Anxiety-related interventions in rodent defense behaviors:

systematic review and meta-analyses

Authors

Farhan Mohammad¹, Joses Ho², Chun Lei Lim², Jia Hern Woo², Dennis Jun Jie Poon², Bhumika Lamba² & Adam Claridge-Chang^{1, 2, 3, *}

Affiliations

- 1. Program in Neuroscience and Behavioral Disorders, Duke-NUS Graduate Medical School, Singapore 138673
- 2. Institute for Molecular and Cell Biology, Agency for Science Technology and Research, Singapore 138673
- 3. Department of Physiology, National University of Singapore, Singapore 138673
- *Corresponding author: claridge-chang.adam@duke-nus.edu.sg

ABSTRACT

Rodent defense behavior assays have been widely used as preclinical models of anxiety to study possible 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 performed 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

Rodent anxiety review 2

1. INTRODUCTION

The anxiety disorders are among the costliest classes of mental disorders, both in 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 anxiety-like behaviors, three anxiety-related defense behavior (ARDEB) assays that specifically aim to measure rodent anxiety have been established, also referred to as 'approach-avoidance conflict tests' (Griebel and Holmes, 2013). The most widely-used ARDEB assays are 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 arena types contain a sheltered domain (e.g., the closed arms in EPM) and an exposed region. It is believed that avoidance of the exposed portions of the chamber reports on anxiety-like states in the rodent brain. The ARDEB assays are accepted as preclinical assays of anxiety disorders, by reference to classic studies that established their validity with a panel 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 for the assessment of clinical anxiety interventions (Baldwin et al., 2014), a solid evidence base of the effect of anxiety interventions in the rodent ARDEB assays is necessary to guide decisions about further basic research and therapeutic development (Vesterinen et al., 2014). To better understand the widespread discordance, 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, known effective anxiotropic interventions could be also adopted as benchmarks against which to validate new rodent assays and/or tests of more genetically tractable model animal species (e.g. Drosophila and zebrafish).

2. MATERIALS AND METHODS

2.1 Literature review

Genes, drugs and environmental interventions that had been proposed to be involved in anxiety were identified by a literature search of review articles on the field of anxiety. 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. The literature for each genetic, pharmacological or environmental intervention was identified by a search on 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) will be the subject of a separate study, currently in preparation.

2.2 Eligibility criteria and study selection

Bibliographic data (including study ID, date of publication, title and abstract) of studies identified by the search phrases were exported 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

Rodent anxiety review 5

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, only experiments using a lifetime loss of function were included in the meta-analyses. 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 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.

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, I² heterogeneity values, and P values using the random effects model, were performed with the metafor package in R (<u>http://CRAN.R-project.org/package=metafor</u>) (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 "Im" 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 Table 3. 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, $l^2 > 75\%$: *Htr1a* overexpression, and physical restraint (Higgins et al., 2003). Three of the meta-analyses, pain and *Htt* Rodent anxiety review 8 knockouts and diazepam, had moderate heterogeneity ($50\% < l^2 < 75\%$). Five metaanalyses had low heterogeneity ($l^2 < 50\%$). 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 metaanalysis 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 points 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 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 shortterm 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., 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; 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* [95Cl -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-and-fill correction indicated a smaller - though still large - effect of -0.85 *g* [95Cl -0.74, -0.96]. The meta-analysis had a moderate level of heterogeneity ($l^2 = 70.4\%$). Subgroup analysis of assay types suggest that assays were not major source of heterogeneity (Figure 3). Additional subgroup analyses of species (rat *versus* mouse) and treatment duration variables 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., Rodent anxiety review 12

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 [95Cl 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 [95Cl -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 ($l^2 = 80\%$). 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; 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 filled the left segment of the funnel plot with ten imputed data points so as to obtain a symmetric funnel plot, reducing 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 Rodent anxiety review 13 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 [95Cl 0.19, 0.93], $P = 2.9 \times 10^{-3}$; 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% Cl 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 rodent 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., 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 [95Cl 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], $P = 3.1 \times 10^{-3}$) 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 Rodent anxiety review 15

(Weninger et al., 1999), which revealed only a small effect (g = 0.30 [95Cl -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 only (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 [95Cl - 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).

