Concordance and incongruence in preclinical anxiety models: systematic review and meta-analyses

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Abstract

Rodent defense behavior assays have been widely used as preclinical models of anxiety to study possibly therapeutic anxiety-reducing interventions. However, some proposed anxiety-modulating factors—genes, drugs and stressors—have had discordant effects across different studies. To reconcile the effect sizes of purported anxiety factors, we conducted systematic review and meta-analyses of the literature on ten anxiety-linked interventions, as examined in the elevated plus maze, open field and light-dark box assays. Diazepam, 5-HT1A receptor gene knockout and overexpression, SERT gene knockout and overexpression, pain, restraint, social isolation, corticotropin-releasing hormone and Crhr1 were selected for review. Eight interventions had statistically significant effects on rodent anxiety, while Htr1a overexpression and Crh knockout did not. Evidence for publication bias was found in the diazepam, Htt knockout, and social isolation literatures. The Htr1a and Crhr1 results indicate a disconnect between preclinical science and clinical research. Furthermore, the meta-analytic data confirmed that genetic SERT anxiety effects were paradoxical in the context of the clinical use of SERT inhibitors to reduce anxiety.

Keywords

anxiety, defense, behavior, rodent, stress, serotonin, meta-analysis, pain, isolation, receptor, transporter, corticotropin releasing hormone

Highlights

- Meta-analysis shows eight rodent anxiety factors have at least moderate effects.
- Publication bias affects four of the anxiety interventions.
- Preclinical rodent anxiety results appear disconnected from clinical efforts.
- Serotonin transporter gene lesion effects are paradoxical with reuptake inhibitors clinical use.

1. Introduction

The anxiety disorders are among the costliest classes of mental disorders, with regard to both morbidity and economic cost (Baldwin et al., 2014; DiLuca and Olesen, 2014). Development of anxiety-reducing (anxiolytic) drugs has been a major focus of the pharmaceutical industry and academic neuropsychiatric research, though no new drug types have been adopted since the introduction of selective serotonin uptake inhibitors (SSRIs) and other antidepressants for the treatment of anxiety disorders (Griebel and Holmes, 2013; Tone, 2009). Anxiety research relies on similarities between human emotional behavior and behaviors in animals (Darwin, 1998), specifically rat and mouse (Prut and Belzung, 2003). While there are many rodent behavioral paradigms that aim to model anxious behavior, three **a**nxiety-**r**elated **de**fense **b**ehavior (ARDEB) assays that specifically aim to measure rodent anxiety have been widely adopted, also referred to as 'approach-avoidance conflict tests': the elevated plus maze (EPM), the light-dark box (LD) and the open field (OF), the first, second and fifth most widely used rodent anxiety assays, respectively (Griebel and Holmes, 2013). All three assays use an arena that contains a sheltered domain (e.g., the closed arms in EPM) and an exposed region. It is believed that an animal's avoidance of the exposed portions of the chamber reports on anxiety-like brain states. The ARDEB assays are accepted as preclinical assays of anxiety disorders, by reference to classic studies that tested their predictive validity with panels of drugs known to have anxiety-modulating effects in humans (Crawley and Goodwin, 1980; Pellow et al., 1985; Simon et al., 1994).

Rodent research has been implicated in the largely frustrated efforts to develop new types of anxiolytics (Griebel and Holmes, 2013). The literature regarding defense behaviors is contradictory about the size and even the direction of many interventions that are proposed to be anxiolytic or anxiogenic (together 'anxiotropic') (Griebel and Holmes, 2013; Prut and Belzung, 2003). This is true even for some anxiety-related factors with major clinical relevance, such as the serotonin transporter (SERT/*Htt*), the target of the SSRIs. As with the assessment of clinical anxiety interventions (Baldwin et al., 2014), a solid preclinical evidence base is necessary to guide decisions about further research and therapeutic development (Vesterinen et al., 2014). To better understand the widespread discordance in rodent anxiety studies, we conducted a quantitative review of the effect of purported anxiety factors on rodent ARDEB. The primary aim of this study was to examine the relevance of these factors and to estimate the magnitude of their effects on rodent anxiety. A secondary goal of this analysis was to examine patterns in ARDEB factor evidence: gaps in the literature, the extent of standardization/heterogeneity and publication bias. Synthesizing the data on anxiety-targeted interventions might also assist in understanding why these assays have not led to new therapies. Once confirmed by meta-analysis, effective anxiotropic interventions can be adopted as benchmarks against which to validate

new rodent assays and/or more tractable model animal species (e.g. *Drosophila* and zebrafish).

2. Materials and Methods

2.1 Literature review

We identified genes, drugs and environmental interventions that had been proposed to be involved in anxiety with a literature search of anxiety review articles. From two histories of anxiety research (Griebel and Holmes, 2013; Tone, 2009), a list of ten anxiotropic interventions were chosen to be included in the systematic review, either due to their clinical relevance (e.g., diazepam, Htt), their role as an example of a class of proposed anxiety-related factors (e.g., isolation), or their connection to possible forthcoming therapeutics (e.g. Crh). A systematic review was conducted to identify published articles addressing experimental outcomes in rodents from the EPM, OF, or LD assays for these interventions (Figure 1). The literature for each genetic, pharmacological or environmental intervention was identified by a search of PubMed and EMBASE using specific search phrases (Table 1). The selective serotonin reuptake inhibitors (SSRIs), which have clinical importance (Baldwin et al., 2014), a very large number of studies conducted on them (Griebel and Holmes, 2013), and controversial efficacy (Kirsch et al., 2008) are the subject of a separate meta-analytic study, currently in preparation.

2.2 Eligibility criteria and study selection

The search phrases in Table 1 were used to identify lists of studies. We exported the articles' bibliographic data (including study ID, date of publication, title and abstract) of to a spreadsheet. Each article on this list was then reviewed at one or more of four levels of detail (title, abstract, full text and a detailed review of experimental design) to determine their eligibility for the review. Studies were required to be written in English and to have reported ARDEB in adult rats or mice. We required that each included study contain (1) primary behavior data from either an OF, EPM, or LD experiment for at least one of the interventions of interest, (2) suitable control data and (3) the relevant statistics (mean, standard error or standard deviation, and sample sizes of both control and intervention groups). Experiments that used combination treatments were excluded. Only studies in which adult rodents were assayed were included. For gene knockout and overexpression interventions, we included only experiments that used a lifetime loss of function throughout the entire animal. All eligible experiments from all eligible studies were included in the ten meta-analyses (Table 1).

2.3 Data items and extraction

The following data were collected from each of the included studies: authors, year of publication, figure and panel numbers, species, genotype, and mean, standard error of the mean and sample size (N) of each intervention and its related control group. Graphically presented data were extracted from Portable Document Format (PDF) files with

Table 1. Summary of systematic reviews of anxiety-related interventions in mouse and rat. The PubMed and Embase query phrases used to identify articles that might contain data relevant to the interventions and assays of interest are detailed. Title, abstract and full-text searches were performed to identify articles meeting the selected criteria.

Intervention	Query phrase used in PubMed and Embase for study selection	Articles identified by phrase	Articles meeting meta-analysis criteria
Diazepam	(diazepam OR valium) AND anxiety AND (open field OR exploratory) AND (rodent OR rat OR rats OR mouse OR mice OR Mus)	540	172
***Ht1a* knockout**	(serotonin1A receptor OR 5-HT1A receptor) AND knockout AND anxiety	85	12
***Ht1a* over-expression**	(serotonin1A receptor OR 5-HT1A receptor) AND (overexpression OR over-expression OR overexpressing) AND anxiety	13	3
***Htt* knockout**	(serotonin transporter) AND (knockout OR knockdown OR deletion OR antisense) AND anxiety AND (elevated plus maze OR open field OR light-dark) AND (rats OR rat OR mice OR mouse OR *Mus*)	37	13
***Htt* over-expression**	serotonin transporter AND anxiety AND (elevated plus maze OR open field OR light-dark) AND (increased OR over-expression OR overexpressing OR transgenic) AND (rats OR rat OR mice OR mouse OR *Mus*)	65	2
***Crh* knockout**	((corticotropin-releasing) AND (stress-induced behaviors OR stress-related behaviors or Behavioral responses to stress OR Behavioral responses to stress)) AND (CRH-deficient mice OR lacking the CRH gene OR CRFko)	12	2
***Crhr1* knockout**	(corticotropin releasing factor receptor 1-deficient mice) OR CRH1 receptor antisense oligodeoxynucleotide OR Crhr1 null mutants OR Corticotropin-releasing hormone receptor antisense	62	6
Pain	(inflammatory pain OR neuropathic pain) AND anxiety AND (elevated plus maze OR open field OR light-dark) AND (rats OR rat OR mice OR mouse OR *Mus*)	73	7
Restraint	acute restraint AND anxiety AND (elevated plus maze OR open field OR light-dark) AND (rats OR rat OR mice OR mouse OR *Mus*)	87	15
Isolation	(social isolation OR single housing) AND anxiety AND (elevated plus maze OR plus-maze OR open field OR OFT OR light-dark) AND (rats OR rat OR mice OR mouse OR *Mus*)	167	50

the Measuring Tool in Adobe Acrobat Pro. All extracted data were checked by a second researcher. For values extracted from tables, the check consisted of ensuring the values were identical. For values extracted from graphical data (e.g. bar plots), the check consisted of a visual inspection to ensure that the extracted value matched the graphical data. Extraction discrepancies were reconciled by conference between the primary extractor and the researcher who identified the discrepancy.

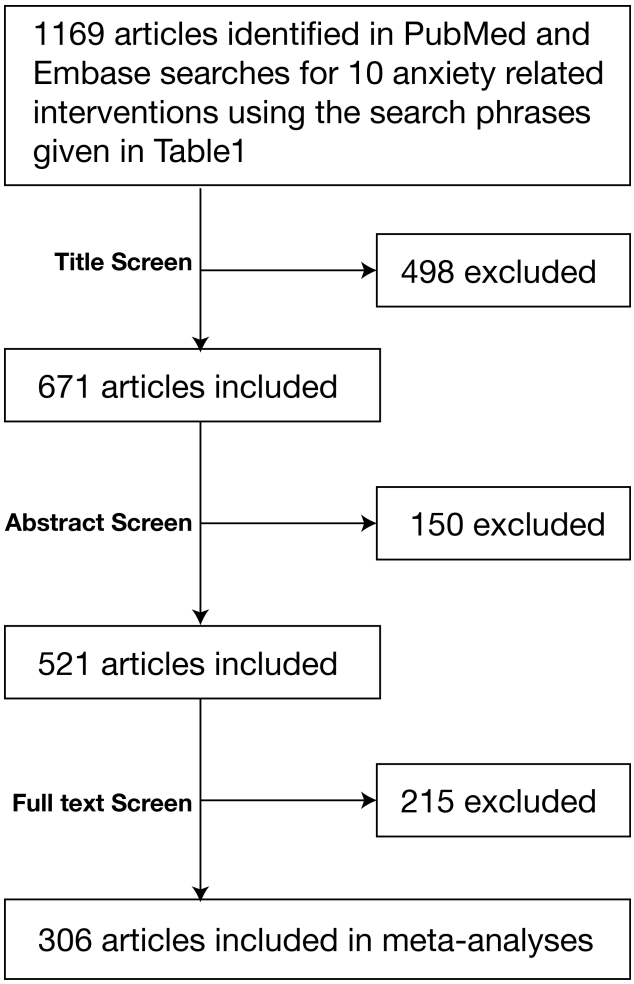


Figure 1. Flow chart of the systematic literature review of 10 anxiotropic interventions.
The literature was reviewed in a four-stage process, starting with searches of the Pubmed and EMBASE databases that yielded 1169 articles, followed by three screens of increasing detail, reviewing the article title, abstract, and full text for experimental design. A total of 306 articles were used in the meta-analysis. Further details are given in Table 1 and the Methods section.

2.4 Summary measures

The following behavioral metrics were extracted from the articles: in OF studies, percent or total time spent at the center; in EPM studies, percent or total time spent on the open arm; in LD studies, percent or total time spent in the bright area. To synthesize these time-based metrics from the three assays, all estimates were standardized to

Hedges' g, a preferred variant of Cohen's d that uses the pooled standard deviation and is corrected for bias using Hedges' method (Borenstein et al., 2011; Cumming, 2012). The conventional adjectives to describe effect size—trivial, small, moderate, large—are used for effect sizes $g < 0.2$, $g < 0.5$, $g < 0.8$ and $g > 0.8$ SD respectively (Cumming, 2012).

2.5 Synthesis of results

Meta-analyses of experimental outcomes, including the calculation of weighted mean effect sizes (Hedges' g), 95% confidence intervals, I^2 heterogeneity values, and P values using the random effects model, were performed with the metafor package in R (http://CRAN.R-project.org/package=metafor) (Viechtbauer, 2010). All error bars in forest plots are 95% confidence intervals; forest plots were generated with custom R scripts.

2.6 Assessment of bias across studies

Publication bias was assessed with funnel plots and Egger's linear regression test of funnel plot asymmetry (Egger et al., 1997). The standard normal deviate (Hedges' g / standard error) for each study was regressed against the study's precision (1 / standard error) using the "lm" function in R (http://www.R-project.org/). For studies that showed publication bias (P-value ≤ 0.05), the trim-and-fill method (Duval and Tweedie, 2000) was employed to estimate the effects of publication bias on the effect size estimate. Funnel plots and trim-and-fill adjustments were performed with the 'metafor' package in R (Viechtbauer, 2010).

3. Results

3.1 Review selection criteria identified 306 eligible articles

The flow-chart in Figure 1 summarizes the study selection process. In total, 1169 articles were identified by the initial search in PubMed and EMBASE databases. According to the selection criteria described above, 498 studies were excluded based on their titles and a further 150 were excluded based on their abstracts. The full text of the remaining 521 articles were screened for criteria related to experimental paradigm, methods, and relevant variables, resulting in the exclusion of a further 215 studies. A total of 306 articles were considered eligible for inclusion in the review.

3.2 Characteristics of included experiments

The characteristics of all included studies are given in Supplementary Table 1. In brief, 582 experiments from 306 studies comprising 411 EPM experiments, 84 OF experiments and 87 LD experiments were identified. Studies were published between 1985 and 2015 and included data from 318 experiments conducted on mice and 264 experiments on rats. Studies reported 515 experiments conducted on male animals, 29 on female, 35 on mixed and 3 experiments with no gender information reported. ARDEB studies of diazepam used a median dosage of 1 mg/kg, with minimum and maximum dosages of

0.01 mg/kg and 20 mg/kg respectively, a dose range is similar to or higher than commonly used by patients.

3.3 Heterogeneity

Statistically significant heterogeneity was found in (8/10) of the meta-analyses. Only two meta-analyses had high heterogeneity, $I^2 > 75\%$: *Htr1a* overexpression, and physical restraint (Higgins et al., 2003).

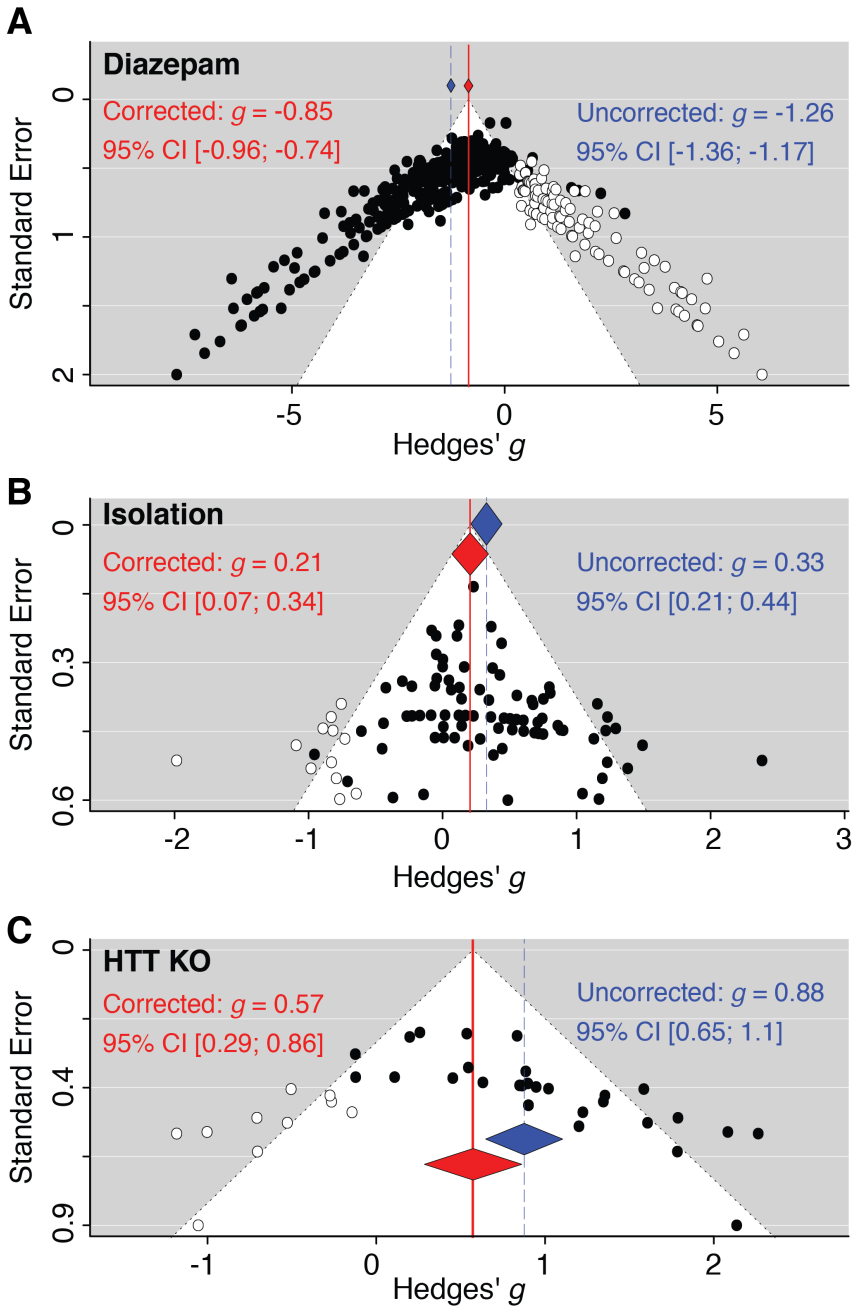


Figure 2. Funnel plots of three meta-analyses with evidence for publication bias.
Where at least ten experiments were available for meta-analysis, the effect sizes (Hedges' g) of the experiments are plotted against their respective standard errors. Points on each plot represent individual experiments. The triangle bounded by dotted lines indicates the area where 95% of studies are expected to fall, in the absence of both publication bias and study heterogeneity. Shown here are funnel plots for experiments on (A) diazepam, (B) social isolation, --and (C) *Htt* knockout.

