hylobatidae/ or hominidae/	6324472
2	Drug Evaluation, Preclinical/	47105
3	((animal\$1 or chordata or vertebrate* or fish\$2 or amphibian* or amphibium* or reptile\$1 or bird\$1 or mammal* or dog or dogs or canine\$1 or cat or cats or hyrax* or marsupial* or monotrem* or scandentia or bat or bats or carnivor* or cetacea or edentata* or elephant* or insect or insects or insectivore or lagomorph* or rodent\$2 or mouse or mice or murine or murinae or muridae or rat or rats or pig or pigs or piglet\$1 or swine or rabbit\$1 or sheep\$1 or goat\$1 or horse\$1 or equus or cow or cows or cattle or calf or calves or bovine or sirenia or ungulate\$1 or primate\$1 or prosimian* or haplorhini* or tarsiiform* or simian* or platyrrhini or catarrhini or cercopithecidae or ape or apes or hylobatidae or hominid* or chimpanzee* or gorilla* or orangutan* or monkey or monkeys or ape or apes) adj5 (trial\$1 or study or studies or experiment* or laborator\$3 or research*)).tw.	609521
4	(preclinical* or pre-clinical*).tw.	94241
5	or/1-4	6478259

6	(pregabalin or lyrica or "CI 1008" or CI-1008 or CI1008 or "3-isobutyl GABA").mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	3461
7	5 and 6	585
8	remove duplicates from 7	585

Database(s): **Embase Classic+Embase** 1947 to 2018 November 05

Search Strategy:

#	Searches	Results
1	exp animal experiment/ or exp animal model/ or animal/ or exp invertebrate Chordata/ or exp experimental animal/ or exp transgenic animal/ or exp male animal/ or exp female animal/ or exp juvenile animal/ or vertebrate/ or exp fish/ or exp amphibia/ or exp reptile/ or exp bird/ or mammal/ or exp hyrax/ or exp marsupial/ or exp monotremate/ or exp scandentia/ or placental mammals/ or exp bat/ or exp carnivora/ or exp cetacea/ or exp edentata/ or exp elephant/ or exp insectivora/ or exp lagomorph/ or exp rodent/ or exp sirenia/ or exp ungulate/ or primate/ or exp prosimian/ or haplorhini/ or exp tarsiiform/ or simian/ or exp platyrrhini/ or catarrhini/ or exp cercopithecidae/ or ape/ or exp hylobatidae/ or hominid/ or exp chimpanzee/ or exp gorilla/ or exp orangutan/	6338583
2	((animal\$1 or chordata or vertebrate* or fish\$2 or amphibian* or amphibium* or reptile\$1 or bird\$1 or mammal* or dog or dogs or canine\$1 or cat or cats or hyrax* or marsupial* or monotrem* or scandentia or bat or bats or carnivor* or cetacea or edentata* or elephant* or insect or insects or insectivore or lagomorph* or rodent\$2 or mouse or mice or murine or murinae or muridae or rat or rats or pig or pigs or piglet\$1 or swine or rabbit\$1 or sheep\$1 or goat\$1 or horse\$1 or equus or cow or cows or cattle or calf or calves or bovine or sirenia or ungulate\$1 or primate\$1 or prosimian* or haplorhini* or tarsiiform* or simian* or platyrrhini or catarrhini or cercopithecidae or ape or apes or hylobatidae or hominid* or chimpanzee* or gorilla* or orangutan* or monkey or monkeys or ape or apes) adj5 (trial\$1 or study or studies or experiment* or laborator\$3 or research*)).tw.	820571
3	(preclinical* or pre-clinical*).tw.	145198

4	or/1-3	6566708
5	(pregabalin or lyrica or "CI 1008" or CI-1008 or CI1008 or "3-isobutyl GABA").mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]	13052
6	4 and 5	1043
7	remove duplicates from 6	1020

Database(s): **BIOSIS Previews** 1969 to 2017

Search Strategy:

#	Searches	Results
1	((animal\$ adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (chordata adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (vertebrate* adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (fish adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (fishes adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (amphibian* adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (reptile\$ adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (bird\$ adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (mammal* adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (dog adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (dogs adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (canine\$ adj5 (trial\$1 or study or studies or experiment* or laborator* or research*))).ti,ab,bo,mc,cc,st,ds,cb,gn,sq,ge,tm,mq,ps,mi.	255763
2	((cat adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (cats adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (hyrax* adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (marsupial* adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (monotrem* adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (scandentia adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (bat adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (bats adj5 (trial\$1 or study or studies or experiment* or laborator* or	13672