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). Of the moderate effect size factors, one (*Htr1a* overexpression) had a non-statistically significant test result. 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 those studies were selected for meta-analysis which report 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 (e.g. unpublished) data to the smaller meta-analyses would be

expected, on average, to lower these effect sizes as well. Heterogeneity was at least moderate ($l^2 > 50\%$) 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 a variable role across factors. Assays could also benefit from increased standardization (Crabbe et al., 1999). Future work will include the implementation of mixed regression models that attempt to account for the unexplained variance (Yildizoglu et al., 2015).

4.3 Disconnects between Htr1a and 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.

4.4 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 the inhibitors' clinical use, it is surprising 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 paradox 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). 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 anxietyrelated effects of conditionally 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 verified anxiety-related interventions (diazepam, Htr1a gene knockout, Htt gene knockout, Htt gene overexpression, acute pain, restraint and Crhr1 gene knockout) verify the validity of the ARDEB tests and can be used as reference manipulations when establishing other anxiety models. The rodent anxiety literature is affected by publication bias that amplifies effect sizes. The meta-analytic results bring several preclinical-clinical disconnects 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 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 Rodent anxiety review 20

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 flexible genetic tools to complement rodent experiments.

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.

Funding Information

The authors were supported by Biomedical Research Council block grants to the Neuroscience Research Partnership and the Institute of Molecular and Cell Biology. FM and ACC also received support from Duke-NUS Graduate Medical School. JH received support from the A*STAR Graduate Academy. ACC received additional support from a Nuffield Department of Medicine Fellowship, a Wellcome Trust block grant to the University of Oxford, A*STAR Joint Council Office grant 1231AFG030 and NARSAD Young Investigator Award 17741. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

REFERENCES

- Abramov, U., Raud, S., Kõks, S., Innos, J., Kurrikoff, K., Matsui, T., Vasar, E., 2004. Targeted mutation of CCK(2) receptor gene antagonises behavioural changes induced by social isolation in female, but not in male mice. Behav Brain Res 155, 1–11. doi:10.1016/j.bbr.2004.03.027
- Anand, R., Gulati, K., Ray, A., 2012. Pharmacological evidence for the role of nitric oxide in the modulation of stress-induced anxiety by morphine in rats. Eur J Pharmacol 676, 71– 74. doi:10.1016/j.ejphar.2011.11.032
- Assie, M.B., Chopin, P., Stenger, A., Palmier, C., Briley, M., 1993. Neuropharmacology of a new potential anxiolytic compound, F 2692, 1-(3'-trifluoromethyl phenyl) 1,4-dihydro 3-amino 4-oxo 6-methyl pyridazine. 1. Acute and in vitro effects. Psychopharmacology (Berl) 110, 13–18.
- Audero, E., Mlinar, B., Baccini, G., Skachokova, Z.K., Corradetti, R., Gross, C., 2013.
 Suppression of serotonin neuron firing increases aggression in mice. J Neurosci 33, 8678–8688. doi:10.1523/JNEUROSCI.2067-12.2013
- Bahi, A., Schwed, J.S., Walter, M., Stark, H., Sadek, B., 2014. Anxiolytic and antidepressant-like activities of the novel and potent non-imidazole histamine H(3) receptor antagonist ST-1283. Drug Des Devel Ther 8, 627–637.
- Baldwin, D.S., Anderson, I.M., Nutt, D.J., Allgulander, C., Bandelow, B., Boer, den, J.A., Christmas, D.M., Davies, S., Fineberg, N., Lidbetter, N., Malizia, A., McCrone, P., Nabarro, D., O'Neill, C., Scott, J., van der Wee, N., Wittchen, H.-U., 2014. Evidencebased pharmacological treatment of anxiety disorders, post-traumatic stress disorder and obsessive-compulsive disorder: A revision of the 2005 guidelines from the British

Association for Psychopharmacology. J Psychopharmacol (Oxford) 28, 403–439. doi:10.1177/0269881114525674