Three of the meta-analyses, pain and *Htt* knockouts and diazepam, had moderate heterogeneity (50% < I^2 < 75%). Five meta-analyses had low heterogeneity (I^2 < 50%). As most of these syntheses contained data from more than one assay type, it is encouraging that half had low or moderate heterogeneity, and outcome that is compatible with the idea that the three ARDEB assays are testing similar aspects of rodent anxiety.

3.4 Substantial publication bias in four anxiety factors

Censorship of non-statistically significant experimental results and selective publication of statistically significant 'positive' results can cause a literature (and meta-analysis thereof) to overstate effect sizes. This effect, termed 'publication bias,' has a profound influence on the literature on rodent models of stroke, and may affect other animal models (Sena et al., 2010). Publication bias in the ARDEB literature was assessed for the six meta-analyses that had at least 20 experiments (Table 2) (Sterne et al., 2011). Funnel plots of these data showed pronounced asymmetry (Figure 2), which pointed to publication bias in these literatures (Sterne et al., 2011). Egger's asymmetry test indicated that four of these literatures showed statistically significant bias (Table 2). For the biased data sets, we applied trim-and-fill adjustment to estimate the number of hypothesized missing studies and to correct the bias (Duval and Tweedie, 2000) (Figure 2). These data support the idea that the literatures of diazepam, *Htt* knockout, social isolation

Table 2. Results of Egger's linear regression test for funnel plot asymmetry across six meta-analyses

Where at least twenty experiments were available for meta-analysis, Egger's linear regression test for funnel plot asymmetry was performed. For each meta-analysis, the number of included studies, the vertical intercept of the linear regression, and the P-values of Egger's test are listed.

Intervention	Experiments	No. of Studies	Hedges' g	P-value	I2 [95% CI]	Egger's Test p-value	Expts Added By Trim-and-Fill	Adjusted Hedges' g [95% CI]	Adjusted P-value
Diazepam	386	172	-1	1.36E-144	1	2.35E-56	102	-1	4.72E-56
Isolation	83	50	0	3.37E-08	0	0	12	0	0
HTT KO	29	13	1	5.18E-14	1	6.72E-06	10	1	9.71E-05
Restraint	21	16	1	0	1	0	NA	NA	NA
Pain	21	7	1	0	1	1	NA	NA	NA
5HT1A KO	20	11	1	3.15E-10	0	1	NA	NA	NA
Ht1a OE	8	3	-1	0	1	NA	NA	NA	NA
Crhr1 KO	8	6	-1	6.64E-11	0	NA	NA	NA	NA
Htt OE	4	2	-1	0	1	NA	NA	NA	NA
Crh KO	2	2	0	0	0	NA	NA	NA	NA

Figure 3 part 1.

	Control N_c	Control Mean	Control SD	Treated N_T	Treated Mean	Treated SD	SMD	95%–CI	Weight
Assay = EPM									
Gonzalez–Trujano ME 2012 Mice 0.01mg/kg Male	6	25.05	9.349	6	36.72	6.15	−1.36	[−2.67; −0.053]	0.2%
Gonzalez–Trujano ME 2012 Mice 0.03mg/kg Male	6	25.05	9.349	6	31.72	14.18	−0.51	[−1.67; 0.646]	0.3%
Gonzalez–Trujano ME 2015 Mice 0.1mg/kg Male	6	15.68	2.402	6	31.84	9.97	−2.06	[−3.56; −0.552]	0.2%
Leggio 2011 Mice 0.1mg/kg Male	7	0.61	0.108	7	0.94	0.22	−1.79	[−3.09; −0.484]	0.2%
Gonzalez–Trujano ME 2012 Mice 0.1mg/kg Male	6	25.05	9.349	6	40.89	10.66	−1.46	[−2.79; −0.126]	0.2%
Harada K 2006 Rats 0.1mg/kg Male	16	9.11	4.224	16	11.88	7.39	−0.45	[−1.15; 0.254]	0.3%
Griebel G 1999 Rats 0.1mg/kg Male	11	15.20	16.915	11	20.10	23.55	−0.23	[−1.07; 0.609]	0.3%
Ponten 2011 Mice 0.1mg/kg Male	7	17.29	1.608	7	15.46	4.54	0.50	[−0.57; 1.572]	0.3%
Leggio 2011 Mice 0.1mg/kg Male	7	1.96	0.217	7	1.27	1.73	0.53	[−0.54; 1.600]	0.3%
Leggio 2011 Mice 0.1mg/kg Male	7	1.27	0.541	7	0.61	0.11	1.57	[0.32; 2.818]	0.2%
Leggio 2011 Mice 0.1mg/kg Male	7	0.61	0.108	7	0.28	0.11	2.83	[1.22; 4.440]	0.2%
Jones GH 1994 Mice 0.2mg/kg Male	8	20.17	6.223	8	23.43	8.58	−0.41	[−1.41; 0.581]	0.3%
Satyan 1998 Rats 0.25mg/kg Female and Male	10	2.76	1.581	5	14.13	9.49	−1.97	[−3.32; −0.626]	0.2%
Ene HM 2015 Mice 0.25mg/kg Male	5	2.97	1.056	7	14.83	7.58	−1.85	[−3.31; −0.399]	0.2%
Ramanathan M 1998 Rats 0.25mg/kg Female and Male	7	3.34	2.333	7	5.97	2.33	−1.05	[−2.19; 0.090]	0.3%
Griebel G 1998 Rats 0.25mg/kg Male	11	14.65	18.511	11	25.81	15.62	−0.63	[−1.49; 0.233]	0.3%
Yasumatsu H 1994 Rats 0.25mg/kg Male	20	6.07	3.737	20	8.40	5.11	−0.51	[−1.14; 0.121]	0.3%
Gonzalez–Trujano ME 2012 Mice 0.3mg/kg Male	6	25.05	9.349	6	55.89	12.78	−2.54	[−4.21; −0.874]	0.2%
Wolfman C 1994 Mice 0.3mg/kg Male	51	5.21	11.102	21	20.95	15.62	−1.24	[−1.79; −0.691]	0.4%
Assi.. MB 1993 Mice 0.3mg/kg Male	10	22.80	4.322	10	31.47	8.64	−1.21	[−2.19; −0.244]	0.3%
Griebel G 1999 Rats 0.3mg/kg Male	11	15.20	16.915	11	17.90	25.87	−0.12	[−0.96; 0.718]	0.3%
Dunn 1998 Rats 0.3mg/kg Male	6	8.21	4.012	6	7.46	7.86	0.11	[−1.02; 1.243]	0.3%
Harada K 2006 Rats 0.32mg/kg Male	16	9.11	4.224	16	11.75	5.81	−0.51	[−1.21; 0.199]	0.3%
Jones GH 1994 Mice 0.39mg/kg Male	8	20.17	6.223	7	29.33	9.43	−1.10	[−2.21; 0.014]	0.3%
Brioni 1994 Rats 0.484mg/kg Male	8	7.00	3.488	6	13.50	27.48	−0.34	[−1.41; 0.729]	0.3%
Mahendra P 2011 Mice 0.5mg/kg Male	6	15.61	3.731	6	46.17	4.25	−7.05	[−10.64; −3.472]	0.1%
Leggio 2011 Mice 0.5mg/kg Male	7	1.39	0.217	7	3.72	0.43	−6.38	[−9.33; −3.426]	0.1%
Leggio 2011 Mice 0.5mg/kg Male	7	1.72	0.210	7	4.38	0.54	−6.06	[−8.88; −3.236]	0.1%
Leggio 2011 Mice 0.5mg/kg Male	7	0.70	0.109	7	1.06	0.11	−3.18	[−4.91; −1.450]	0.2%
Ramanathan M 1998 Rats 0.5mg/kg Female and Male	7	3.34	2.333	7	10.70	2.33	−2.95	[−4.60; −1.300]	0.2%
Ognibene E 2008 Mice 0.5mg/kg Male	8	9.80	3.576	8	20.02	4.47	−2.39	[−3.75; −1.026]	0.2%
Ene HM 2015 Mice 0.5mg/kg Male	5	2.97	1.056	6	10.03	4.60	−1.84	[−3.36; −0.325]	0.2%
Srinivasan J 2003 Rats 0.5mg/kg Male	6	4.07	1.306	6	5.77	0.33	−1.65	[−3.03; −0.265]	0.2%
Ponten 2011 Mice 0.5mg/kg Male	7	17.29	1.608	7	30.36	15.48	−1.11	[−2.26; 0.041]	0.3%
Peng WH 2004 Mice 0.5mg/kg Male	12	2.39	1.409	12	5.28	3.33	−1.09	[−1.96; −0.223]	0.3%
Yasumatsu H 1994 Rats 0.5mg/kg Male	20	6.07	3.737	20	11.92	6.88	−1.04	[−1.70; −0.371]	0.3%
Yamada K 2000 Mice 0.5mg/kg Not specified	6	18.69	3.910	6	23.29	5.06	−0.94	[−2.16; 0.280]	0.2%
Drapier D 2007 Rats 0.5mg/kg Male	11	29.45	11.209	12	43.34	18.43	−0.87	[−1.73; −0.005]	0.3%
Mesfin M 2014 Mice 0.5mg/kg Male	6	14.72	16.485	6	41.22	41.37	−0.78	[−1.97; 0.415]	0.3%
Lepicard EM 2000 Mice 0.5mg/kg Male	18	17.54	9.498	18	30.22	20.58	−0.77	[−1.45; −0.094]	0.3%
Lepicard EM 2000 Mice 0.5mg/kg Male	15	16.42	26.013	18	36.94	28.90	−0.72	[−1.43; −0.015]	0.3%
Griebel G 1999 Rats 0.5mg/kg Male	10	18.18	15.245	10	27.13	19.17	−0.50	[−1.39; 0.398]	0.3%
Rochford J 1997 Mice 0.5mg/kg Male	8	3.27	3.081	8	5.01	3.70	−0.48	[−1.48; 0.514]	0.3%
Fraser LM 2010 Mice 0.5mg/kg Female and Male	15	17.19	14.012	15	21.26	16.35	−0.26	[−0.98; 0.459]	0.3%
Sakaue M1 2003 Mice 0.5mg/kg Male	17	9.60	13.606	17	12.60	10.31	−0.24	[−0.92; 0.432]	0.3%
Jain 2005 Rats 0.5mg/kg Male	6	21.55	4.432	6	21.87	7.89	−0.05	[−1.18; 1.084]	0.3%
Griebel G 1998 Rats 0.5mg/kg Male	11	14.65	18.511	11	14.30	11.57	0.02	[−0.81; 0.858]	0.3%
Leggio 2011 Mice 0.5mg/kg Male	7	1.39	0.217	7	1.06	0.13	1.73	[0.44; 3.016]	0.2%
Wolfman C 1994 Mice 0.6mg/kg Male	51	5.21	11.102	22	16.45	27.91	−0.62	[−1.14; −0.114]	0.4%
Baretta IP 2012 Mice 0.75mg/kg Male	10	7.58	5.124	10	40.99	22.28	−1.98	[−3.09; −0.870]	0.3%
Tolardo R 2010 Mice 0.75mg/kg Male	9	48.42	11.513	9	68.80	8.86	−1.89	[−3.04; −0.734]	0.3%
Da Silva NL 1996 Rats 0.75mg/kg Male	12	39.61	27.444	12	36.84	16.92	0.12	[−0.68; 0.918]	0.3%
R..go L 1988 Mice 0.75mg/kg Male	18	22.50	12.374	8	20.00	14.14	0.19	[−0.65; 1.022]	0.3%
Da Silva NL 1996 Rats 0.75mg/kg Female	12	44.50	10.978	12	37.63	9.15	0.66	[−0.17; 1.481]	0.3%
Jones GH 1994 Mice 0.78mg/kg Male	8	20.17	6.223	8	36.53	8.30	−2.11	[−3.40; −0.822]	0.2%
Brioni 1994 Rats 0.997mg/kg Male	8	7.00	3.488	8	17.03	29.60	−0.45	[−1.45; 0.546]	0.3%
Brioni 1994 Mice 0.997mg/kg Male	8	11.60	6.788	8	24.40	40.80	−0.41	[−1.41; 0.580]	0.3%
Ramanathan M 1998 Rats 1mg/kg Female and Male	7	3.34	2.333	7	21.50	2.33	−7.28	[−10.60; −3.963]	0.1%
Micale 2009 Mice 1mg/kg Male	9	9.29	3.182	9	32.32	3.64	−6.42	[−8.95; −3.889]	0.1%
Martinez AL 2006 Mice 1mg/kg Male	6	14.22	6.834	6	66.83	8.74	−6.19	[−9.37; −2.998]	0.1%
Leggio 2011 Mice 1mg/kg Male	7	1.96	0.216	7	9.63	1.73	−5.83	[−8.57; −3.102]	0.1%
Leggio 2011 Mice 1mg/kg Male	7	2.29	0.324	7	13.27	2.48	−5.80	[−8.52; −3.083]	0.1%
Sugiyama A 2012 Rats 1mg/kg Male	6	13.06	12.711	6	89.29	11.72	−5.75	[−8.75; −2.759]	0.1%
Saitoh A 2013 Rats 1mg/kg Male	5	27.38	14.456	6	93.73	8.52	−5.25	[−8.20; −2.305]	0.1%
Kumar D 2014 Rats 1mg/kg Female and Male	6	14.41	4.190	6	40.51	5.28	−5.06	[−7.74; −2.371]	0.1%
Gonzalez–Trujano ME 2012 Mice 1mg/kg Male	6	25.05	9.349	6	69.94	8.18	−4.72	[−7.25; −2.177]	0.1%
Karim N 2011 Mice 1mg/kg Male	8	16.84	5.380	8	38.39	5.38	−3.79	[−5.58; −1.996]	0.2%
Carro–Juarez M 2012 Rats 1mg/kg Male	7	11.49	5.242	7	29.88	4.19	−3.63	[−5.51; −1.740]	0.2%
Silva 2007 Mice 1mg/kg Male	13	21.63	8.312	13	65.85	14.89	−3.55	[−4.85; −2.255]	0.2%
Melo FH 2010 Mice 1mg/kg Male	12	23.78	10.917	12	61.41	11.11	−3.30	[−4.59; −2.006]	0.2%
Yamada K 2000 Mice 1mg/kg Not specified	6	18.69	3.910	6	32.49	3.91	−3.26	[−5.19; −1.322]	0.2%
Ven..ncio ET 2011 Rats 1mg/kg Male	9	24.27	8.800	9	60.75	13.20	−3.10	[−4.56; −1.632]	0.2%
Yadav AV 2008 Mice 1mg/kg Male	6	10.27	5.226	6	35.67	9.80	−2.98	[−4.81; −1.155]	0.2%
Kumar D 2014 Rats 1mg/kg Female and Male	6	13.96	5.155	6	38.93	9.96	−2.90	[−4.70; −1.105]	0.2%
Ochoa–Sanchez R 2012 Rats 1mg/kg Male	12	11.62	6.396	7	62.85	27.94	−2.82	[−4.18; −1.450]	0.2%
Thippeswamy BS 2011 Mice 1mg/kg Male	6	14.27	7.887	6	37.73	7.67	−2.78	[−4.54; −1.029]	0.2%
Lolli LF 2007 Mice 1mg/kg Male	10	15.46	9.781	10	50.08	13.75	−2.78	[−4.07; −1.482]	0.2%
Lolli LF 2007 Mice 1mg/kg Male	10	7.42	5.866	10	47.01	19.50	−2.63	[−3.89; −1.373]	0.2%
Peng WH 2004 Mice 1mg/kg Male	15	2.40	2.262	15	8.42	2.26	−2.58	[−3.59; −1.584]	0.3%
Fajemiroye JO 2014 Mice 1mg/kg Male	9	22.33	8.693	9	51.99	13.81	−2.45	[−3.74; −1.159]	0.2%
Girish C 2013 Mice 1mg/kg Male	6	25.66	6.699	6	57.31	15.80	−2.41	[−4.03; −0.787]	0.2%
Girish C 2013 Mice 1mg/kg Male	6	25.66	6.700	6	57.31	15.80	−2.41	[−4.03; −0.787]	0.2%

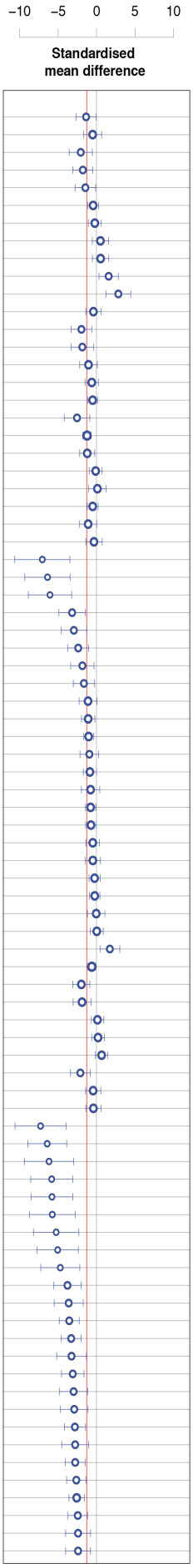


Figure 3 part 2.