	research*)) or (carnivor* adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (cetacea adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (edentata* adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (elephant* adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)))).ti,ab,bo,mc,cc,st,ds,cb,gn,sq,ge,tm,mq,ps,mi.	
3	((insect adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (insects adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (insectivore adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (lagomorph* adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (rodent adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (rodents adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (rodentia adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (mouse adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (mice adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (murine adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (murinae adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (muridae adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)))).ti,ab,bo,mc,cc,st,ds,cb,gn,sq,ge,tm,mq,ps,mi.	132175
4	((rat adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (rats adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (pig adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (pigs adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (piglet\$ adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (swine adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (rabbit\$ adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (bovine adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (sirenia adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (ungulate adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (primate adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (prosimian* adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (haplorhini* adj5 (trial\$1	178844

	or study or studies or experiment* or laborator* or research*)) or (tarsiiform* adj5 (trial\$1 or study or studies or experiment* or laborator* or research*))).ti,ab,bo,mc,cc,st,ds,cb,gn,sq,ge,tm,mq,ps,mi.	
5	((simian* adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (platyrrhini adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (sheep\$ adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (goat\$ adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (horse\$ adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (equus adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (cow adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (cows adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (cattle adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (calf adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (calves adj5 (trial\$1 or study or studies or experiment* or laborator* or research*))).ti,ab,bo,mc,cc,st,ds,cb,gn,sq,ge,tm,mq,ps,mi.	38588
6	((platyrrhini adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (catarrhini adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (cercopithecidae adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (ape adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (apes adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (hylobatidae adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (hominid* adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (chimpanzee* adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (gorilla* adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (orangutan* adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (monkey adj5 (trial\$1 or study or studies or experiment* or laborator* or research*)) or (monkeys adj5 (trial\$1 or study or studies or experiment* or laborator* or research*))).ti,ab,bo,mc,cc,st,ds,cb,gn,sq,ge,tm,mq,ps,mi.	11228
7	1 or 2 or 3 or 4 or 5 or 6	593703
8	(preclinical\$ or "pre-clinical\$").ti,ab,bo,mc,cc,st,ds,cb,gn,sq,ge,tm,mq,ps,mi.	68176
9	7 or 8	653082

10	(pregabalin or lyrica or "CI 1008" or CI-1008 or CI1008 or "3-isobutyl GABA").mp. [mp=abstract, original language book title (non-english), book title (english), title, heading words]	2427
11	9 and 10	131
12	remove duplicates from 11	131

## Protocol

The following protocol was prespecified and timestamped on March 4, 2017 (<https://originstamp.org/s/b8d6abcb70cfd7aa953bbb648d7df3a953edff35518c924599f81c77d fb7fe58>).

### TITLE

Validity Threats in Preclinical Pain Research: A Systematic Review and Meta-Analysis of Pregabalin Studies

### INTRODUCTION

Approximately one in five adults suffer from chronic pain worldwide, and another one in ten adults are diagnosed with chronic pain each year. Clinical trials testing pain medications are practically and scientifically challenging; they are also expensive and potentially burdensome for patients. Preclinical experiments thus provide a key evidentiary basis for launching trials.

Despite greatly increased academic and industry resources aimed at basic, translational, and clinical pain research in the recent past, only a handful of drugs have been approved for use as analgesics. This has led many commentators to suggest areas needing improvement in pain research. For example, systematic reviews of pain preclinical research suggest deficient reporting and the use of study designs associated with random variation and high risk of bias. There has been a call for the use of novel models and readouts and more emphasis on pharmacokinetic-pharmacodynamic relationships. While the poor predictive validity of animal behavior models used in preclinical pain research might be one explanation, the design and conduct of clinical trials, and limited testing of clinical toxicity might also be to blame for translational failure.

### PRIMARY OBJECTIVE

Our primary objective is to explore the influence of study quality and design characteristics on the efficacy of pregabalin in behavioral models of pain.

### SECONDARY OBJECTIVE

We will also test for the presence of publication bias in pregabalin preclinical pain studies.

## HYPOTHESIS

We expect to see inconsistent use of experimental practices aimed at a) minimizing bias and strengthening causal inferences (internal validity), and b) maximizing the correspondence between experimental setup and clinical scenarios (construct validity).

We expect to see limited replication in different models of pain (external validity).

We expect to identify great variability in pooled preclinical effect sizes.

We expect to identify the presence of publication bias in preclinical pain studies and a corresponding overestimation of effect.

## METHOD OVERVIEW

We will conduct a systematic review and meta-analysis of pregabalin (Lyrica) preclinical studies.

### *Systematic Review of Full Publications*

We will search biomedical databases (MEDLINE, Embase, BIOSIS) and manually screen the results to identify all published, *in vivo* animal studies testing the analgesic properties of pregabalin that meet the inclusion criteria outlined below.