- Barbosa, P.R., Valvassori, S.S., Bordignon, C.L.J., Kappel, V.D., Martins, M.R., Gavioli,
 E.C., Quevedo, J., Reginatto, F.H., 2008. The aqueous extracts of Passiflora alata and
 Passiflora edulis reduce anxiety-related behaviors without affecting memory process in
 rats. J Med Food 11, 282–288. doi:10.1089/jmf.2007.722
- Baretta, I.P., Felizardo, R.A., Bimbato, V.F., Santos, dos, M.G.J., Kassuya, C.A.L.,
 Gasparotto Junior, A., da Silva, C.R., de Oliveira, S.M., Ferreira, J., Andreatini, R., 2012.
 Anxiolytic-like effects of acute and chronic treatment with Achillea millefolium L.
 extract. J Ethnopharmacol 140, 46–54. doi:10.1016/j.jep.2011.11.047
- Barnes, N.M., Costall, B., Kelly, M.E., Onaivi, E.S., Naylor, R.J., 1990. Ketotifen and its analogues reduce aversive responding in the rodent. Pharmacol Biochem Behav 37, 785–793.
- Bellavite, P., Magnani, P., Zanolin, E., Conforti, A., 2011. Homeopathic Doses of Gelsemium sempervirens Improve the Behavior of Mice in Response to Novel Environments. Evid Based Complement Alternat Med 2011, 362517.
- Belzung, C., Agmo, A., 1997. Naloxone blocks anxiolytic-like effects of benzodiazepines in Swiss but not in Balb/c mice. Psychopharmacology (Berl) 132, 195–201.
- Benbouzid, M., Pallage, V., Rajalu, M., Waltisperger, E., Doridot, S., Poisbeau, P., Freund-Mercier, M.J., Barrot, M., 2008. Sciatic nerve cuffing in mice: a model of sustained neuropathic pain. Eur J Pain 12, 591–599. doi:10.1016/j.ejpain.2007.10.002
- Bert, B., Fink, H., Hörtnagl, H., Veh, R.W., Ben Davies, Theuring, F., Kusserow, H., 2006.
 Mice over-expressing the 5-HT(1A) receptor in cortex and dentate gyrus display
 exaggerated locomotor and hypothermic response to 8-OH-DPAT. Behav Brain Res
 167, 328–341. doi:10.1016/j.bbr.2005.09.020

- Bhatt, S., Mahesh, R., Devadoss, T., Jindal, A.K., 2013. Anxiolytic-like effect of (4benzylpiperazin-1-yl)(3-methoxyquinoxalin-2-yl)methanone (6g) in experimental mouse models of anxiety. Indian J Pharmacol 45, 248–251.
- Bhattacharya, S.K., Mitra, S.K., 1991. Anxiolytic activity of Panax ginseng roots: an experimental study. J Ethnopharmacol 34, 87–92.
- Birkett, M.A., Shinday, N.M., Kessler, E.J., Meyer, J.S., Ritchie, S., Rowlett, J.K., 2011.
 Acute anxiogenic-like effects of selective serotonin reuptake inhibitors are attenuated
 by the benzodiazepine diazepam in BALB/c mice. Pharmacol Biochem Behav 98, 544–
 551. doi:10.1016/j.pbb.2011.03.006
- Blainski, A., Piccolo, V.K., Mello, J.C.P., de Oliveira, R.M.W., 2010. Dual effects of crude extracts obtained from Petiveria alliacea L. (Phytolaccaceae) on experimental anxiety in mice. J Ethnopharmacol 128, 541–544. doi:10.1016/j.jep.2010.01.012
- Blakley, G., Pohorecky, L.A., 2006. Psychosocial stress alters ethanol's effect on open field behaviors. Pharmacol Biochem Behav 84, 51–61. doi:10.1016/j.pbb.2006.04.005
- Blednov, Y.A., Stoffel, M., Chang, S.R., Harris, R.A., 2001. GIRK2 deficient mice. Evidence for hyperactivity and reduced anxiety. Physiol Behav 74, 109–117.
- Bledsoe, A.C., Oliver, K.M., Scholl, J.L., Forster, G.L., 2011. Anxiety states induced by post-weaning social isolation are mediated by CRF receptors in the dorsal raphe nucleus. Brain Res Bull 85, 117–122. doi:10.1016/j.brainresbull.2011.03.003
- Borenstein, M., Hedges, L.V., Higgins, J., Rothstein, H.R., 2011. Introduction to metaanalysis. John Wiley & Sons, Ltd.
- Borsini, F., Brambilla, A., Cesana, R., Donetti, A., 1993. The effect of DAU 6215, a novel 5HT-3 antagonist, in animal models of anxiety. Pharmacol Res 27, 151–164. doi:10.1006/phrs.1993.1015