	Control N_c	Control Mean	Control SD	Treated N_T	Treated Mean	Treated SD	SMD	95%–CI	Weight
Assay = EPM (continued)									
Micale 2008 Mice 1mg/kg Male	10	12.05	3.536	10	27.05	7.79	−2.38	[−3.57; −1.178]	0.3%
Saiyudthong 2011 Rats 1mg/kg Male	10	22.48	11.439	10	53.29	13.40	−2.37	[−3.56; −1.172]	0.3%
Consoli D 2007 Mice 1mg/kg Male	10	7.60	5.481	10	18.87	3.79	−2.29	[−3.47; −1.111]	0.3%
Nagaraja TS 2012 Mice 1mg/kg Male	6	11.50	3.919	6	21.70	4.41	−2.26	[−3.83; −0.687]	0.2%
Girish C 2013 Mice 1mg/kg Male	6	26.93	7.321	6	57.24	15.96	−2.25	[−3.82; −0.685]	0.2%
Taiwo AE 2012 Rats 1mg/kg Female	10	4.41	4.912	10	25.00	11.39	−2.25	[−3.42; −1.079]	0.3%
Micale 2009 Mice 1mg/kg Male	10	15.72	3.373	10	45.27	17.69	−2.22	[−3.39; −1.060]	0.3%
de Sousa FC 2007 Mice 1mg/kg Male	12	33.29	10.049	12	59.58	13.31	−2.15	[−3.19; −1.114]	0.3%
Gomes PB 2010 Mice 1mg/kg Male	12	36.90	10.288	12	59.75	10.29	−2.14	[−3.18; −1.107]	0.3%
Bahi A 2014 Mice 1mg/kg Male	9	27.69	14.703	8	65.79	20.21	−2.07	[−3.30; −0.832]	0.2%
Harada K 2006 Rats 1mg/kg Male	16	9.11	4.224	16	22.18	7.92	−2.01	[−2.88; −1.139]	0.3%
Barbosa PR 2008 Rats 1mg/kg Male	15	19.29	14.942	15	58.38	22.91	−1.97	[−2.86; −1.075]	0.3%
Mansouri MT 2014 Rats 1mg/kg Male	10	12.96	6.633	10	35.37	13.94	−1.97	[−3.07; −0.859]	0.3%
Blainski A 2010 Mice 1mg/kg Male	9	16.43	9.998	9	40.71	13.93	−1.91	[−3.07; −0.748]	0.3%
Wang X 2015 Mice 1mg/kg Male	10	8.02	7.485	10	20.38	4.76	−1.89	[−2.98; −0.796]	0.3%
Mansouri MT 2014 Rats 1mg/kg Male	10	13.46	5.788	10	35.17	14.56	−1.88	[−2.96; −0.788]	0.3%
de−Paris F 2000 Rats 1mg/kg Male	15	21.38	13.052	10	61.30	28.93	−1.86	[−2.84; −0.883]	0.3%
Nagaraja TS 2012 Mice 1mg/kg Male	6	7.90	2.939	6	15.30	4.41	−1.82	[−3.25; −0.390]	0.2%
Rejon−Orantes JC 2013 Mice 1mg/kg Male	5	4.03	2.907	5	12.63	5.44	−1.78	[−3.36; −0.197]	0.2%
de Sousa FC 2007 Mice 1mg/kg Male	12	36.29	14.063	12	59.02	10.47	−1.77	[−2.74; −0.802]	0.3%
Kebebew Z 2013 Mice 1mg/kg Male	8	14.67	18.051	8	46.92	17.27	−1.73	[−2.92; −0.531]	0.3%
Ponten 2011 Mice 1mg/kg Male	7	17.29	1.608	7	32.31	11.73	−1.68	[−2.95; −0.404]	0.2%
de Melo CT 2006 Mice 1mg/kg Male	13	38.44	11.468	13	64.55	18.38	−1.65	[−2.56; −0.741]	0.3%
Cechin EM 2003 Rats 1mg/kg Male	13	23.87	18.148	13	61.30	26.92	−1.58	[−2.48; −0.681]	0.3%
Sorra K 2014 Mice 1mg/kg Male	4	31.44	7.235	4	44.67	7.98	−1.51	[−3.22; 0.205]	0.2%
Taiwo AE 2012 Rats 1mg/kg Male	10	8.66	7.751	10	28.91	17.01	−1.47	[−2.48; −0.456]	0.3%
Rochford J 1997 Mice 1mg/kg Male	8	3.27	3.081	8	11.11	6.47	−1.46	[−2.60; −0.325]	0.3%
Sugiyama A 2012 Rats 1mg/kg Male	5	28.72	20.342	5	69.02	28.84	−1.46	[−2.94; 0.021]	0.2%
Dunn 1998 Rats 1mg/kg Male	6	8.21	4.012	6	23.98	13.63	−1.45	[−2.78; −0.119]	0.2%
Lepicard EM 2000 Mice 1mg/kg Male	15	16.42	26.013	19	50.75	23.12	−1.37	[−2.13; −0.612]	0.3%
Kuribara 2000 Mice 1mg/kg Male	10	1.07	1.370	10	7.37	6.11	−1.36	[−2.36; −0.368]	0.3%
Huerta−Reyes M 2013 Mice 1mg/kg Female and Male	8	27.04	12.677	8	80.85	51.79	−1.35	[−2.47; −0.233]	0.3%
Taiwo AE 2012 Rats 1mg/kg Male	10	10.15	8.027	10	22.11	9.73	−1.28	[−2.27; −0.303]	0.3%
Kalouda T 2015 Rats 1mg/kg Male	10	23.22	6.859	10	43.13	20.58	−1.24	[−2.22; −0.268]	0.3%
Taiwo 2012 Rats 1mg/kg Male	10	9.76	7.134	10	21.44	10.60	−1.24	[−2.21; −0.264]	0.3%
Thongsaard W 1996 Rats 1mg/kg Male	10	0.87	1.095	10	16.62	18.07	−1.18	[−2.14; −0.213]	0.3%
Yasumatsu H 1994 Rats 1mg/kg Male	20	6.07	3.737	20	10.77	4.33	−1.14	[−1.81; −0.468]	0.3%
Raquibul Hasan SM 2009 Mice 1mg/kg Male	5	27.47	30.320	5	66.05	31.61	−1.12	[−2.51; 0.262]	0.2%
Hasen..hrl RU 1996 Rats 1mg/kg Male	9	16.25	24.795	10	49.04	30.58	−1.12	[−2.10; −0.134]	0.3%
Bhattacharya 1991 Mice 1mg/kg Male	24	14.27	15.350	10	33.27	20.58	−1.09	[−1.88; −0.305]	0.3%
Mechan AO 2002 Rats 1mg/kg Male	9	20.38	13.506	9	38.13	17.44	−1.08	[−2.09; −0.077]	0.3%
Pellow S 1985 Rats 1mg/kg Male	7	18.88	4.371	7	24.14	5.48	−0.99	[−2.13; 0.138]	0.3%
Parent AJ 2012 Rats 1mg/kg Male	11	23.68	11.378	11	39.46	19.35	−0.96	[−1.85; −0.065]	0.3%
Thongsaard W 1996 Rats 1mg/kg Male	10	0.87	1.095	10	16.28	21.90	−0.95	[−1.89; −0.016]	0.3%
Mora S 2005 Rats 1mg/kg Female and Male	16	11.11	10.230	16	32.07	30.30	−0.90	[−1.64; −0.171]	0.3%
Griebel G 1999 Rats 1mg/kg Male	11	15.20	16.915	11	33.20	25.21	−0.81	[−1.68; 0.069]	0.3%
Cole 1995 Mice 1mg/kg Male	19	12.00	12.641	19	23.80	17.44	−0.76	[−1.42; −0.098]	0.3%
Lepicard EM 2000 Mice 1mg/kg Male	18	17.54	9.498	17	29.85	20.58	−0.76	[−1.45; −0.069]	0.3%
Paine TA 2002 Rats 1mg/kg Male	8	36.74	12.226	8	44.05	7.28	−0.69	[−1.70; 0.330]	0.3%
Mechan AO 2002 Rats 1mg/kg Male	9	19.59	10.321	9	33.88	28.59	−0.63	[−1.59; 0.319]	0.3%
Frassetto 2010 Rats 1mg/kg Male	11	51.28	14.909	11	69.87	41.07	−0.58	[−1.44; 0.278]	0.3%
Hui 2002 Mice 1mg/kg Male	16	44.32	8.056	16	62.73	46.05	−0.54	[−1.25; 0.164]	0.3%
Sakaue M1 2003 Mice 1mg/kg Male	17	9.60	13.606	17	16.00	9.48	−0.53	[−1.22; 0.153]	0.3%
Chen 2004 Mice 1mg/kg Male	10	35.00	17.707	10	58.33	60.66	−0.50	[−1.39; 0.393]	0.3%
Faria 1997 Rats 1mg/kg Male	8	11.68	4.526	10	24.16	31.48	−0.50	[−1.45; 0.448]	0.3%
Griebel G 1998 Rats 1mg/kg Male	11	14.65	18.511	11	23.72	25.45	−0.39	[−1.24; 0.453]	0.3%
Taiwo AE 2012 Rats 1mg/kg Female	10	18.08	16.062	10	23.38	12.07	−0.36	[−1.24; 0.528]	0.3%
Taiwo 2012 Rats 1mg/kg Female	10	17.29	16.644	10	22.56	13.08	−0.34	[−1.22; 0.547]	0.3%
Griebel G 2002 Rats 1mg/kg Male	11	12.40	6.633	11	16.50	15.92	−0.32	[−1.17; 0.519]	0.3%
Jaszberenyi M 2007 Rats 1mg/kg Male	7	4.08	2.622	7	4.96	2.44	−0.32	[−1.38; 0.734]	0.3%
Griebel G 1999 Rats 1mg/kg Male	10	18.18	15.245	10	23.00	14.81	−0.31	[−1.19; 0.576]	0.3%
Okuyama S 1999 Rats 1mg/kg Male	14	12.10	5.543	14	13.70	7.85	−0.23	[−0.97; 0.514]	0.3%
Wanasuntronwong 2012 Mice 1mg/kg Male	9	4.94	2.910	9	8.28	21.00	−0.21	[−1.14; 0.715]	0.3%
Da Silva NL 1996 Rats 1mg/kg Male	12	39.61	27.444	12	43.71	6.86	−0.20	[−1.00; 0.605]	0.3%
Fernandez SP 2008 Mice 1mg/kg Male	12	13.20	10.446	12	14.96	13.06	−0.14	[−0.95; 0.657]	0.3%
Jaszberenyi 2009 Rats 1mg/kg Male	10	22.63	4.993	10	24.74	59.65	−0.05	[−0.92; 0.829]	0.3%
Steiner MA 2012 Rats 1mg/kg Male	12	22.73	8.998	12	23.05	12.37	−0.03	[−0.83; 0.771]	0.3%
Leggio 2011 Mice 1mg/kg Male	7	0.98	0.108	7	0.98	0.11	0.00	[−1.05; 1.048]	0.3%
Leggio 2011 Mice 1mg/kg Male	7	1.63	0.216	7	1.63	0.33	0.00	[−1.05; 1.048]	0.3%
Da Silva NL 1996 Rats 1mg/kg Female	12	44.50	10.978	12	42.78	10.06	0.16	[−0.64; 0.959]	0.3%
Meyer L 2013 Rats 1.25mg/kg Male	12	8.99	5.738	12	15.86	3.45	−1.40	[−2.31; −0.492]	0.3%
Dunn RW 1989 Rats 1.25mg/kg Male	8	16.40	5.091	8	24.13	5.75	−1.35	[−2.46; −0.231]	0.3%
Meyer L 2013 Rats 1.25mg/kg Male	12	19.33	7.420	12	25.68	5.14	−0.96	[−1.81; −0.108]	0.3%
Klodzinska 2004 Rats 1.25mg/kg Male	7	10.75	4.267	7	20.70	15.65	−0.81	[−1.92; 0.293]	0.3%
Klodzinska 2004 Rats 1.25mg/kg Male	7	3.61	1.368	7	6.72	4.92	−0.80	[−1.91; 0.300]	0.3%
Tatarczynska E 2004 Rats 1.25mg/kg Male	6	10.90	3.429	7	20.60	15.19	−0.79	[−1.94; 0.360]	0.3%
Wesolowska A 2007 Rats 1.25mg/kg Male	8	10.90	3.960	8	20.60	17.54	−0.72	[−1.74; 0.299]	0.3%
Jastrzebska−Wi..sek M 2014 Rats 1.25mg/kg Male	8	26.21	21.847	8	43.31	28.09	−0.64	[−1.65; 0.370]	0.3%
Simpson J 2012 Rats 1.25mg/kg Male	6	52.45	17.594	6	52.77	13.87	−0.02	[−1.15; 1.113]	0.3%
Molina−Hernendez M 2004 Rats 1.3mg/kg Male	7	23.20	14.191	7	54.51	10.23	−2.37	[−3.83; −0.904]	0.2%
Zanoli 2002 Rats 1.5mg/kg Male	12	12.03	15.949	12	65.11	34.98	−1.88	[−2.87; −0.896]	0.3%
Griebel G 1997 Rats 1.5mg/kg Male	11	15.03	15.549	11	55.45	30.03	−1.63	[−2.61; −0.638]	0.3%

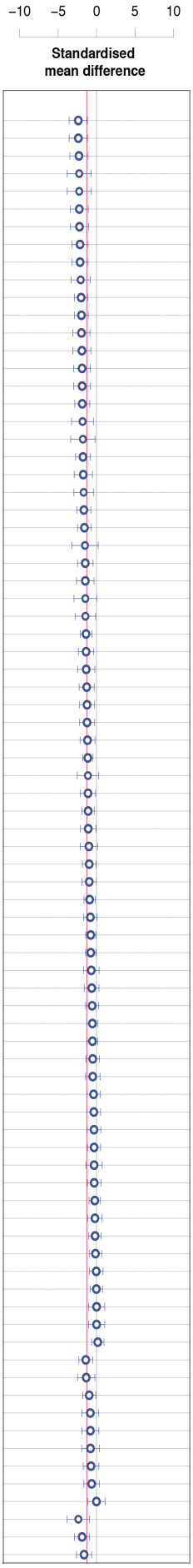


Figure 3 part 3.