## SAMPLE AND SAMPLING METHODS

### Eligibility:

*Inclusion criteria:* a) original reports, b) full-text articles, c) English language, d) containing at least one experiment measuring pain response in live, non-human animals, and e) employing pregabalin in a control, comparator, or experimental context. For inclusion in the quantitative meta-analysis, additional criteria for preclinical experiments were a) reported sample size, b) included time-course data, and c) measured variance as SD of the mean or SEM.

### Sample size rationale / calculations:

Our sample will be saturated and include all published, full-text preclinical efficacy studies testing pregabalin against pain.

## TREATMENT / DATA COLLECTION

### *Extraction*

Our extractions have been adapted from methods previously described. The following variables will be extracted from each eligible publication:

- a) Study-level elements: conflict of interest disclosure, funding type, total number of animals used, publication date, etc.
- b) Experiment-level elements: sample size, randomization of treatment allocation, blinding of outcome assessment, inferential statistic used, removal of animals throughout the experiment, species, sex, weight, age, strain, pain assay, drug administration schedule, and presence of combination and comparator arms.
- c) Effect size elements: sample size, mean measure of treatment effect, and SDM/SEM.

For each experiment, data presented graphically will be extracted using the graph digitizer software GraphClick (Arizona Software).

Our full codebook is available on request.

Two independent coders will extract a subsample of eligible studies and inter-coder reliability will be assessed. Both coders will undergo a period of training before extraction to ensure consistent quality. Disagreements will be reconciled by discussion between coders. A single coder will then carry out the remaining extractions. Extractions will be completed using Numbat Meta-Analysis Extraction Manager. All graphs will be plotted, and all statistical inferences will be performed using R v.3.3.1 or higher.

## OUTCOME

We will calculate preclinical effect size, and its associated variance, across a number of experimental conditions, using group-level data presented in publication figures. We have developed a technique for calculating preclinical effect size by measuring the area under the time-course curve (AUC) using the trapezoidal rule, testing all reported doses of pregabalin at 30, 60, 120 and 180 minutes after drug administration.

## PRIMARY ANALYSIS

### I. Meta-Analysis

The percentage of maximum possible effect (MPE) of pregabalin will be calculated for each experiment, at each reported dose and across a number of experimental durations (30, 60, 120 and 180). Pooled effect sizes will then be calculated using a random-effects model.

Exploratory sub-analyses will be performed to test the impact of experimental design practices on pooled effect sizes. Experiments will be stratified by internal and construct validity practices, and pooled effect sizes will be compared. We will also stratify studies by external validity score (i.e., the number of

etiology/assay combinations tested per study) to rule out the possibility that treatment effects are attributable to idiosyncrasies in model systems.

A number of additional exploratory sub-analyses will be performed:

*A. Species*

Pooled effect sizes for rats vs. mice will be compared.

*B. Sex*

Pooled effect sizes for male vs. female vs. mixed animal populations will be compared.

*C. Schedule*

Pooled effect sizes for preventive vs. ameliorative drug schedules will be compared.

*D. Pain Etiology*

Pooled effect sizes for neuropathic etiology vs. inflammatory vs. nociceptive vs. disease model will be compared.

*E. Pain Assay*

Pooled effect sizes for mechanical vs. thermal vs. cold vs. spontaneous pain measures will be compared.

*E. Industry Funding*

Pooled effect sizes for industry funded vs. non-industry funded studies will be compared.

*F. Conflict of Interest*

Pooled effect sizes for studies with and without a conflict of interest will be compared.

*G. Dose*

Pooled effect sizes for studies of increasing dose will be compared.

*H. Pregabalin as Experimental vs. Research Reagent*

We will compare effect sizes when pregabalin is used as an experimental agent vs. when it is used as research reagent (i.e., as an active comparator).

## SECONDARY ANALYSIS

*I. Study Demographics*

We will present basic demographic information, including total number of animals tested, sponsor, publication date, location of corresponding author, presence of conflict of interest, etc., for each preclinical study.

## *II. Experimental Practices*

We will extract and present key information about internal, construct and external validity design elements for each preclinical experiment.

## *III. AERO Diagrams*

We will track the preclinical development of pregabalin over time by producing a series of AERO (Accumulation of Evidence and Research Organization) diagrams organized by publication date. AERO nodes will be colored based on whether the preclinical study is positive, inconclusive or negative. Different shapes will also be used to indicate pain etiology, assay used, or sponsor.

## *IV. Publication Bias*

Publication bias will be evaluated using funnel plots with Duval and Tweedie's trim and fill method of estimating missing studies and adjusting the point estimate. Funnel plots take advantage of the fact that smaller studies are prone to large random variation. Under-representation of smaller studies showing nonpositive effects can suggest publication bias.

## *V. Post-Hoc Analysis*

A number of exploratory post-hoc analyses may also be performed.

## ETHICS

This study is based on published reports and does not involve human subjects.