Brenes, J.C., Padilla, M., Fornaguera, J., 2009. A detailed analysis of open-field habituation

and behavioral and neurochemical antidepressant-like effects in postweaning enriched rats. Behav Brain Res 197, 125–137. doi:10.1016/j.bbr.2008.08.014

Brioni, J.D., O'Neill, A.B., Kim, D.J., Buckley, M.J., Decker, M.W., Arneric, S.P., 1994. Anxiolytic-like effects of the novel cholinergic channel activator ABT-418. J Pharmacol Exp Ther 271, 353–361.

Busnardo, C., Alves, F.H.F., Crestani, C.C., Scopinho, A.A., Resstel, L.B.M., Corrêa, F.M.A.,
2013. Paraventricular nucleus of the hypothalamus glutamate neurotransmission
modulates autonomic, neuroendocrine and behavioral responses to acute restraint
stress in rats. Eur Neuropsychopharmacol 23, 1611–1622.
doi:10.1016/j.euroneuro.2012.11.002

- Button, K.S., Ioannidis, J.P.A., Mokrysz, C., Nosek, B.A., Flint, J., Robinson, E.S.J., Munafò,
 M.R., 2013. Power failure: why small sample size undermines the reliability of
 neuroscience. Nat Rev Neurosci 14, 365–376. doi:10.1038/nrn3475
- Carneiro, L.M.V., Diogenes, J.P.L., Vasconcelos, S.M.M., Aragao, G.F., Noronha, E.C.,
 Gomes, P.B., Viana, G.S.B., 2005. Behavioral and neurochemical effects on rat
 offspring after prenatal exposure to ethanol. Neurotoxicol Teratol 27, 585–592.
 doi:10.1016/j.ntt.2005.06.006
- Carrier, N., Kabbaj, M., 2012. Testosterone and imipramine have antidepressant effects in socially isolated male but not female rats. Horm Behav 61, 678–685. doi:10.1016/j.yhbeh.2012.03.001

Carro-Juarez, M., Rodriguez-Landa, J.F., Rodriguez-Pena, M. de L., Rovirosa-Hernandez,
M. de J., Garcia-Orduna, F., 2012. The aqueous crude extract of Montanoa frutescens
produces anxiolytic-like effects similarly to diazepam in Wistar rats: involvement of
GABAA receptor. J Ethnopharmacol 143, 592–598. doi:10.1016/j.jep.2012.07.022
Carroll, J.C., Boyce-Rustay, J.M., Millstein, R., Yang, R., Wiedholz, L.M., Murphy, D.L.,

Rodent anxiety review 26

Holmes, A., 2007. Effects of mild early life stress on abnormal emotion-related behaviors in 5-HTT knockout mice. Behav Genet 37, 214–222. doi:10.1007/s10519-006-9129-9