	Control N_c	Control Mean	Control SD	Treated N_T	Treated Mean	Treated SD	SMD	95%–CI	Weight
Assay = EPM (continued)									
Dalvi A 2001 Mice 1.5mg/kg Male	13	8.50	7.572	13	23.60	11.90	−1.47	[−2.35; −0.585]	0.3%
Rago L 1988 Mice 1.5mg/kg Male	18	22.50	12.374	8	39.17	15.91	−1.20	[−2.10; −0.292]	0.3%
Sherif F 1994 Rats 1.5mg/kg Male	24	5.42	6.532	8	19.58	24.49	−1.05	[−1.90; −0.205]	0.3%
Dalvi A 1999 Mice 1.5mg/kg Male	10	9.85	10.904	10	19.21	9.35	−0.88	[−1.81; 0.045]	0.3%
Drapier D 2007 Rats 1.5mg/kg Male	11	29.45	11.209	11	47.23	25.80	−0.86	[−1.74; 0.021]	0.3%
Stankevicius D 2008 Mice 1.5mg/kg Not specified	10	20.02	7.605	10	34.23	44.18	−0.43	[−1.32; 0.460]	0.3%
LaBuda 2001 Mice 1.5mg/kg Female and Male	10	3.75	3.953	10	15.47	42.11	−0.38	[−1.26; 0.511]	0.3%
Jones GH 1994 Mice 1.56mg/kg Male	8	20.17	6.223	7	43.53	14.61	−2.01	[−3.32; −0.701]	0.2%
Brioni 1994 Rats 1.99mg/kg Male	8	7.00	3.488	8	21.67	28.53	−0.68	[−1.70; 0.334]	0.3%
Gonzalez−Trujano ME 2012 Mice 2mg/kg Male	6	25.05	9.349	6	81.89	2.26	−7.71	[−11.60; −3.827]	0.1%
Gupta D 2014 Mice 2mg/kg Male	6	17.19	2.275	6	55.85	7.19	−6.69	[−10.11; −3.275]	0.1%
Gupta D 2015 Mice 2mg/kg Female and Male	6	16.59	2.370	6	52.12	7.11	−6.19	[−9.37; −2.997]	0.1%
Kurhe YV 2014 Mice 2mg/kg Male	6	2.33	0.416	6	8.24	1.24	−5.89	[−8.94; −2.834]	0.1%
de Almeida 2012 Mice 2mg/kg Male	7	38.90	3.122	7	68.95	6.31	−5.65	[−8.31; −2.993]	0.1%
Karim N 2011 Mice 2mg/kg Male	8	16.84	5.380	8	55.48	7.84	−5.43	[−7.80; −3.070]	0.1%
Carro−Ju..rez M 2012 Rats 2mg/kg Male	7	11.49	5.242	7	46.76	7.86	−4.94	[−7.32; −2.560]	0.1%
Swami SU 2014 Mice 2mg/kg Male	6	13.05	5.590	6	70.98	16.02	−4.46	[−6.88; −2.029]	0.1%
Fortes AC 2013 Mice 2mg/kg Male	11	24.85	2.885	11	37.55	2.89	−4.23	[−5.84; −2.626]	0.2%
Carneiro LM 2005 Rats 2mg/kg Female and Male	14	11.83	6.623	8	74.97	34.66	−2.87	[−4.14; −1.594]	0.2%
Bhatt S 2013 Mice 2mg/kg Male	8	2.00	1.923	8	11.17	4.16	−2.68	[−4.12; −1.233]	0.2%
Carro−Juarez M 2012 Rats 2mg/kg Male	7	14.94	10.444	7	52.76	16.35	−2.58	[−4.11; −1.050]	0.2%
Han H 2009 Mice 2mg/kg Male	11	12.78	3.980	11	36.27	13.02	−2.35	[−3.48; −1.217]	0.3%
Lepicard EM 2000 Mice 2mg/kg Male	15	16.42	26.013	20	65.30	15.90	−2.30	[−3.18; −1.418]	0.3%
Yao Y 2010 Mice 2mg/kg Male	10	22.35	6.966	10	40.91	9.05	−2.20	[−3.36; −1.044]	0.3%
Rochford J 1997 Mice 2mg/kg Male	8	3.27	3.081	8	10.24	3.08	−2.14	[−3.43; −0.844]	0.2%
Karim N 2011 Mice 2mg/kg Male	8	13.68	16.683	8	50.07	16.18	−2.09	[−3.38; −0.810]	0.2%
Pellow S 1985 Rats 2mg/kg Male	7	18.88	4.371	7	29.97	5.93	−1.99	[−3.35; −0.636]	0.2%
Faturi CB 2010 Rats 2mg/kg Male	10	8.55	10.724	10	47.03	25.64	−1.87	[−2.96; −0.787]	0.3%
Chen SW 2005 Mice 2mg/kg Male	10	16.05	10.973	10	44.96	18.29	−1.84	[−2.92; −0.756]	0.3%
Contreras CM 2011 Rats 2mg/kg Female	6	24.18	15.036	6	58.19	20.48	−1.75	[−3.16; −0.337]	0.2%
de la Pena JB 2013 Mice 2mg/kg Male	9	35.82	10.014	9	57.47	13.84	−1.71	[−2.82; −0.591]	0.3%
Fernandez SP 2008 Mice 2mg/kg Male	12	13.20	10.446	12	36.81	16.29	−1.67	[−2.62; −0.716]	0.3%
Karim N 2011 Mice 2mg/kg Male	8	15.97	24.554	8	50.70	13.75	−1.65	[−2.83; −0.472]	0.3%
Schmitt 2002 Rats 2mg/kg Male	12	5.53	4.704	9	43.36	33.61	−1.64	[−2.67; −0.619]	0.3%
Volke V 1998 Rats 2mg/kg Male	9	12.88	10.815	9	35.02	14.94	−1.62	[−2.72; −0.519]	0.3%
Chen SW 2005 Mice 2mg/kg Male	10	12.97	8.749	10	42.67	24.45	−1.55	[−2.57; −0.523]	0.3%
Molander AC 2011 Rats 2mg/kg Male	8	37.43	11.240	8	56.46	12.03	−1.55	[−2.70; −0.390]	0.3%
Schmitt 2001 Rats 2mg/kg Male	10	3.62	5.010	8	40.46	33.95	−1.54	[−2.63; −0.452]	0.3%
Mi XJ 2005 Mice 2mg/kg Male	10	0.17	0.129	10	0.37	0.13	−1.48	[−2.50; −0.470]	0.3%
Contreras CM 2011 Rats 2mg/kg Male	10	24.18	19.411	6	58.19	26.44	−1.45	[−2.61; −0.286]	0.3%
Santos Rosa D 2012 Rats 2mg/kg Male	6	16.18	8.472	6	47.89	28.34	−1.40	[−2.72; −0.082]	0.2%
Zhang LM 2014 Mice 2mg/kg Male	10	12.76	8.431	10	32.38	17.62	−1.36	[−2.35; −0.367]	0.3%
Griebel G 1999 Rats 2mg/kg Male	10	18.18	15.245	10	38.02	13.50	−1.32	[−2.31; −0.332]	0.3%
Colla AR 2015 Mice 2mg/kg Male	8	5.99	5.126	8	22.86	16.90	−1.28	[−2.38; −0.174]	0.3%
Molander AC 2011 Rats 2mg/kg Male	7	42.08	18.381	7	62.71	12.66	−1.22	[−2.40; −0.050]	0.3%
Griebel G 1998 Rats 2mg/kg Male	11	14.65	18.511	11	43.26	26.03	−1.22	[−2.14; −0.294]	0.3%
Cha HY 2005 Mice 2mg/kg Male	10	1.99	1.571	10	29.55	30.63	−1.22	[−2.19; −0.246]	0.3%
Cha HY 2005 Mice 2mg/kg Male	10	2.53	1.600	10	29.60	30.66	−1.19	[−2.16; −0.226]	0.3%
Cha HY 2005 Mice 2mg/kg Male	10	3.99	2.894	10	29.53	30.52	−1.13	[−2.09; −0.170]	0.3%
Cha HY 2005 Mice 2mg/kg Male	10	4.94	3.502	10	29.81	30.44	−1.10	[−2.05; −0.145]	0.3%
Fernandez F 2004 Rats 2mg/kg Male	10	31.94	23.231	10	56.37	20.03	−1.08	[−2.03; −0.127]	0.3%
Cha HY 2005 Mice 2mg/kg Male	10	5.07	3.472	10	29.65	30.71	−1.08	[−2.03; −0.125]	0.3%
Liu J 2015 Mice 2mg/kg Male	15	0.22	0.086	15	0.31	0.08	−1.05	[−1.82; −0.280]	0.3%
Engin E 2009 Rats 2mg/kg Male	10	18.30	15.832	10	44.16	34.77	−0.92	[−1.85; 0.015]	0.3%
Lepicard EM 2000 Mice 2mg/kg Male	18	17.54	9.498	20	30.60	19.00	−0.84	[−1.50; −0.171]	0.3%
Wanasuntronwong 2012 Mice 2mg/kg Male	9	4.94	2.910	9	10.35	8.36	−0.82	[−1.80; 0.149]	0.3%
Sakaue M1 2003 Mice 2mg/kg Male	17	9.60	13.606	17	25.60	24.33	−0.79	[−1.49; −0.091]	0.3%
Fraser LM 2010 Mice 2mg/kg Female and Male	15	17.19	14.012	15	31.21	22.77	−0.72	[−1.46; 0.020]	0.3%
Liu J 2015 Mice 2mg/kg Male	15	0.24	0.099	15	0.31	0.10	−0.69	[−1.43; 0.047]	0.3%
Pain L 1999 Rats 2mg/kg Male	8	0.16	0.129	8	0.42	0.78	−0.44	[−1.43; 0.557]	0.3%
Paine TA 2002 Rats 2mg/kg Male	8	36.74	12.226	8	40.75	14.26	−0.29	[−1.27; 0.701]	0.3%
Boerngen−Lacerda 2000 Mice 2mg/kg Male	12	6.48	7.918	8	12.76	32.66	−0.28	[−1.18; 0.617]	0.3%
Karaka.. A 2011 Rats 2mg/kg Male	5	0.17	0.046	5	0.18	0.03	−0.28	[−1.53; 0.971]	0.2%
Tatarczynska E 2004 Rats 2.5mg/kg Male	6	10.90	3.429	6	47.20	11.02	−4.10	[−6.38; −1.825]	0.1%
Wesolowska A 2007 Rats 2.5mg/kg Male	8	10.90	3.960	8	47.20	12.73	−3.64	[−5.38; −1.898]	0.2%
Klodzinska A 2004 Rats 2.5mg/kg Male	7	10.75	4.267	7	47.31	13.51	−3.41	[−5.23; −1.604]	0.2%
Klodzinska 2004 Rats 2.5mg/kg Male	7	3.61	1.368	7	15.71	5.20	−2.98	[−4.64; −1.319]	0.2%
Dunn RW 1989 Rats 2.5mg/kg Male	8	16.40	5.091	8	33.70	7.17	−2.63	[−4.06; −1.201]	0.2%
de Castro PC 2007 Rats 2.5mg/kg Male	10	41.00	8.433	10	73.00	24.24	−1.69	[−2.74; −0.637]	0.3%
Jastrzebska−Wiesek M 2014 Rats 2.5mg/kg Male	8	26.21	21.847	8	57.38	24.97	−1.26	[−2.35; −0.157]	0.3%
Drapier D 2007 Rats 2.5mg/kg Male	11	29.45	11.209	11	57.78	29.48	−1.22	[−2.15; −0.297]	0.3%
Yasumatsu H 1994 Rats 2.5mg/kg Male	20	6.07	3.737	20	12.62	7.87	−1.04	[−1.71; −0.378]	0.3%
Flores JA 2006 Rats 2.5mg/kg Male	7	36.60	13.229	7	52.20	18.52	−0.91	[−2.03; 0.211]	0.3%
Simpson J 2012 Rats 2.5mg/kg Male	6	52.45	17.594	6	69.42	17.23	−0.90	[−2.11; 0.313]	0.2%
Harro J 1990 Mice 2.5mg/kg Male	9	17.08	11.250	9	36.67	33.75	−0.74	[−1.70; 0.222]	0.3%
Popik P 2006 Rats 2.5mg/kg Male	24	27.81	37.684	24	55.62	49.28	−0.62	[−1.20; −0.043]	0.4%
Wada T 1991 Rats 2.5mg/kg Male	12	6.78	5.939	12	7.73	8.97	−0.12	[−0.92; 0.680]	0.3%
Brioni 1994 Rats 2.99mg/kg Male	8	7.00	3.488	8	14.97	24.00	−0.44	[−1.43; 0.556]	0.3%
Dunn 1998 Rats 3mg/kg Male	6	8.21	4.012	6	24.54	11.29	−1.78	[−3.20; −0.360]	0.2%
Stemmelin J 2008 Rats 3mg/kg Male	13	1.45	2.653	13	11.95	8.90	−1.55	[−2.44; −0.655]	0.3%
Griebel G 2002 Rats 3mg/kg Male	11	12.40	6.633	11	32.90	20.89	−1.27	[−2.20; −0.340]	0.3%
Griebel G 1999 Rats 3mg/kg Male	11	15.20	16.915	11	47.80	31.51	−1.24	[−2.17; −0.313]	0.3%

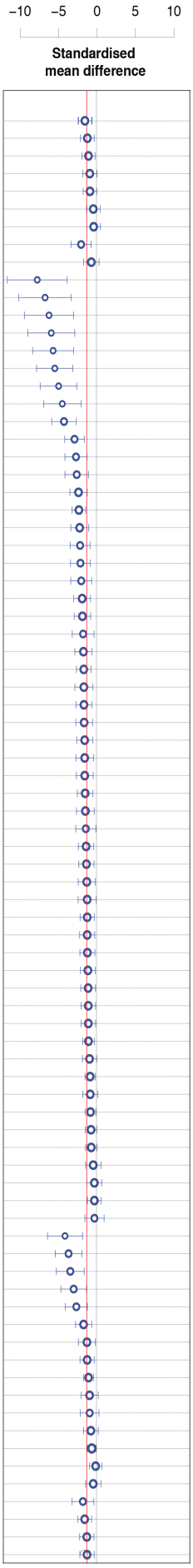
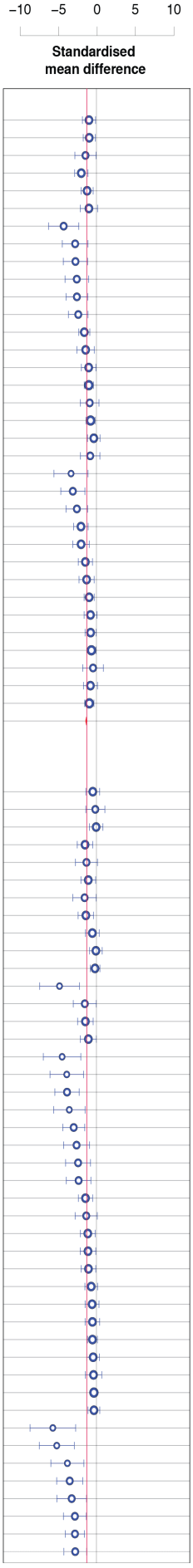


Figure 3 part 4.

	Control N_c	Control Mean	Control SD	Treated N_T	Treated Mean	Treated SD	SMD	95%–CI	Weight
Assay = EPM (continued)									
Steiner MA 2012 Rats 3mg/kg Male	12	22.73	8.998	12	35.06	14.49	−0.99	[−1.84; −0.131]	0.3%
Okuyama S 1999 Rats 3mg/kg Male	14	12.10	5.543	14	19.88	9.70	−0.96	[−1.74; −0.168]	0.3%
Jones GH 1994 Mice 3.13mg/kg Male	8	20.17	6.223	4	59.33	44.78	−1.44	[−2.83; −0.055]	0.2%
Harada K 2006 Rats 3.2mg/kg Male	16	9.11	4.224	16	29.83	13.73	−1.99	[−2.85; −1.123]	0.3%
Fraser LM 2010 Mice 4mg/kg Female and Male	15	17.19	14.012	15	44.32	26.86	−1.23	[−2.02; −0.443]	0.3%
Carro–Juarez M 2012 Rats 4mg/kg Male	7	11.49	5.242	7	19.62	9.44	−1.00	[−2.13; 0.137]	0.3%
Moreira MR 2014 Rats 5mg/kg Male	8	15.93	3.357	8	33.15	4.20	−4.28	[−6.24; −2.324]	0.2%
Tatarczynska E 2004 Rats 5mg/kg Male	6	10.90	3.429	7	70.40	26.70	−2.79	[−4.46; −1.114]	0.2%
Klodzinska A 2004 Rats 5mg/kg Male	7	10.75	4.267	7	70.43	28.45	−2.75	[−4.33; −1.162]	0.2%
Klodzinska 2004 Rats 5mg/kg Male	7	3.61	1.368	7	23.46	10.12	−2.57	[−4.10; −1.044]	0.2%
Weso..owska A 2007 Rats 5mg/kg Male	8	10.90	3.960	8	70.40	30.83	−2.56	[−3.97; −1.150]	0.2%
Larissa FdA 2013 Mice 5mg/kg Male	9	32.37	16.189	9	72.84	16.19	−2.38	[−3.65; −1.109]	0.2%
Yasumatsu H 1994 Rats 5mg/kg Male	20	6.07	3.737	20	15.48	7.28	−1.59	[−2.31; −0.874]	0.3%
Jastrzebska–Wiesek M 2014 Rats 5mg/kg Male	8	26.21	21.847	8	59.59	22.63	−1.42	[−2.55; −0.289]	0.3%
Harro J 1990 Rats 5mg/kg Male	10	31.30	27.512	10	74.70	50.28	−1.03	[−1.97; −0.081]	0.3%
Wada T 1991 Rats 5mg/kg Male	24	6.78	8.399	24	16.23	9.89	−1.01	[−1.62; −0.409]	0.4%
Simpson J 2012 Rats 5mg/kg Male	6	52.45	17.594	6	72.71	23.65	−0.90	[−2.11; 0.315]	0.2%
Popik P 2006 Rats 5mg/kg Male	24	27.81	37.684	24	64.50	53.63	−0.78	[−1.37; −0.190]	0.4%
Langen B 2005 Rats 6mg/kg Male	12	13.09	28.018	12	28.38	50.94	−0.36	[−1.17; 0.449]	0.3%
Jones GH 1994 Mice 6.25mg/kg Male	8	20.17	6.223	4	44.00	47.52	−0.83	[−2.09; 0.436]	0.2%
Tanaka M 2013 Mice 10mg/kg Male	5	12.69	3.153	5	45.97	12.41	−3.32	[−5.53; −1.104]	0.1%
Hazim AI 2014 Rats 10mg/kg Male	8	7.88	7.800	8	46.61	14.85	−3.09	[−4.65; −1.520]	0.2%
Hazim AI 2014 Rats 10mg/kg Male	8	14.30	8.932	8	38.51	8.93	−2.56	[−3.97; −1.152]	0.2%
Okuyama S 1999 Rats 10mg/kg Male	14	12.10	5.543	14	40.00	18.02	−2.03	[−2.97; −1.096]	0.3%
Griebel G 2002 Rats 10mg/kg Male	11	12.40	6.633	11	49.10	23.88	−2.01	[−3.08; −0.954]	0.3%
Wada T 1991 Rats 10mg/kg Male	12	6.78	5.939	12	19.66	10.49	−1.46	[−2.38; −0.541]	0.3%
Wada T 1991 Rats 10mg/kg Male	10	3.75	5.558	10	21.21	17.40	−1.29	[−2.28; −0.312]	0.3%
Yasumatsu H 1994 Rats 10mg/kg Male	20	6.07	3.737	20	12.27	8.06	−0.97	[−1.63; −0.309]	0.3%
Steiner MA 2012 Rats 10mg/kg Male	12	22.73	8.998	12	32.14	13.50	−0.79	[−1.63; 0.044]	0.3%
Harada K 2006 Rats 10mg/kg Male	16	9.11	4.224	16	28.51	34.32	−0.77	[−1.50; −0.052]	0.3%
Popik P 2006 Rats 10mg/kg Male	24	27.81	37.684	24	58.88	52.18	−0.67	[−1.25; −0.089]	0.4%
Jones GH 1994 Mice 12.5mg/kg Male	8	20.17	6.223	3	33.77	56.10	−0.46	[−1.81; 0.888]	0.2%
Rex 2002 Rats 15mg/kg Male	10	8.43	5.376	10	18.57	16.34	−0.80	[−1.72; 0.121]	0.3%
Popik P 2006 Rats 20mg/kg Male	24	27.81	37.684	24	77.22	62.32	−0.94	[−1.54; −0.345]	0.4%
Random effects model	2901			2763			−1.33	[−1.44; −1.219]	72.2%
Heterogeneity: I–squared=67.8%, tau–squared=0.5613, p<0.0001									
Assay = LD									
Wikinski SI 2001 Rats 0.03mg/kg Male	10	11.50	5.493	10	18.28	17.79	−0.49	[−1.39; 0.399]	0.3%
Costall 1990 Mice 0.063mg/kg Male	5	47.81	10.795	5	49.84	11.66	−0.16	[−1.41; 1.080]	0.2%
Barnes NM 1990 Mice 0.063mg/kg Male	10	43.51	18.244	10	44.47	13.68	−0.06	[−0.93; 0.820]	0.3%
Wikinski SI 2001 Rats 0.1mg/kg Male	10	11.50	5.493	10	22.33	8.11	−1.50	[−2.51; −0.482]	0.3%
Costall 1990 Mice 0.125mg/kg Male	5	47.81	10.795	5	67.09	15.29	−1.31	[−2.75; 0.122]	0.2%
Barnes NM 1990 Mice 0.125mg/kg Male	10	43.51	18.244	10	68.75	25.85	−1.08	[−2.03; −0.129]	0.3%
Costall 1990 Mice 0.25mg/kg Male	5	47.81	10.795	5	71.52	16.19	−1.55	[−3.06; −0.046]	0.2%
Barnes NM 1990 Mice 0.25mg/kg Male	10	43.51	18.244	10	72.36	21.28	−1.39	[−2.39; −0.395]	0.3%
Wikinski SI 2001 Rats 0.3mg/kg Male	10	11.50	5.493	10	16.13	9.94	−0.55	[−1.45; 0.344]	0.3%
Birkett 2011 Mice 0.3mg/kg Male	14	11.70	5.332	10	13.04	22.48	−0.09	[−0.90; 0.726]	0.3%
Harada K 2006 Mice 0.32mg/kg Male	19	0.90	1.744	20	1.40	2.91	−0.20	[−0.83; 0.427]	0.3%
Mahendra P 2011 Mice 0.5mg/kg Male	6	20.17	3.707	6	43.33	5.06	−4.82	[−7.40; −2.236]	0.1%
Costall 1990 Mice 0.5mg/kg Male	5	47.81	10.795	5	70.14	15.22	−1.53	[−3.03; −0.028]	0.2%
Barnes NM 1990 Mice 0.5mg/kg Male	10	43.51	18.244	10	78.85	27.37	−1.46	[−2.46; −0.446]	0.3%
Zheng 2009 Mice 0.5mg/kg Female and Male	10	11.77	13.377	7	24.24	6.33	−1.07	[−2.11; −0.018]	0.3%
Sugiyama A 2012 Rats 1mg/kg Male	6	16.89	10.343	6	75.32	13.51	−4.48	[−6.92; −2.043]	0.1%
Yadav AV 2008 Mice 1mg/kg Male	6	26.67	6.859	6	55.83	7.02	−3.88	[−6.06; −1.693]	0.1%
Pires LF 2013 Mice 1mg/kg Male	10	20.64	5.084	10	46.95	7.76	−3.84	[−5.43; −2.256]	0.2%
Saitoh A 2013 Rats 1mg/kg Male	6	24.12	13.634	6	78.91	14.85	−3.55	[−5.60; −1.498]	0.1%
Fajemiroye JO 2014 Mice 1mg/kg Male	9	30.17	3.500	9	45.50	6.00	−2.97	[−4.40; −1.543]	0.2%
Ishaq H 2014 Rats 1mg/kg Male	6	9.30	3.466	6	24.58	6.83	−2.60	[−4.29; −0.914]	0.2%
Galeotti N 2013 Mice 1mg/kg Male	6	33.61	6.579	6	50.21	6.16	−2.40	[−4.02; −0.784]	0.2%
Thippeswamy BS 2011 Mice 1mg/kg Male	6	39.73	10.476	6	66.07	10.32	−2.34	[−3.93; −0.740]	0.2%
Fernandez SP 2008 Mice 1mg/kg Male	12	26.46	9.388	12	42.87	12.38	−1.44	[−2.36; −0.527]	0.3%
Costall 1990 Mice 1mg/kg Male	5	47.81	10.795	5	68.30	16.19	−1.34	[−2.79; 0.102]	0.2%
Barnes NM 1990 Mice 1mg/kg Male	10	43.51	18.244	10	71.15	27.37	−1.14	[−2.10; −0.178]	0.3%
Ven..ncio ET 2011 Rats 1mg/kg Male	9	32.61	10.916	9	47.45	14.18	−1.12	[−2.13; −0.106]	0.3%
Belzung C 1997 Mice 1mg/kg Male	10	24.74	6.766	10	36.24	13.11	−1.06	[−2.00; −0.107]	0.3%
Okuyama S 1999 Mice 1mg/kg Male	12	12.96	10.264	12	22.41	15.40	−0.70	[−1.53; 0.132]	0.3%
Wikinski SI 2001 Rats 1mg/kg Male	10	11.50	5.493	10	17.29	12.29	−0.58	[−1.48; 0.317]	0.3%
Wanasuntronwong 2012 Mice 1mg/kg Male	9	29.29	9.520	9	39.62	24.00	−0.54	[−1.48; 0.406]	0.3%
Harada K 2006 Mice 1mg/kg Male	19	0.90	1.744	20	5.27	11.04	−0.53	[−1.17; 0.106]	0.3%
Belzung C 1997 Mice 1mg/kg Male	12	6.76	10.246	12	11.83	11.71	−0.44	[−1.26; 0.367]	0.3%
Costa 2011 Mice 1mg/kg Male	7	21.29	4.987	7	29.77	27.71	−0.40	[−1.46; 0.663]	0.3%
Bellavite P 2011 Mice 1mg/kg Male	71	15.98	10.870	69	19.91	11.71	−0.35	[−0.68; −0.012]	0.4%
Birkett 2011 Mice 1mg/kg Male	14	11.70	5.332	13	17.70	24.08	−0.34	[−1.10; 0.421]	0.3%
Kurhe YV 2014 Mice 2mg/kg Male	6	1.56	0.498	6	7.06	1.16	−5.69	[−8.65; −2.723]	0.1%
Gupta D 2014 Mice 2mg/kg Male	8	20.92	3.444	8	57.59	8.82	−5.18	[−7.45; −2.906]	0.1%
Swami SU 2014 Mice 2mg/kg Male	6	36.93	6.726	6	61.64	5.19	−3.79	[−5.95; −1.645]	0.1%
Bhatt S 2013 Mice 2mg/kg Male	8	16.56	4.620	8	33.94	4.79	−3.49	[−5.19; −1.799]	0.2%
Gupta D 2015 Mice 2mg/kg Female and Male	6	22.94	4.164	6	58.47	13.72	−3.23	[−5.16; −1.308]	0.2%
Karim N 2011 Mice 2mg/kg Male	8	24.17	10.608	8	47.39	2.97	−2.82	[−4.30; −1.334]	0.2%
Fortes AC 2013 Mice 2mg/kg Male	11	26.21	3.471	11	33.90	1.34	−2.81	[−4.05; −1.574]	0.2%
Karim N 2011 Mice 2mg/kg Male	8	25.04	10.990	8	50.18	5.08	−2.78	[−4.25; −1.304]	0.2%