- Carvajal, C.C., Vercauteren, F., Dumont, Y., Michalkiewicz, M., Quirion, R., 2004. Aged neuropeptide Y transgenic rats are resistant to acute stress but maintain spatial and non-spatial learning. Behav Brain Res 153, 471–480. doi:10.1016/j.bbr.2004.01.004
- Cechin, E.M., Quevedo, J., Barichello, T., Machado-Vieira, R., Gentil, V., Kapczinski, F., 2003. Dose-related effects of propericiazine in rats. Braz J Med Biol Res 36, 227–231.
- Cha, H.-Y., Park, J.-H., Hong, J.T., Yoo, H.-S., Song, S., Hwang, B.-Y., Eun, J.-S., Oh, K.-W., 2005. Anxiolytic-like effects of ginsenosides on the elevated plus-maze model in mice. Biol Pharm Bull 28, 1621–1625.
- Chappell, A.M., Carter, E., McCool, B.A., Weiner, J.L., 2013. Adolescent rearing conditions influence the relationship between initial anxiety-like behavior and ethanol drinking in male Long Evans rats. Alcohol Clin Exp Res 37 Suppl 1, E394–403. doi:10.1111/j.1530-0277.2012.01926.x
- Cheeta, S., Irvine, E., File, S.E., 2001. Social isolation modifies nicotine's effects in animal tests of anxiety. Br J Pharmacol 132, 1389–1395. doi:10.1038/sj.bjp.0703991
- Chen, S.W., Kong, W.X., Zhang, Y.J., Li, Y.L., Mi, X.J., Mu, X.S., 2004. Possible anxiolytic effects of taurine in the mouse elevated plus-maze. Life Sci. 75, 1503–1511. doi:10.1016/j.lfs.2004.03.010
- Chen, S.W., Mi, X.J., Wang, R., Wang, W.J., Kong, W.X., Zhang, Y.J., Li, Y.L., 2005. Behavioral effects of sinomenine in murine models of anxiety. Life Sci. 78, 232–238. doi:10.1016/j.lfs.2005.04.056
- Chessick, C.A., Allen, M.H., Thase, M., Batista Miralha da Cunha, A.B.C., Kapczinski, F.F.K., de Lima, M.S.M.L., Santos Souza, dos, J.J.S.S., 2006. Azapirones for

generalized anxiety disorder. Cochrane database of systematic reviews (Online) CD006115. doi:10.1002/14651858.CD006115

- Chesworth, R., Yulyaningsih, E., Cappas, E., Arnold, J., Sainsbury, A., Karl, T., 2012. The response of neuregulin 1 mutant mice to acute restraint stress. Neurosci Lett 515, 82–86. doi:10.1016/j.neulet.2012.03.024
- Choleris, E., Thomas, A.W., Kavaliers, M., Prato, F.S., 2001. A detailed ethological analysis of the mouse open field test: effects of diazepam, chlordiazepoxide and an extremely low frequency pulsed magnetic field. Neurosci Biobehav Rev 25, 235–260.
- Cole, J.C., Rodgers, R.J., 1995. Ethological comparison of the effects of diazepam and acute/chronic imipramine on the behaviour of mice in the elevated plus-maze. Pharmacol Biochem Behav 52, 473–478.
- Colla, A.R.S., Rosa, J.M., Cunha, M.P., Rodrigues, A.L.S., 2015. Anxiolytic-like effects of ursolic acid in mice. Eur J Pharmacol 758, 171–176. doi:10.1016/j.ejphar.2015.03.077
- Conrad, K.L., Louderback, K.M., Gessner, C.P., Winder, D.G., 2011. Stress-induced alterations in anxiety-like behavior and adaptations in plasticity in the bed nucleus of the stria terminalis. Physiol Behav 104, 248–256. doi:10.1016/j.physbeh.2011.03.001
- Consoli, D., Leggio, G.M., Mazzola, C., Micale, V., Drago, F., 2007. Behavioral effects of the beta3 adrenoceptor agonist SR58611A: is it the putative prototype of a new class of antidepressant/anxiolytic drugs? Eur J Pharmacol 573, 139–147. doi:10.1016/j.ejphar.2007.06.048
- Contreras, C.M., Rodriguez-Landa, J.F., Gutierrez-Garcia, A.G., Mendoza-Lopez, M.R., Garcia-Rios, R.I., Cueto-Escobedo, J., 2011. Anxiolytic-like effects of human amniotic fluid and its fatty acids in Wistar rats. Behav Pharmacol 22, 655–662.
- Coric, V., Feldman, H.H., Oren, D.A., Shekhar, A., Pultz, J., Dockens, R.C., Wu, X., Gentile, K.A., Huang, S.-P., Emison, E., Delmonte, T., D'Souza, B.B., Zimbroff, D.L., Grebb,

J.A., Goddard, A.W., Stock, E.G., 2010. Multicenter, randomized, double-blind, active comparator and placebo-controlled trial of a corticotropin-releasing factor receptor-1 antagonist in generalized anxiety disorder. Depress Anxiety 27, 417–425. doi:10.1002/da.20695