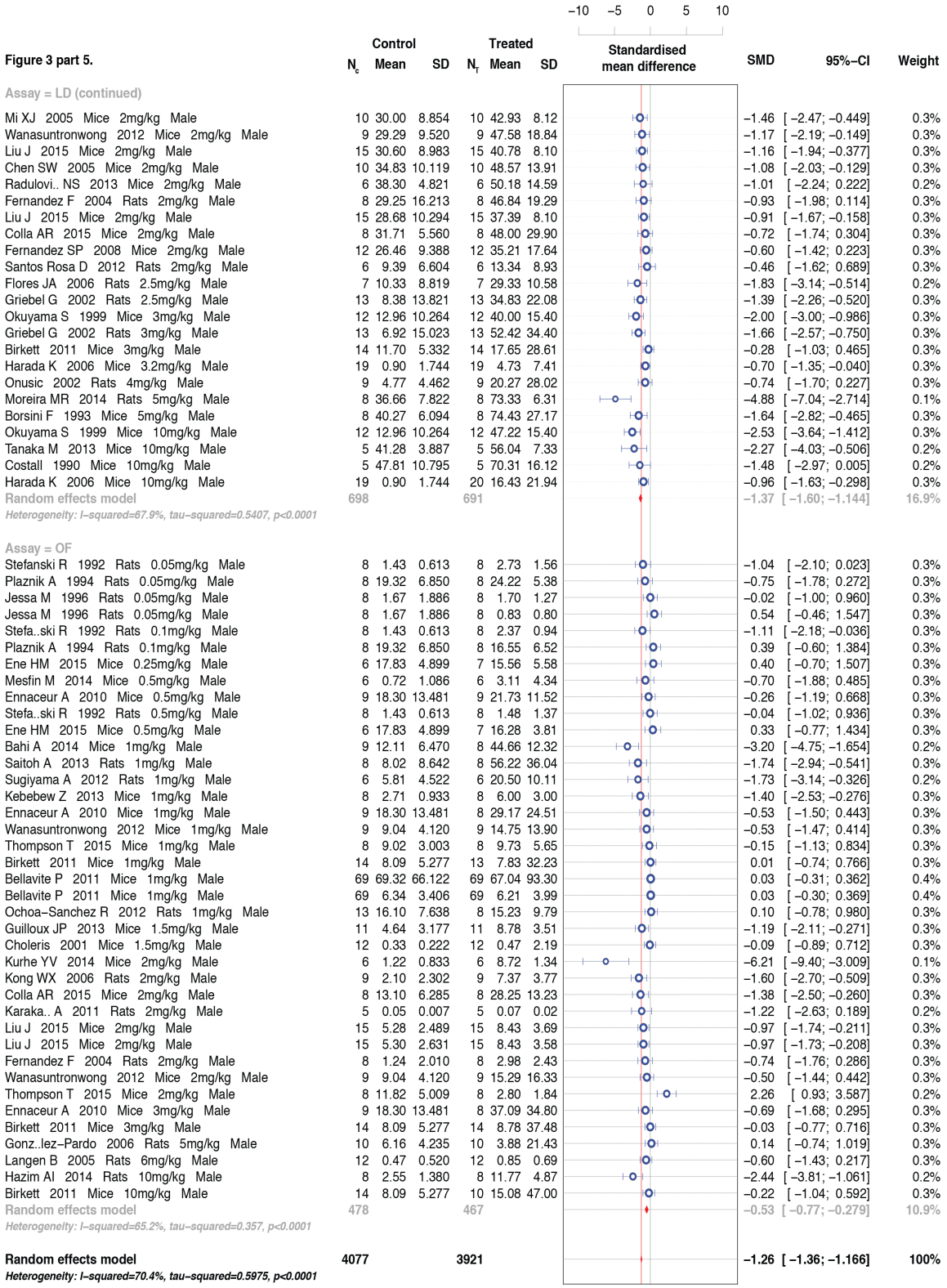


Figure 3. Meta-analysis of diazepam on rodent anxiety related behavior.

Meta-analysis of rodent diazepam effect sizes, shown as a forest plot of standardized effect sizes (Hedges' g). The meta-analysis is sub-grouped by animal species. Error bars indicate the 95% confidence intervals of standardized mean difference. The weighted average mean effect size of subgroups and all studies is represented by the central vertices of a red diamond; the outer vertices indicate the 95% confidence intervals. Control and treatment samples sizes are given in the columns listed as N_C and N_T respectively.

and restraint effects on ARDEB are strongly affected by publication bias.

3.5 Diazepam reduces anxiety-related defense behaviors

Diazepam is an important minor tranquilizer that was used for decades as the first line of treatment for anxiety disorders (Tone, 2009) and, along with other benzodiazepines, is still used extensively to control anxiety (Baldwin et al., 2014). Recent clinical meta-analysis studies have found support for the efficacy of benzodiazepines in the short-term treatment of anxiety disorders (Baldwin et al., 2014). However, a review of diazepam effects in open field studies revealed widespread disagreement between with 29 studies supporting an anxiolytic effect and 23 supporting either an anxiogenic effect or no effect (Prut and Belzung, 2003). We reviewed the available literature on diazepam for the three major rodent ARDEB assays: EPM, OF and LD. This review identified 172 articles containing relevant data (Assie et al., 1993; Bahi et al., 2014; Barbosa et al., 2008; Baretta et al., 2012; Barnes et al., 1990; Bellavite et al., 2011; Belzung and Agmo, 1997; Bhatt et al., 2013; Bhattacharya and Mitra, 1991; Birkett et al., 2011; Blainski et al., 2010; Borsini et al., 1993; Brioni et al., 1994; Carneiro et al., 2005; Carro-Juarez et al., 2012; Cechin et al., 2003; Cha et al., 2005; Chen et al., 2004; 2005; Choleris et al., 2001; Cole and Rodgers, 1995; Colla et al., 2015; Consoli et al., 2007; Contreras et al., 2011; Costa et al., 2011; Costall et al., 1990; Da Silva et al., 1996; Dalvi and Rodgers, 2001; 1999; de A Vieira et al., 2013; de Almeida et al., 2012; de Castro et al., 2007; de Melo et al., 2006; de Sousa et al., 2007; de-Paris et al., 2000; Drapier et al., 2007; R. W. Dunn et al., 1989; 1998; Ene et al., 2015; Engin et al., 2009; Ennaceur et al., 2010; Fajemiroye et al., 2014; Faria et al., 1997; Faturi et al., 2010; F. Fernandez et al., 2004; S. P. Fernandez et al., 2008; Flores et al., 2006; Fortes et al., 2013; Fraser et al., 2010; Frassetto et al., 2010; Galeotti et al., 2013; Girish et al., 2013; Gomes et al., 2010; Ma Eva Gonzalez-Trujano et al., 2006; Maria Eva Gonzalez-Trujano et al., 2015; González-Pardo et al., 2006; Griebel et al., 1998; 1997; 1999a; 1999b; 2002; Guilloux et al., 2013; Gupta et al., 2014; 2015; Han et al., 2009; Harada et al., 2006; Hasenohrl et al., 1996; Hazim et al., 2014; Huerta-Reyes et al., 2013; Hui et al., 2002; Ishaq, 2014; N. S. Jain et al., 2005; Jastrzebska-Wiesek et al., 2014; Jászberényi et al., 2009; 2007; Jessa et al., 1996; Jones et al., 1994; Kalouda and Pitsikas, 2015; Karakas et al., 2011; Karim et al., 2011; Kebebew and Shibeshi, 2013; Klodzinska et al., 2004a; 2004b; Kong et al., 2006; Kumar and Bhat, 2014; Kurhe et al., 2014; Kuribara et al., 2000; la Pena et al., 2013; LaBuda and Fuchs, 2001; Langen et al., 2005; Leggio et al., 2011; Lepicard et al., 2000; Jie Liu et al., 2015; Lolli et al., 2007; Mahendra and Bisht, 2011; Mansouri et al., 2014; Martinez et al., 2006; Mechan et al., 2002; Melo et al., 2010; Mesfin et al., 2014; Meyer et al., 2013; Mi et al., 2005; Micale et al., 2009; 2008; Molander et al., 2011; Molina-Hernandez et al., 2004; Mora et al., 2005; Moreira et al., 2014; Nagaraja et al., 2012; Ochoa-Sanchez et al., 2012; Ognibene et al., 2008; Okuyama et al., 1999; Onusic et al., 2002; Pain et al.,

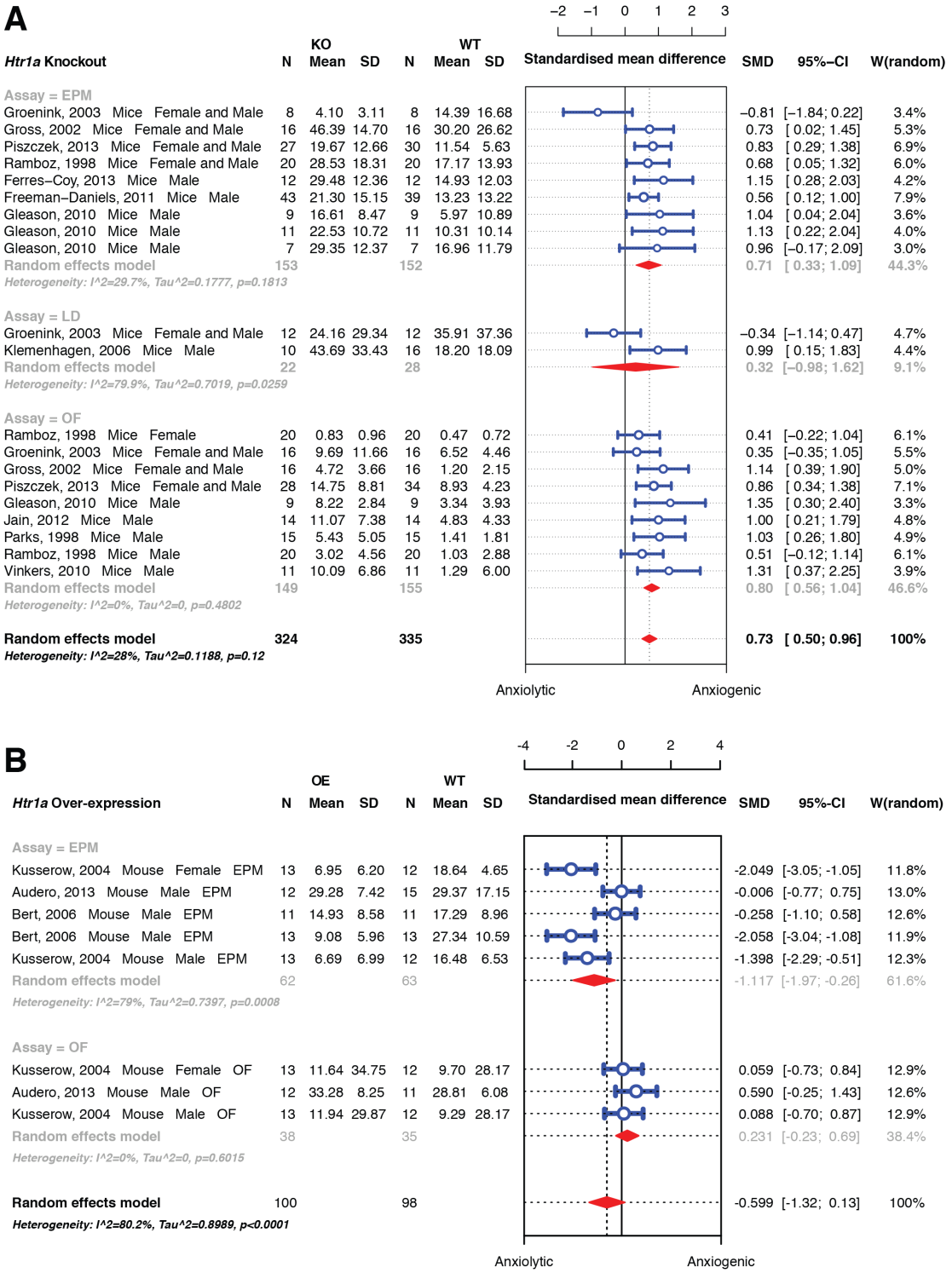


Figure 4. Meta-analyses of serotonin receptor 1A interventions on rodent anxiety-related behaviors.

Meta-analysis of effect sizes of serotonin-targeted interventions is shown as a forest plot of standardized effect sizes (Hedges' g). Error bars indicate the 95% confidence intervals of g. The weighted average mean effect size of all studies is represented by the central vertices of a red diamond; the outer vertices indicate the 95% confidence intervals. Control and treatment samples sizes (N_C, N_T) and the assay types of the studies are given; elevated plus maze (EPM), open field (OF) and light-dark box (LD). Effects of: A. Serotonin receptor gene Htr1a knockout models. B. Htr1a overexpression.

1999; Paine et al., 2002; Parent et al., 2012; Pellow et al., 1985; Peng et al., 2004; Pires et al., 2013; Plaznik et al., 1994; Ponten et al., 2011; Popik et al., 2006; Radulovic et al., 2013; Rago et al., 1988;

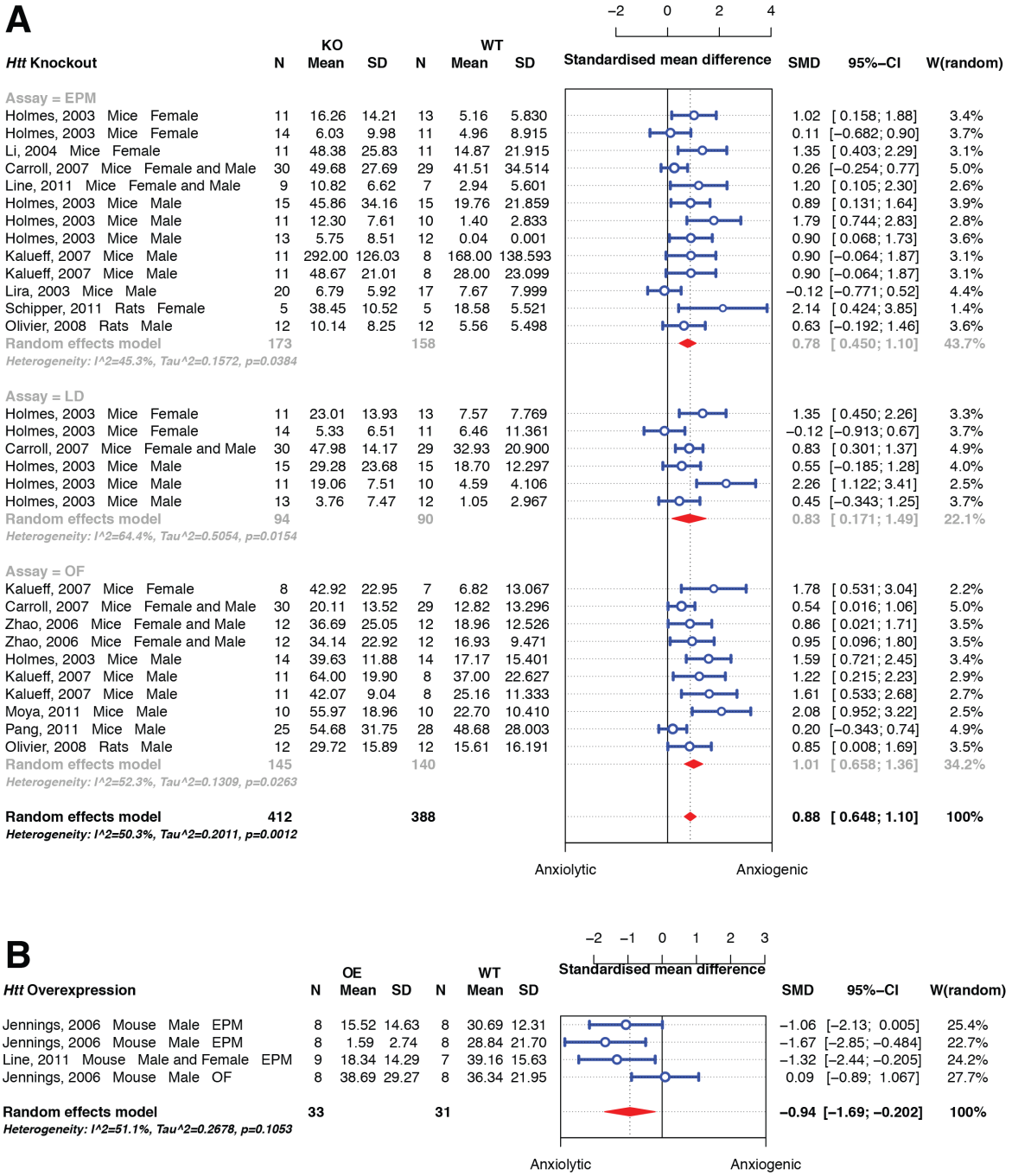


Figure 5. Meta-analyses of serotonin transporter interventions on rodent anxiety-related behaviors.