- Costa, C.A.R. de A., Kohn, D.O., de Lima, V.M., Gargano, A.C., Flório, J.C., Costa, M., 2011. The GABAergic system contributes to the anxiolytic-like effect of essential oil from Cymbopogon citratus (lemongrass). J Ethnopharmacol 137, 828–836. doi:10.1016/j.jep.2011.07.003
- Costall, B., Domeney, A.M., Gerrard, P.A., Horovitz, Z.P., Kelly, M.E., Naylor, R.J., Tomkins, D.M., 1990. Effects of captopril and SQ29,852 on anxiety-related behaviours in rodent and marmoset. Pharmacol Biochem Behav 36, 13–20.
- Crabbe, J.C., Wahlsten, D., Dudek, B.C., 1999. Genetics of mouse behavior: interactions with laboratory environment. Science 284, 1670–1672.
- Crawley, J., Goodwin, F.K., 1980. Preliminary report of a simple animal behavior model for the anxiolytic effects of benzodiazepines. Pharmacol Biochem Behav 13, 167–170.

Cuenya, L., Fosacheca, S., Mustaca, A., Kamenetzky, G., 2012. Effects of isolation in adulthood on frustration and anxiety. Behav Processes 90, 155–160. doi:10.1016/j.beproc.2012.01.003

- Cumming, G., 2012. Understanding the New Statistics: Effect Sizes, Confidence Intervals, and Meta-Analysis, Multivariate Applications Series.
- Da Silva, N.L., Ferreira, V.M., Carobrez, A. de P., Morato, G.S., 1996. Individual housing from rearing modifies the performance of young rats on the elevated plus-maze apparatus. Physiol Behav 60, 1391–1396.
- Dalvi, A., Rodgers, R.J., 2001. Anxiolytic effects of valproate and diazepam in mice are differentially sensitive to picrotoxin antagonism. Pharmacol Biochem Behav 68, 23–32.

Dalvi, A., Rodgers, R.J., 1999. Behavioral effects of diazepam in the murine plus-maze: flumazenil antagonism of enhanced head dipping but not the disinhibition of open-arm avoidance. Pharmacol Biochem Behav 62, 727–734.

Darwin, C., 1998. The Expression of the Emotions in Man and Animals. Project Gutenberg.

- Das, S.K., Barhwal, K., Hota, S.K., Thakur, M.K., Srivastava, R.B., 2014. Disrupting monotony during social isolation stress prevents early development of anxiety and depression like traits in male rats. BMC Neurosci 16, 2–2. doi:10.1186/s12868-015-0141-y
- de A Vieira, L.F., S Reis, dos, M.D., Brandão, A.R.A., Viana, I.M.M.N., da Silva, J.P., Barreto, E., Smaniotto, S., 2013. Anxiolytic-like effect of the extract from Bowdichia virgilioides in mice. Revista Brasileira de Farmacognosia 23, 680–686. doi:10.1590/S0102-695X2013005000044
- de Almeida, A.A.C., Costa, J.P., de Carvalho, R.B.F., de Sousa, D.P., de Freitas, R.M., 2012. Evaluation of acute toxicity of a natural compound (+)-limonene epoxide and its anxiolytic-like action. Brain Res 1448, 56–62. doi:10.1016/j.brainres.2012.01.070
- de Castro, P.C.F., Hoshino, A., da Silva, J.C., Mendes, F.R., 2007. Possible anxiolytic effect of two extracts of Passiflora quadrangularis L. in experimental models. Phytother Res 21, 481–484. doi:10.1002/ptr.2079
- de Melo, C.T.V., Monteiro, A.P., Leite, C.P., de Araujo, F.L.O., Lima, V.T.M., Barbosa-Filho, J.M., de Franca Fonteles, M.M., de Vasconcelos, S.M.M., de Barros Viana, G.S., de Sousa, F.C.F., 2006. Anxiolytic-like effects of (O-methyl)-N-2,6-dihydroxybenzoyl-tyramine (riparin III) from Aniba riparia (Nees) Mez (Lauraceae) in mice. Biol Pharm Bull 29, 451–454.
- de Sousa, F.C.F., Leite, C.P., de Melo, C.T.V., de Araujo, F.L.O., Gutierrez, S.J.C., Barbosa-Filho, J.M., Fonteles, M.M. de F., de Vasconcelos, S.M.M., de Barros Viana, G.S., 2007.