Meta-analysis of effect sizes of serotonin-targeted interventions is shown as a forest plot of standardized effect sizes (Hedges' g). Error bars indicate the 95% confidence intervals of g. The weighted average mean effect size of all studies is represented by the central vertices of a red diamond; the outer vertices indicate the 95% confidence intervals. Control and treatment samples sizes (N_C, N_T) and the assay types of the studies are given; elevated plus maze (EPM), open field (OF) and light-dark box (LD). Effects of: A. Serotonin transporter gene (Htt) knockout models B. Htt overexpression models.

Ramanathan et al., 1998; Raquibul Hasan et al., 2009; Rejon-Orantes et al., 2013; Rex et al., 2002; Rochford et al., 1997; Saiyudthong and Marsden, 2011; Sakaue et al., 2003; Santos Rosa et al., 2012; Satyan et al., 1998; Schmitt et al., 2002; 2001; Sherif et al., 1994; Silva et al., 2007; Simpson and Kelly, 2012; Sorra et al., 2014; Srinivasan et al., 2003; Stankevicius et al., 2008; Stefanski et al., 1992; Steiner et al., 2012; Stemmelin et al., 2008; Sugiyama et al., 2012; Swami et al., 2014; Taiwo et al., 2012; Tanaka et al., 2013; Tatarczynska et al., 2004; Thippeswamy et al., 2011; Thompson et al., 2015; Thongsaard et al., 1996; Tolardo et al., 2010; Varty et al., 2002; Venancio et al., 2011; Volke et al., 1998; Wada and Fukuda, 1991; Wanasuntronwong et al., 2012; Wang et al., 2015; Wesolowska and Nikiforuk, 2007; Wikinski et al., 2001; Wolfman et al., 1994; Yadav et al., 2008; Yamada et al., 2000; Yao et al., 2010; Yasumatsu et al., 1994; Zanoli et al., 2002; L.-M. Zhang et al., 2014; Zheng et al., 2009). Calculation of an average Hedges' g (Cumming, 2012) for the 386 experiments contained therein indicated that diazepam had a very large effect on ARDEB, with a -1.26 *g* [95CI -1.36, -1.17] reduction compared with untreated control animals (Figure 3, Table 2). However, as Egger's regression indicated the source literature was affected by publication bias, trim-

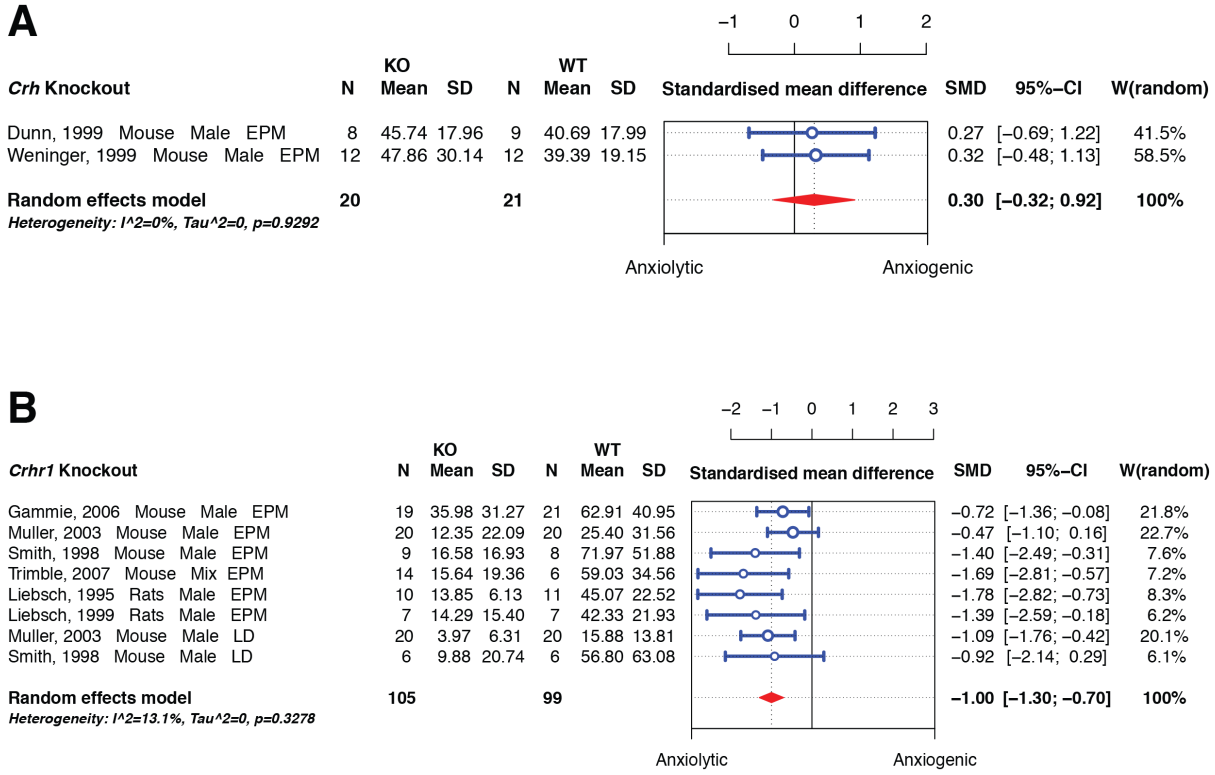


Figure 6. Meta-analyses of the effects of stress signaling genes on anxiety-related behaviors. Meta-analysis of effect sizes of stress signaling genes, shown as a forest plot of standardized effect sizes (Hedges' g). Error bars indicate the 95% confidence intervals of g. The weighted average mean effect size of all studies is represented by the central vertices of a red diamond; the outer vertices indicate the 95% confidence intervals. Control and treatment samples sizes (N_C, N_T) and the assay types of the studies are given; elevated plus maze (EPM), open field (OF) and light-dark box (LD). Effects of: A. Crh gene knockout models. B. Crhr1 gene knockout models.

and-fill correction indicated a smaller—though still large—effect of -0.85 *g* [95CI -0.74, -0.96]. The meta-analysis had a moderate level of heterogeneity (I^2 = 70.4%). Subgroup analysis of assay types suggest that assays were not major source of heterogeneity (Figure 3). Subgroup analysis of the assay types showed that EPM and LD yielded very similar diazepam effect sizes (g = -1.33 and -1.37 respectively, Figure 3). However, the diazepam effect size in OF was found to be substantially smaller (g = -0.53, Figure 3). Additional subgroup analyses of treatment duration variables, dosages and route of treatments revealed that these factors were also only minor sources of heterogeneity (data not shown), indicating that laboratory, dosage, strain and other possible sources of experimental variation likely play a role. As diazepam is universally accepted to be an effective anxiolytic, and it has a robust (bias-corrected) effect in the rodent ARDEB assays, this meta-analytic result verifies the validity of the defense behavior tests.

3.6 5-HT1A receptor function influences ARDEB

Following negative publicity regarding the adverse effects of benzodiazepines (Tone, 2009), pharmaceutical companies focused on the serotonergic system (Griebel and Holmes, 2013). Of the fourteen mammalian serotonergic receptors, much investigation has centered on the serotonin receptor 5-HT1A and its proposed influence on anxiety disorders and depression (Samuels et al., 2014). More than 1200 articles describe experiments connecting 5-HT1A agonism with rodent anxiety (Griebel and Holmes, 2013). However, a substantial proportion of those articles reported that 5-HT1A agonists or knockout of the *Htr1a* gene either produced no effect on anxiety or an effect that was opposite to the receptor's proposed mode of action (Griebel and Holmes, 2013). We systematically reviewed the literature on gene manipulations of *Htr1a* and identified 11 knockout articles (Ferrés-Coy et al., 2013; Freeman-Daniels et al., 2011; Gleason et al., 2010; Groenink et al., 2003; Gross et al., 2002; A. Jain et al., 2012; Klemenhagen et al., 2006; Parks et al., 1998; Piszczek et al., 2013; Ramboz et al., 1998; Vinkers et al., 2010). Meta-analysis of the knockout data revealed that removal of *Htr1a* produced a moderate increase (Hedges' g = 0.73 [95CI 0.50, 0.96] , P = 3.5×10^{-10}) in ARDEB phenotypes (Figure 4A). The three studies of *Htr1a* overexpression found by the review (Audero et al., 2013; Bert et al., 2006; Kusserow et al., 2004) indicated that this intervention moderately decreased ARDEB (g = -0.6 [95CI -1.3, 0.13], P = 0.11; Figure 4B). The cumulative sample size of *Htr1a* overexpression is quite substantial (N = 100, 98), though the moderate effect size observed was not statistically significant and had high heterogeneity (I^2 = 80%). These results confirm that *Htr1a* function has a moderate effect on rodent anxiety.

3.7 Anxiotropic effects of the serotonin transporter

The serotonin transporter (SERT) is the target for the selective serotonin reuptake inhibitors (SSRIs), a class of drugs used to treat depression and anxiety (Baldwin et al., 2014). Meta-analysis of thirteen knockout studies (Carroll et al., 2007; Holmes et al., 2003a; 2003b;

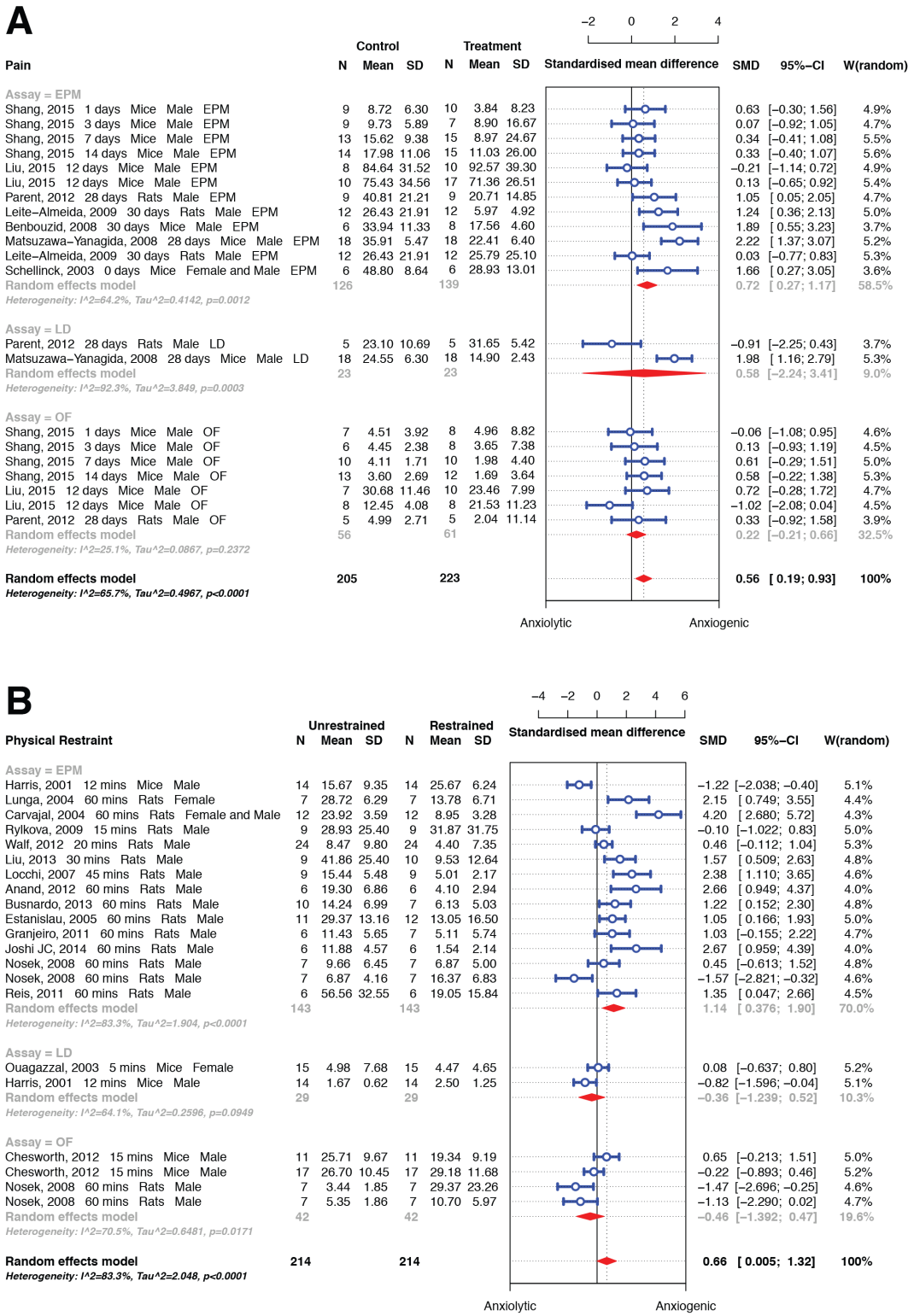
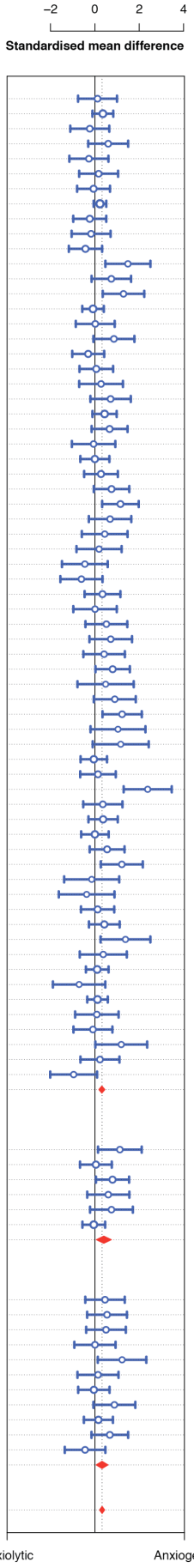


Figure 7. Meta-analyses of experiments on the stress-anxiety relationship in rodents.
Meta-analysis of effect sizes of stress-anxiety interventions, shown as a forest plot of standardized effect sizes (Hedges' g). Error bars indicate the 95% confidence intervals of g. The weighted average mean effect size of all studies is represented by the central vertices of a red diamond; the outer vertices indicate the 95% confidence intervals. Control and treatment samples sizes (N_C, N_T) and the assay types of the studies are given; elevated plus maze (EPM), open field (OF) and light-dark box (LD). Effects of: A. Acute pain. B. Restraint stress (immobilization). C. Social isolation.

C

Figure 7 part 2.