Evaluation of effects of N-(2-hydroxybenzoyl) tyramine (riparin II) from Aniba riparia (NEES) MEZ (Lauracea) in anxiety models in mice. Biol Pharm Bull 30, 1212–1216.

- de-Paris, F., Busnello, J.V., Vianna, M.R., Salgueiro, J.B., Quevedo, J., Izquierdo, I., Kapczinski, F., 2000. The anticonvulsant compound gabapentin possesses anxiolytic but not amnesic effects in rats. Behav Pharmacol 11, 169–173.
- DiLuca, M., Olesen, J., 2014. The Cost of Brain Diseases: A Burden or a Challenge? Neuron 82, 1205–1208. doi:10.1016/j.neuron.2014.05.044
- Djordjevic, J., Djordjevic, A., Adzic, M., Radojcic, M.B., 2012. Effects of chronic social isolation on Wistar rat behavior and brain plasticity markers. Neuropsychobiology 66, 112–119. doi:10.1159/000338605
- Doremus-Fitzwater, T.L., Varlinskaya, E.I., Spear, L.P., 2009. Effects of pretest manipulation on elevated plus-maze behavior in adolescent and adult male and female Sprague-Dawley rats. Pharmacol Biochem Behav 92, 413–423. doi:10.1016/j.pbb.2009.01.006
- Drapier, D., Bentue-Ferrer, D., Laviolle, B., Millet, B., Allain, H., Bourin, M., Reymann, J.-M., 2007. Effects of acute fluoxetine, paroxetine and desipramine on rats tested on the elevated plus-maze. Behav Brain Res 176, 202–209. doi:10.1016/j.bbr.2006.10.002
- Dunn, A.J., Swiergiel, A.H., 1999. Behavioral responses to stress are intact in CRF-deficient mice. Brain Res 845, 14–20.
- Dunn, R.W., Corbett, R., Fielding, S., 1989. Effects of 5-HT1A receptor agonists and NMDA receptor antagonists in the social interaction test and the elevated plus maze. Eur J Pharmacol 169, 1–10.
- Dunn, R.W., Reed, T.A., Copeland, P.D., Frye, C.A., 1998. The nitric oxide synthase inhibitor 7-nitroindazole displays enhanced anxiolytic efficacy without tolerance in rats following subchronic administration. Neuropharmacology 37, 899–904.

Duval, S., Tweedie, R., 2000. Trim and fill: A simple funnel-plot-based method of testing

and adjusting for publication bias in meta-analysis. Biometrics 56, 455-463.

- Egger, M., Davey Smith, G., Schneider, M., Minder, C., 1997. Bias in meta-analysis detected by a simple, graphical test. BMJ 315, 629–634.
- Ene, H.M., Kara, N.Z., Barak, N., Reshef Ben-Mordechai, T., Einat, H., 2015. Effects of repeated asenapine in a battery of tests for anxiety-like behaviours in mice. Acta Neuropsychiatr 1–7. doi:10.1017/neu.2015.53
- Engin, E., Treit, D., Dickson, C.T., 2009. Anxiolytic- and antidepressant-like properties of ketamine in behavioral and neurophysiological animal models. Neuroscience 161, 359–369. doi:10.1016/j.neuroscience.2009.03.038
- Ennaceur, A., Michalikova, S., van Rensburg, R., Chazot, P.L., 2010. Distinguishing anxiolysis and hyperactivity in an open space behavioral test. Behav Brain Res 207, 84– 98. doi:10.1016/j.bbr.2009.09.042
- Estanislau, C., Morato, S., 2005. Prenatal stress produces more behavioral alterations than maternal separation in the elevated plus-maze and in the elevated T-maze. Behav Brain Res 163, 70–77. doi:10.1016/j.bbr.2005.04.003
- Estelles, J., Lluch, J., Rodriguez-Arias, M., Aguilar, M.A., Minarro, J., 2007. Cocaine exposure during adolescence affects anxiety in adult mice. Brain Res Bull 71, 393–403. doi:10.1016/j.brainresbull.2006.10.008
- Fajemiroye, J.O., Galdino, P.M., Florentino, I.F., Da Rocha, F.F., Ghedini, P.C., Polepally,
 P.R., Zjawiony, J.K., Costa, E.A., 2014. Plurality of anxiety and depression alteration
 mechanism by oleanolic acid. J Psychopharmacol (Oxford) 28, 923–934.
 doi:10.1177/0269881114536789
- Faria, M.S., Muscará, M.N., Moreno Júnior, H., Teixeira, S.A., Dias, H.B., De Oliveira, B., Graeff, F.G., De Nucci, G., 1997. Acute inhibition of nitric oxide synthesis induces anxiolysis in the plus maze test. Eur J Pharmacol 323, 37–43.