Social Isolation	Group–housed N	Group–housed Mean	Group–housed SD	Isolated N	Isolated Mean	Isolated SD	SMD	95%–CI	W(random)
Assay = EPM									
Abramov, 2004 21 days Mice Female	10	5.40	18.41	10	3.03	18.96	1e−01	[−0.76; 1.00]	1.1%
Blednov YA, 2001 6 days Mice Female and Male	32	10.58	9.31	40	6.94	10.41	4e−01	[−0.11; 0.83]	2.1%
Majercsik, 2003 7 days Mice Male	10	14.95	10.97	10	17.50	10.37	−2e−01	[−1.11; 0.65]	1.1%
Majercsik, 2003 7 days Mice Male	10	22.12	6.58	10	15.40	13.60	6e−01	[−0.30; 1.50]	1.1%
Majercsik, 2003 7 days Mice Male	10	19.43	7.24	10	22.13	11.61	−3e−01	[−1.15; 0.61]	1.1%
Majercsik, 2003 7 days Mice Male	10	15.47	6.96	10	13.42	14.70	2e−01	[−0.71; 1.05]	1.1%
Rodgers, 1993 7 days Mice Male	14	4.67	2.99	14	4.83	2.62	−6e−02	[−0.80; 0.68]	1.4%
Estelles J, 2007 14 days Mice Male	97	15.08	18.53	94	4.36	63.27	2e−01	[−0.05; 0.52]	2.7%
Rodgers, 1993 14 days Mice Male	14	4.67	2.99	14	5.53	4.24	−2e−01	[−0.97; 0.51]	1.4%
Abramov, 2004 21 days Mice Male	10	2.71	19.34	10	8.00	37.53	−2e−01	[−1.05; 0.71]	1.1%
Rodgers, 1993 21 days Mice Male	14	4.67	2.99	14	6.00	3.12	−4e−01	[−1.17; 0.33]	1.4%
Wei XY, 2007 21 days Mice Male	10	29.25	15.78	10	11.07	4.84	1e+00	[0.48; 2.51]	0.9%
Koike H, 2009 28 days Mice Male	10	13.41	7.81	11	8.20	5.56	7e−01	[−0.15; 1.64]	1.1%
Koike H, 2009 28 days Mice Male	10	41.80	27.61	12	14.11	12.22	1e+00	[0.35; 2.23]	1.0%
Moragrega , 2003 30 days Mice Male	32	38.10	16.97	33	39.40	14.36	−8e−02	[−0.57; 0.40]	2.1%
Liu X, 2013 42 days Mice Male	10	8.09	53.49	10	7.12	49.05	2e−02	[−0.86; 0.89]	1.1%
Workman JL, 2011 42 days Mice Male	10	31.46	20.09	10	17.50	8.92	9e−01	[−0.07; 1.78]	1.1%
Voikar V, 2005 49 days Mice Male	15	2.43	17.04	15	15.97	59.64	−3e−01	[−1.02; 0.42]	1.4%
Imanaka A, 2006 7 days Rats Female	15	24.37	32.79	12	22.50	20.37	6e−02	[−0.69; 0.82]	1.4%
Leussis MP, 2008 7 days Rats Female	8	16.17	45.73	8	4.88	28.27	3e−01	[−0.71; 1.27]	1.0%
Da Silva NL, 1997 28 days Rats Female	10	43.89	23.41	10	20.03	39.00	7e−01	[−0.20; 1.62]	1.1%
Pisu MG, 2013 28 days Rats Female	27	19.24	31.10	26	7.55	19.73	4e−01	[−0.11; 0.99]	1.9%
Imanaka A, 2009 35 days Rats Female	12	25.80	17.67	13	14.27	15.86	7e−01	[−0.14; 1.48]	1.3%
Doremus−Fitzwater TL, 2009 49 days Rats Female	8	3.94	23.70	8	5.36	24.07	−6e−02	[−1.04; 0.92]	1.0%
Knuth ED, 2007 7 days Rats Female and Male	18	0.06	76.83	18	0.04	46.90	3e−04	[−0.65; 0.65]	1.6%
Reis FM, 2012 0.02 days Rats Male	14	27.94	23.77	13	22.18	15.26	3e−01	[−0.48; 1.04]	1.4%
Reis FM, 2012 0.08 days Rats Male	14	27.94	23.77	12	12.33	14.63	8e−01	[−0.05; 1.55]	1.3%
Reis FM, 2012 1 days Rats Male	14	27.94	23.77	13	6.57	7.63	1e+00	[0.33; 1.98]	1.2%
Haller J, 1999 4 days Rats Male	9	13.83	21.17	9	2.70	5.29	7e−01	[−0.27; 1.64]	1.0%
Cheeta S, 2001 7 days Rats Male	7	36.57	17.11	8	29.69	11.89	4e−01	[−0.59; 1.48]	0.9%
Cheeta S, 2001 7 days Rats Male	7	33.62	9.47	8	31.66	9.85	2e−01	[−0.83; 1.21]	0.9%
Cheeta S, 2001 7 days Rats Male	7	21.96	13.50	8	31.24	23.23	−5e−01	[−1.48; 0.58]	0.9%
Haller J, 2000 7 days Rats Male	9	7.06	3.17	9	11.88	10.23	−6e−01	[−1.56; 0.34]	1.0%
Haller J, 2000 7 days Rats Male	12	14.46	13.85	12	10.35	8.55	3e−01	[−0.46; 1.15]	1.3%
Leussis MP, 2008 7 days Rats Male	8	7.63	45.90	8	7.29	37.92	8e−03	[−0.97; 0.99]	1.0%
Majercsik, 2003 7 days Rats Male	9	14.36	12.12	9	8.62	8.61	5e−01	[−0.42; 1.46]	1.0%
Majercsik, 2003 7 days Rats Male	9	12.66	13.20	9	4.81	6.60	7e−01	[−0.24; 1.68]	1.0%
Majercsik, 2003 7 days Rats Male	9	9.75	8.10	9	6.82	4.89	4e−01	[−0.52; 1.35]	1.0%
Reis FM, 2012 7 days Rats Male	14	27.94	23.77	14	11.23	15.84	8e−01	[0.03; 1.58]	1.3%
Wright, 2001 7 days Rats Male	5	10.57	12.22	5	5.53	4.99	5e−01	[−0.78; 1.76]	0.7%
Das SK, 2015 14 days Rats Male	12	28.51	6.32	8	23.24	4.29	9e−01	[−0.05; 1.85]	1.0%
dos Santos L, 2010 14 days Rats Male	12	9.08	7.33	12	2.12	2.45	1e+00	[0.35; 2.12]	1.1%
Lapiz MD, 2001 14 days Rats Male	6	29.75	14.40	6	17.17	6.34	1e+00	[−0.19; 2.28]	0.7%
Lapiz MD, 2001 14 days Rats Male	6	17.19	7.79	6	9.82	2.71	1e+00	[−0.10; 2.43]	0.7%
Quintino−dos−Santos JW, 2014 20 days Rats Male	18	16.10	29.90	27	18.39	56.63	−5e−02	[−0.64; 0.55]	1.7%
Bledsoe AC, 2011 21 days Rats Male	12	13.31	48.84	12	6.43	45.16	1e−01	[−0.66; 0.94]	1.3%
Brenes JC, 2009 28 days Rats Male	12	7.68	1.43	12	4.51	1.12	2e+00	[1.30; 3.47]	0.8%
Da Silva NL, 1996 28 days Rats Male	10	38.92	62.39	10	18.18	46.79	4e−01	[−0.53; 1.25]	1.1%
Pisu MG, 2011 28 days Rats Male	18	19.89	68.76	18	1.18	8.96	4e−01	[−0.29; 1.03]	1.6%
Pisu MG, 2014 28 days Rats Male	20	12.77	39.44	20	12.68	40.96	2e−03	[−0.62; 0.62]	1.7%
Serra M, 2000 28 days Rats Male	13	30.92	41.87	13	13.96	1.27	6e−01	[−0.23; 1.34]	1.3%
Cuenya L, 2012 30 days Rats Male	10	9.97	4.46	11	5.38	2.63	1e+00	[0.27; 2.16]	1.0%
Fone KC, 1996 35 days Rats Male	5	10.58	28.36	5	14.93	26.87	−1e−01	[−1.38; 1.10]	0.7%
Fone KC, 1996 35 days Rats Male	5	12.47	33.08	5	24.14	22.81	−4e−01	[−1.63; 0.89]	0.7%
Imanaka A, 2008 35 days Rats Male	12	18.57	14.20	16	16.13	21.60	1e−01	[−0.62; 0.87]	1.4%
Chappell AM, 2013 42 days Rats Male	16	23.62	41.14	17	8.27	28.26	4e−01	[−0.26; 1.12]	1.5%
Hirani K, 2005 42 days Rats Male	8	18.47	6.43	8	9.28	6.15	1e+00	[0.26; 2.50]	0.8%
Kokare DM, 2010 42 days Rats Male	7	13.55	20.53	7	6.28	14.90	4e−01	[−0.68; 1.44]	0.9%
McCool BA, 2009 42 days Rats Male	28	21.50	56.09	31	15.67	51.09	1e−01	[−0.40; 0.62]	2.0%
Pritchard LM, 2008 42 days Rats Male	6	23.88	12.08	6	33.85	13.82	−7e−01	[−1.89; 0.47]	0.7%
Ryu V, 2009 42 days Rats Male	36	12.83	80.30	36	4.96	42.25	1e−01	[−0.34; 0.58]	2.1%
Doremus−Fitzwater TL, 2009 49 days Rats Male	8	6.30	68.85	8	1.60	12.97	9e−02	[−0.89; 1.07]	1.0%
Yildirim E, 2012 49 days Rats Male	10	27.34	20.43	10	29.81	33.19	−9e−02	[−0.96; 0.79]	1.1%
Yorgason JT, 2013 49 days Rats Male	7	20.77	11.19	7	7.65	9.33	1e+00	[0.02; 2.36]	0.8%
Zhang Y, 2012 56 days Rats Male	10	13.10	2.78	10	12.10	5.19	2e−01	[−0.65; 1.11]	1.1%
Thorsell A, 2006 84 days Rats Male	9	18.40	20.11	7	39.20	21.16	−1e+00	[−2.01; 0.10]	0.9%
Random effects model	889			906			3e−01	[0.18; 0.45]	79.5%
Heterogeneity: I^2=31%, Tau^2=0.1354, p=0.0103									
Assay = LD									
Wei XY, 2007 21 days Mice Male	9	47.62	17.99	10	26.22	18.22	1e+00	[0.14; 2.11]	1.0%
Voikar V, 2005 49 days Mice Male	15	43.10	22.08	15	42.20	14.33	5e−02	[−0.67; 0.76]	1.5%
Voikar V, 2005 49 days Mice Male	15	46.40	17.82	15	31.00	19.75	8e−01	[0.05; 1.54]	1.4%
Carrier N, 2012 21 days Rats Female	9	31.61	27.47	9	17.35	15.84	6e−01	[−0.34; 1.56]	1.0%
Carrier N, 2012 21 days Rats Male	9	29.45	21.16	9	14.01	17.97	7e−01	[−0.21; 1.71]	1.0%
McCool BA, 2009 42 days Rats Male	28	40.47	47.62	31	42.40	31.18	−5e−02	[−0.56; 0.46]	2.0%
Random effects model	85			89			4e−01	[0.06; 0.75]	7.8%
Heterogeneity: I^2=35.1%, Tau^2=0.0301, p=0.1735									
Assay = OF									
Linge R, 2013 28 days Mice Male	10	13.65	25.65	10	4.19	11.15	5e−01	[−0.43; 1.35]	1.1%
Liu X, 2013 42 days Mice Male	10	22.13	35.67	10	5.93	17.84	6e−01	[−0.35; 1.45]	1.1%
Conrad KL, 2011 49 days Mice Male	10	23.43	21.13	10	12.04	22.24	5e−01	[−0.39; 1.40]	1.1%
Knuth ED, 2007 7 days Rats Female	8	7.22	19.52	10	7.08	24.67	6e−03	[−0.92; 0.94]	1.1%
Hermes G, 2011 70 days Rats Female	8	43.63	20.29	8	19.52	16.63	1e+00	[0.13; 2.32]	0.8%
Knuth ED, 2007 7 days Rats Male	9	6.77	15.00	9	4.57	14.40	1e−01	[−0.78; 1.07]	1.1%
Blakley G, 2006 14 days Rats Male	33	0.50	3.10	10	0.67	5.31	−4e−02	[−0.75; 0.66]	1.5%
Das SK, 2015 14 days Rats Male	12	20.48	4.48	8	16.84	2.97	9e−01	[−0.06; 1.83]	1.0%
Djordjevic J, 2012 21 days Rats Male	18	16.97	29.92	18	11.38	37.42	2e−01	[−0.49; 0.82]	1.6%
Lukkes JL, 2009 21 days Rats Male	12	48.09	23.22	12	34.92	13.04	7e−01	[−0.15; 1.50]	1.2%
Thorsell A, 2006 84 days Rats Male	10	11.31	13.39	9	20.04	23.64	−4e−01	[−1.35; 0.47]	1.1%
Random effects model	140			114			3e−01	[0.06; 0.59]	12.7%
Heterogeneity: I^2=0%, Tau^2=0.0157, p=0.4675									
Random effects model	**1114**			**1109**			**3e−01**	**[0.21; 0.44]**	**100%**
Heterogeneity: I^2=26.9%, Tau^2=0.1091, p=0.0151									



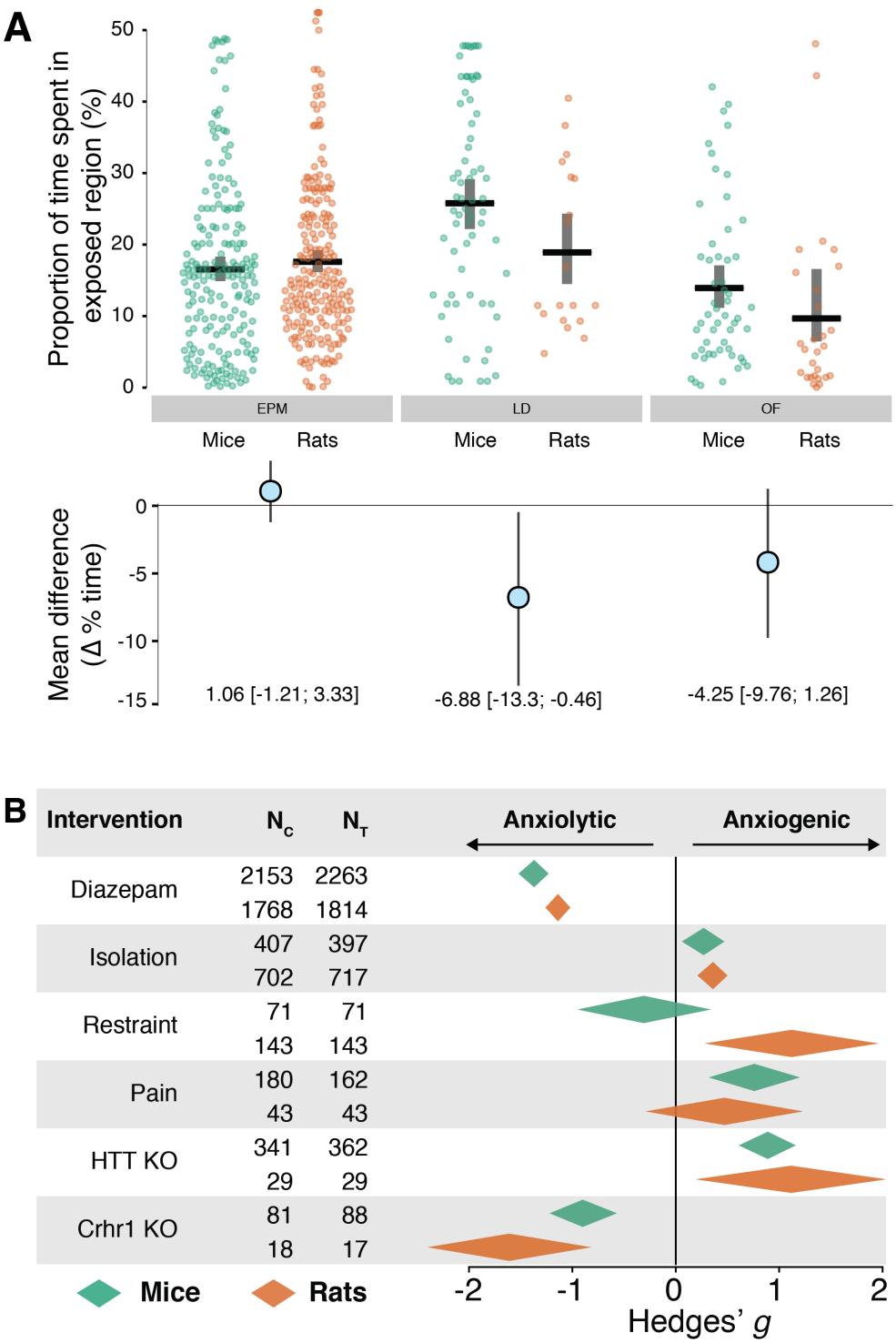


Figure 8. Species differences in ARDEB.
A. Contrasts of mice and rats naive defence behaviors in three different assays. Upper panel shows the means of proportion of time spent in the exposed region, categorized by assay type and species. Each point is the mean value of an experiment. The lower panel shows the contrast means and confidence intervals (mean difference of the percent time spent in exposed region). B. The weighted mean effect sizes of six interventions subgrouped into mice and rats. Color indicates species (green = mice, orange = rats). Each mean effect size is represented by the central vertices of diamond; the outer vertices indicate the 95% confidence interval. The horizontal axis is Hedges' g, the standard deviation change relative to control animals. N_C and N_T indicate control and treatment animal sample sizes respectively.

Kalueff et al., 2007a; 2007b; Li et al., 2004; Line et al., 2011; Lira et al., 2003; Moya et al., 2011; Olivier et al., 2008; Pang et al., 2011; Schipper et al., 2011; Zhao et al., 2006) revealed a large anxiogenic effect (g = 0.88 [95CI 1.26, 0.23] , P = 5.2 x 10^{-14}; Figure 5A) produced by knocking out the SERT gene, *Htt*. However, a funnel plot and Egger's regression revealed a pronounced bias in reported effect sizes (Egger's test P = 6.7 X 10^{-6}, Table 2). Trim-and-fill adjustment added ten imputed data points to the left segment of the funnel plot, lowering the effect size to g = 0.57 [95CI 0.29, 0.86], a moderate effect. Only two articles studying the effect of *Htt* overexpression on ARDEB were found (Jennings et al., 2006; Line et al., 2011). Meta-analysis revealed a large anxiolytic effect (g = -0.94 [95CI -1.69, -0.20], P = 0.013; Figure 5B) in EPM and OF assays (no LD articles were found). The transporter gene knockout and overexpression effects clearly connect *Htt* function to rodent anxiety. However, the direction of effects is the opposite of what would be expected from the clinical application of SERT inhibitors, given that SSRI reduction of SERT function is believed to have a therapeutic, anxiety-reducing effect.

3.8 The effect of acute pain on rodent anxiety

Environmental stressors have physiological effects on animals that promote the anxiety-like state (van Praag, 2003). To survey a range of stress modalities we selected acute pain, bodily restraint and social isolation for review; all three have been found to promote anxiety in humans (Sherif and Oreland, 1995). The systematic review identified seven papers measuring the effect of acute pain on ARDEB (Benbouzid et al., 2008; Leite-Almeida et al., 2012; Yan Liu et al., 2015; Matsuzawa-Yanagida et al., 2008; Parent et al., 2012; Schellinck et al., 2003; Shang et al., 2014). Meta-analysis of the 21 experiments therein indicated a moderate anxiogenic effect (g = 0.56 [95CI 0.19, 0.93], P = 2.9 x 10^{-3}; Figure 6A).

3.9 The effect of bodily restraint on rodent anxiety

Review of 16 studies of rodent bodily restraint (Anand et al., 2012; Busnardo et al., 2013; Carvajal et al., 2004; Chesworth et al., 2012; Estanislau and Morato, 2005; Granjeiro et al., 2011; Harris et al., 2001; Joshi et al., 2014; Jing Liu et al., 2011; Locchi et al., 2008; Lunga and Herbert, 2004; Nosek et al., 2008; Ouagazzal et al., 2003; D. G. Reis et al., 2011; Rylkova et al., 2009; Walf and Frye, 2012) containing 21 experiments indicated that it had an overall moderate anxiogenic effect in EPM and OF assays (g = 0.70 [95% CI 0.82 – 1.32], P = 0.027; Figure 6B). The restraint meta-analysis had a high level of heterogeneity, I^2 = 89%; a subgroup analysis by assay type revealed that the different assays were not the source of this variability (data not shown).

3.10 Social isolation has a small effect on defense behaviors

Systematic review identified 50 articles on social isolation and ARDEB (Abramov et al., 2004; Blakley and Pohorecky, 2006; Blednov et al.,

2001; Bledsoe et al., 2011; Brenes et al., 2009; Carrier and Kabbaj, 2012; Chappell et al., 2013; Cheeta et al., 2001; Conrad et al., 2011; Cuenya et al., 2012; Da Silva et al., 1996; Das et al., 2014; Djordjevic et al., 2012; Doremus-Fitzwater et al., 2009; Estelles et al., 2007; Fone et al., 1996; Haller and Halász, 1999; Haller et al., 2000; Hermes et al., 2011; Hirani et al., 2005; Imanaka et al., 2008; 2006; Knuth and Etgen, 2007; Koike et al., 2009; Kokare et al., 2010; Lapiz et al., 2001; Leussis and Andersen, 2008; Linge et al., 2013; Xiao Liu et al., 2013; Lukkes et al., 2009; Majercsik et al., 2003; McCool and Chappell, 2009; Moragrega et al., 2003; Pisu et al., 2013; 2011; Pritchard et al.,

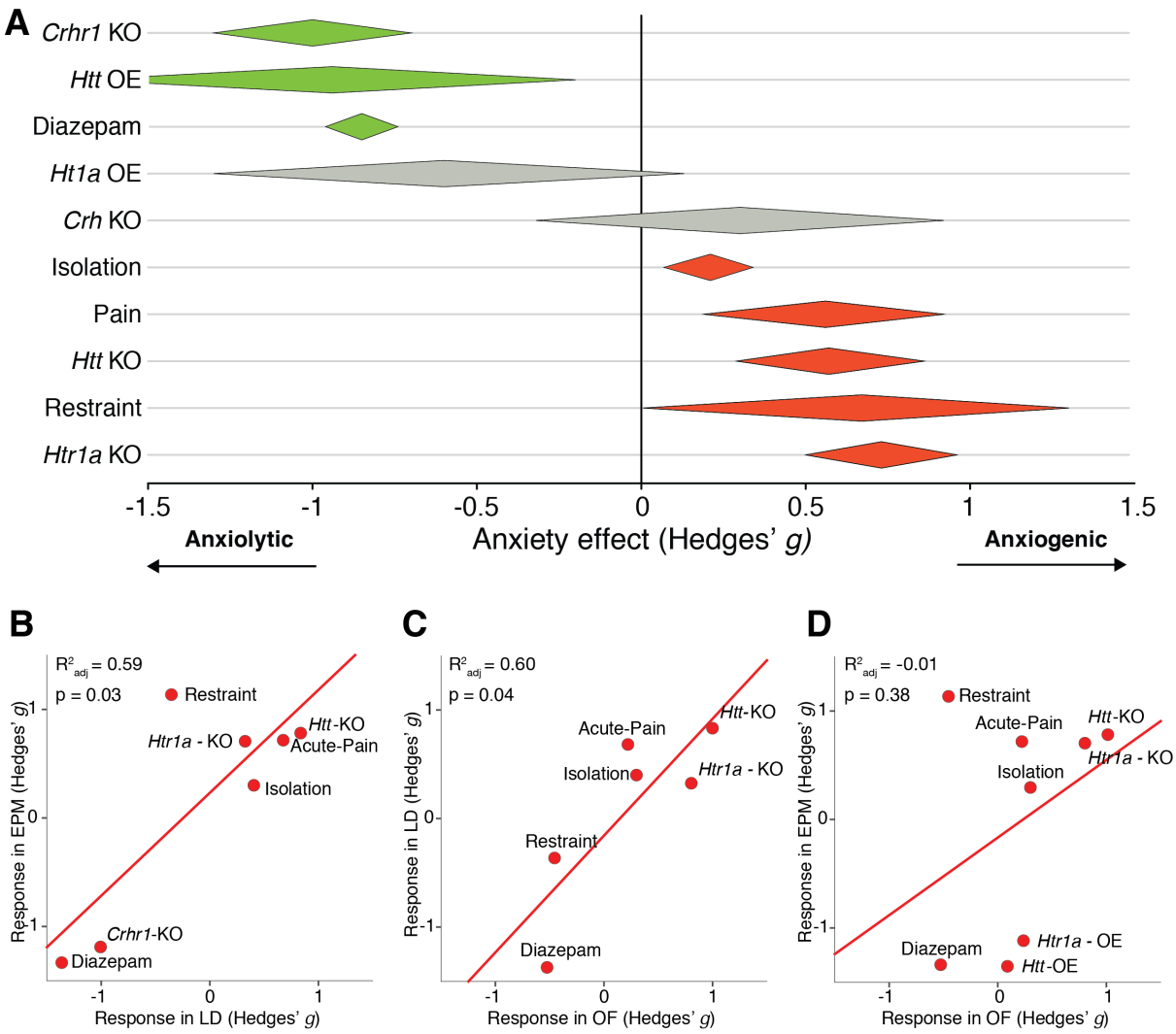


Figure 9. Summary effect sizes of all meta-analyses.

A. The weighted mean effect sizes of all 10 interventions are shown here. Color indicates direction (green = anxiolytic, red = anxiogenic) and statistical significance (grey = statistically non-significant). The diamonds for the diazepam, social isolation, and Htt KO meta-analyses represent the summary effect sizes after trim-and-fill bias correction. Each mean effect size is represented by the central vertices of a diamond; the outer vertices indicate the 95% confidence intervals. The horizontal axis is Hedges' g, the standard deviation change relative to control animals. B. Method comparison of LD and EPM shows that the two methods report ARDEB changes with 59% concordance. C. Method comparison of OF and LD shows that the two methods reports ARDEB changes with 60% concordance. D. Method comparion of OF and EPM shows that there is no concordance between the two methods.

2008; Quintino-dos-Santos et al., 2014; F. M. C. V. Reis et al., 2012; Rodgers and Cole, 1993; Ryu et al., 2009; Santos et al., 2010; Serra et al., 2000; Thorsell et al., 2006; Voikar et al., 2005; Wei et al., 2007; Workman et al., 2011; Wright and Ingenito, 2001; Yildirim et al., 2012; Yorgason et al., 2013; Y. Zhang et al., 2012). Meta-analysis revealed a small anxiogenic effect (g = 0.33 [95CI 0.21, 0.44], P = 3.4 x 10^{-8}; Figure 6C), but this included likely publication bias; the trim-and-fill method corrected the anxiogenic effect to only 0.21 *g* [95CI 0.07, 0.34], P = 3.1 x 10^{-3}) a very small anxiotropic effect (Figure 2B). It appears that, unlike the physical stressors, social isolation has only a modest influence on the ARDEB assays.

3.11 Crh gene knockout has a modest effect on rodent ARDEB

Several neuropeptide-related genes involved in stress signaling have been linked to anxiety, notably the peptide, corticotropin-releasing hormone (CRH; also known as corticotropin-releasing factor) (Kormos and Gaszner, 2013) and its receptor, CRHR1. Two studies that examined the effects of *Crh* knockouts on ARDEB were found (Weninger et al., 1999), which revealed only a small effect (g = 0.30 [95CI -0.32, 0.92], P = 0.34; Figure 7A). This supports the idea that CRH has only a modest effect on the ARDEB. The meta-analytic result may suffer from insufficient precision as the cumulative sample size was small (N = 20, 21). As publication bias appears to affect the literature, this small effect could be an overestimate.

3.12 Crhr1 gene knockout has a large effect on rodent anxiety

CRH exerts its biological action via two receptors known as CRHR1 and CRHR2. The two receptors are pharmacologically distinct and only the former has been widely studied in the context of anxiety (Owens and Nemeroff, 1991; Paez-Pereda et al., 2011). Meta-analysis (Gammie and Stevenson, 2006; Liebsch et al., 1999; 1995; Müller et al., 2003; Smith et al., 1998; Trimble et al., 2007) found that, in contrast to the *Crh* knockout, deletion of *Crhr1* had a large anxiolytic effect on ARDEB (g = -1.0 [95CI -1.30, -0.70], P = 6.64 x 10^{-11}; Figure 7B). The discordance between *Crh* and *Crhr1* knockout effects has previously been attributed to the action of other peptide ligand(s) of *Crhr1*, either urocortin or another, unidentified ligand (A. J. Dunn and Swiergiel, 1999).

3.13 Species and sex differences in ARDEB

Rats and mice have differences in their defensive behavior when exposed to predators or predator cues (Blanchard, 2001). We used the synthetic data to examine species differences in baseline ARDEB prior to anxiotropic manipulation. The LD box showed the most substantial difference between species -6.88 % [-13.3; -0.46], P = 0.04, but in general, inter-species differences in naive ARDEB were minor when compared to the overall variance (Figure 8A).

Where rat data were available, inter-species differences between the effects of anxiety-related interventions were investigated. There were only minor inter-species differences in the meta-analytic effect sizes of

the interventions (Figure 8B). A striking exception to this trend was a large rat-mouse difference in the response to restraint: this treatment appeared strongly anxiogenic for rats, but modestly anxiolytic for mice.

We investigated sex differences in ARDEB, but found that the anxiety studies contained ∼18× fewer experiments on female rodents than males, rendering any meta-analytic estimates of female ARDEB imprecise.

3.14 Validity of the ARDEB assays

Of the interventions analyzed above, only diazepam has extensive clinical evidence supporting its ability to alter human anxiety. The large anxiolytic diazepam effect size observed with the ARDEB assays verifies their validity (Figure 9A). The stressors—isolation, acute pain and restraint—would all be expected to produce increases in human anxiety, and all show anxiogenic effects in the ARDEB, thus also verifying the validity of these assays (Figure 9A), with the exception of the surprisingly small social isolation effect (0.21 *g*). Establishing the validity of animal models relies partly on showing concordance between models (Campbell and Fiske, 1959; van der Staay, 2006). To explore the concordance between the three assays, we conducted regression analyses on all possible two-way comparisons of ΔARDEB (Figure 9B-D). The LD-EPM and OF-LD comparisons of ARDEB changes both showed 60% concordance, supporting the idea that the three assays were measuring similar aspects. However, surprisingly, the OF-EPM comparison revealed that the two methods were discordant (R^2_{adj} = -0.01, Figure 9D).

4. Discussion

4.1 Summary of evidence

Inspection of the forest plots reveals that all of the primary publication sets include experimental effect sizes that are discordant, either in direction (anxiolytic versus anxiogenic) and/or magnitude. The generality of discordance in the literature emphasizes the utility of meta-analysis to behavioral neuroscience to give a quantitative overview and to synthesize the best evidence available. Of ten analyses of putative anxiotropic interventions, eight yielded at least moderate meta-analytic effect sizes and two produced small effect sizes (Figure 9). The synthetic data strongly confirm that diazepam, the serotonergic system, environmental stressors, and *Crhr1* influence an anxiety-like process in the mouse brain.

4.2 Limitations

This study is limited by its exclusive use of English-language published data. Some studies had to be excluded from the meta-analysis during the full text scan because they did not report measures of variance. Only studies that reported time or percent time spent in exposed arena could be selected for meta-analysis. We found no knowledge gaps *per se*, as all ten proposed anxiety-related factors had at least two studies.

Nevertheless, *Htt* overexpression, *Crh* knockouts and the non-anxiety genes had limited cumulative sample sizes ($N_{cumulative} < 64$, 64). Of the six factors for which publication bias was examined, three were affected. The presence of publication bias in the larger data sets suggests that inclusion of further data to the smaller meta-analyses would be expected, on average, to lower these effect sizes as well. Heterogeneity was at least moderate ($I^2 > 50\%$) in five of the meta-analyses, indicating that the random effects model is insufficient to explain the variance in these data sets. Thus, laboratory, strain, assay type and other protocol variations played variable roles across factors. Heterogeneity could in theory be reduced by increased standardization (Crabbe et al., 1999). Multilevel regression models of these data may be able to account for the unexplained variance (Yildizoglu et al., 2015).

4.3 Assay validity

The validity of each ARDEB assay was originally tested with a panel of anxiotropic agents (Crawley and Goodwin, 1980; Pellow et al., 1985; Simon et al., 1994). In the decades since the variability of assay results and the disappointing clinical outcome of compounds identified with these preclinical assays raise new questions about their validity (Griebel and Holmes, 2013). The diazepam, restraint and acute pain synthetic data shown here support the ARDEB assays' validity, though two other results raise doubts: (1) the social isolation effect on ARDEB is weaker than expected (Figure 9A); (2) the failure of EPM and OF to reproduce each other's outcomes (Figure 9D). The EPM-OF meta-regression discordance is an exploratory observation that could be verified with a formal method comparison with animals run through all three assays (Bland and Altman, 1999).

How might the validity of the ARDEB assays be tested further? First, meta-analyses of additional known anxiotropic agents will help assess the assays' strengths and weaknesses. Second, assay validity assessment would be helped by researchers making their video or tracking data available (ideally with experimental metadata in a standard file format), similar to data sharing efforts currently underway in neurophysiology. Anxiety assay validity may also be tested with new instrumentation that allows the estimation of animal pose (Nanjappa et al., 2015; Wiltschko et al., 2015) and that will make complex, ethological relevant anxiety assays (Blanchard et al., 2001) increasingly accessible for routine analysis (Schaefer and Claridge-Chang, 2012). Looking backward (meta-analysis and data sharing) and forward (more refined anxiety assays) are both valuable to rodent anxiety research. It has been suggested that research consortia should form to overcome the cost restrictions of large rodent sample sizes (Button et al., 2013); perhaps a consortium could form around the problem of anxiety assay validation.

4.4 Disconnect between Htr1a & Crhr1 preclinical results and clinical efforts

Meta-analysis of *Htr1a* overexpression revealed it has a moderate anxiotropic effect (-0.6 *g*), smaller than the bias-corrected diazepam effect (-0.85 *g*), suggesting that compounds aiming to increase 5-HT1A function may be a poor strategy to reduce anxiety. This view is supported by clinical meta-analyses that have concluded that drugs targeting 5-HT1A—the azapirones—appear inferior to benzodiazepines for generalized anxiety disorder (Chessick et al., 2006) and that there is insufficient evidence to support azapirone use in panic disorder (Imai et al., 2014). It appears that clinical adoption of the azapirones was/is not informed by the preclinical genetic evidence base. A second type of preclinical-clinical disconnect is observed with the *Crhr1* knockouts. The synthetic preclinical data indicate that *Crhr1* knockout produces a very large reduction of rodent anxiety (g = -1.0 [95Ci -0.7, -1.3], I^2 = 13%, $N_{cumulative}$ = 105, 99). However, at least one clinical trial of a CRHR1 antagonist for generalized anxiety disorder showed no benefit over placebo (Coric et al., 2010). The discrepancy between the efficacy of *Crhr1* knockouts and inefficacy of CRHR1 antagonists in patients remains unexplained.

4.5 A paradox in Htt-SSRI anxiety effects

Drugs that inhibit SERT, the SSRIs, are recommended as the first line of pharmacological treatment for anxiety (Baldwin et al., 2014). Blocking SERT-mediated reuptake of serotonin from the synaptic cleft is the proposed mechanism of SSRI anxiety reduction, although rodent studies of chronic SSRI effects on ARDEB have been inconclusive (Griebel and Holmes, 2013; Perez-Caballero et al., 2014). Given the inhibitors' clinical use, it is paradoxical that *Htt* knockouts have elevated anxiety relative to controls (0.57 *g*) and that *Htt* overexpression dramatically reduces rodent anxiety (-0.94 *g*). The reason for this drug/gene incongruence is not clear. In some cases, the authors of the primary *Htt* knockout studies have not discussed it (Carroll et al., 2007; Kalueff et al., 2007a; Moya et al., 2011; Schipper et al., 2011). Other authors have remarked that the underlying reason remains unclear (Holmes et al., 2003b; Lira et al., 2003) or have called the validity of ARDEB assays into doubt (Pang et al., 2011). As both genetic knockouts and SSRIs are expected to produce monotonic, systemic reductions of SERT function, this incongruence is not easily explained by models of serotonin conflicting action that invoke distinct 5-HT circuits in the brain with opposing effects on defense (Deakin and Graeff, 1991). Others have proposed two explanatory hypotheses. The first is that increased anxiety arises from developmental alterations present in *Htt* knockouts not present in chronically drug-treated animals (Holmes et al., 2003b; Olivier et al., 2008; Zhao et al., 2006). This hypothesis could be tested with conditional knockdown models, i.e. in animals with *Htt* only deleted at the adult stage. While systematic review of PubMed and EMBASE did not identify any published reports of post-developmental *Htt* knockout experiments (e.g., using floxed *Htt*), researchers have analyzed the anxiety-related effects of condi-

tionally ablating the *Pet-1* gene. *Pet-1* is a transcription factor with an expression range that overlaps closely with the expression of *Htt*. In mice with *Pet-1* removed in adulthood, mRNA levels of *Htt* are substantially reduced (Chen Liu et al., 2010). Like *Htt* knockouts, these mice show increased anxiety-like behaviors in multiple ARDEB assays (Chen Liu et al., 2010), eroding confidence in the developmental alteration hypothesis. A second hypothesis to explain the *Htt*/SSRI paradox is that there is a J-shaped relationship between *Htt* function and anxiety, i.e., both wild-type and knockout animals would have higher anxiety relative to animals with intermediate function (Olivier et al., 2008). The SSRI/knockout paradox is also observed in depression assays, though interfering RNA knockdown of *Htt* in adult mice reduced the forced swim test measure of depression (N = 10, 10) (Thakker et al., 2005).

5. Conclusions

This study confirms that diazepam, two environmental stressors and three genes influence rodent anxiety as measured by defense behavior assays. These anxiety-related interventions (diazepam, *Htr1a* gene knockout, *Htt* gene knockout, *Htt* gene overexpression, acute pain, restraint and *Crhr1* gene knockout) can be used as reference manipulations when establishing other anxiety models. The rodent anxiety literature is affected by publication bias that amplifies effect sizes. For the panel of ten interventions, there is strong EPM-LD and OF-LD ARDEB assay concordance, but EPM and OF did not reproduce each other. The meta-analytic results bring several preclinical-clinical incongruencies into sharp relief: the weakness of *Htr1a* overexpression contrasting with the clinical use of azapirones, the potently anxiogenic *Crhr1* knockout contrasting with the clinical failure of CRHR1 antagonists, and the anxiogenic SERT knockout contrasting with the clinical use of SSRIs as anxiolytic drugs. Meta-analysis has the ability to aggregate information and resolve discordance in the primary literature, something of particularly use to behavioral neuroscience where most primary articles describe experiments with poor precision (Button et al., 2013). Precise estimation of effect magnitudes (Claridge-Chang and Assam, 2016) is important both to better understand animal model strengths/weaknesses and to improve the ability of preclinical studies to guide clinical investigation. The formation of multi-lab consortia to coordinate the examination of important hypothesized anxiety factors would be one promising way to increase the reliability of rodent anxiety data (Button et al., 2013). New, automated methods of behavioral imaging will also play a role in better preclinical models (Schaefer and Claridge-Chang, 2012). Another possibility would be to use small animal models (worms, flies, and zebrafish) that allow large sample sizes and powerful genetic tools to complement rodent experiments (Mohammad et al., 2016).

Supplementary Legends

Supplementary Table 1. Characteristics of included experiments.
Characteristics of experiments from included studies are listed with Pubmed ID, year of study, and figure panel. The assay type, assay duration, variable used in experiment, route of injection, drug dosage, treatment duration, species, strain and gender are also detailed. Assay duration and treatment duration are listed in minutes. Dosage is listed in mg per kg body weight of animal. Cells containing NS (Not Specified) indicate that the information was not available in the study.

Supplementary Information 1.
Spreadsheet containing extracted data (.xlsx file). Each dataset is in a separate sheet in the Excel file.

Supplementary Information 2.
Extracted meta-analytic data in R-compatible format (.RData file)

Supplementary Information 3.
R markdown code (.Rmd) used for meta-analysis and plotting

Author Contributions
Conceptualization, FM and ACC; Software Programming , JH and FM; Investigation, FM, JH, CLL, JHW, DJJP and BL; Validation, FM, JH, CLL, JHW, DJJP and BL; Data Curation, FM; Writing – Original Draft, FM, JH and ACC; Writing – Review & Editing, ACC; Visualization, FM, JH and ACC; Supervision, ACC; Project Administration, ACC; Funding Acquisition, ACC. All authors have agreed to the final content.

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