Faturi, C.B., Leite, J.R., Alves, P.B., Canton, A.C., Teixeira-Silva, F., 2010. Anxiolytic-like effect of sweet orange aroma in Wistar rats. Prog Neuropsychopharmacol Biol Psychiatry 34, 605–609. doi:10.1016/j.pnpbp.2010.02.020

Fernandez, F., Misilmeri, M.A., Felger, J.C., Devine, D.P., 2004. Nociceptin/orphanin FQ increases anxiety-related behavior and circulating levels of corticosterone during neophobic tests of anxiety. Neuropsychopharmacology 29, 59–71. doi:10.1038/sj.npp.1300308

- Fernandez, S.P., Mewett, K.N., Hanrahan, J.R., Chebib, M., Johnston, G.A.R., 2008. Flavan-3-ol derivatives are positive modulators of GABA(A) receptors with higher efficacy for the alpha(2) subtype and anxiolytic action in mice. Neuropharmacology 55, 900–907. doi:10.1016/j.neuropharm.2008.06.069
- Ferrés-Coy, A., Santana, N., Castañé, A., Cortés, R., Carmona, M.C., Toth, M., Montefeltro,
 A., Artigas, F., Bortolozzi, A., 2013. Acute 5-HT(1)A autoreceptor knockdown increases
 antidepressant responses and serotonin release in stressful conditions.
 Psychopharmacology (Berl) 225, 61–74. doi:10.1007/s00213-012-2795-9
- Flores, J.A., Galan-Rodriguez, B., Ramiro-Fuentes, S., Fernandez-Espejo, E., 2006. Role for dopamine neurons of the rostral linear nucleus and periaqueductal gray in the rewarding and sensitizing properties of heroin. Neuropsychopharmacology 31, 1475– 1488. doi:10.1038/sj.npp.1300946
- Fone, K.C., Shalders, K., Fox, Z.D., Arthur, R., Marsden, C.A., 1996. Increased 5-HT2C
 receptor responsiveness occurs on rearing rats in social isolation.
 Psychopharmacology (Berl) 123, 346–352.
- Fortes, A.C., Almeida, A.A.C., Mendonca-Junior, F.J.B., Freitas, R.M., Soares-Sobrinho, J.L., La Roca Soares, de, M.F., 2013. Anxiolytic properties of new chemical entity, 5TIO1. Neurochem Res 38, 726–731. doi:10.1007/s11064-013-0970-y

- Fraser, L.M., Brown, R.E., Hussin, A., Fontana, M., Whittaker, A., O'Leary, T.P., Lederle, L.,
 Holmes, A., Ramos, A., 2010. Measuring anxiety- and locomotion-related behaviours in
 mice: a new way of using old tests. Psychopharmacology (Berl) 211, 99–112.
 doi:10.1007/s00213-010-1873-0
- Frassetto, S.S., Alves, I.O., Santos, M.M., Schmidt, A.E.S., Lopes, J.J., Oliveira, P.A., Vinagre, A.S., Pereira, P., 2010. Absence of sibutramine effect on spontaneous anxiety in rats. Arg Bras Endocrinol Metabol 54, 375–380.
- Freeman-Daniels, E., Beck, S.G., Kirby, L.G., 2011. Cellular correlates of anxiety in CA1 hippocampal pyramidal cells of 5-HT1A receptor knockout mice. Psychopharmacology (Berl) 213, 453–463. doi:10.1007/s00213-010-2030-5
- Galeotti, N., Sanna, M.D., Ghelardini, C., 2013. Pleiotropic effect of histamine H4 receptor modulation in the central nervous system. Neuropharmacology 71, 141–147. doi:10.1016/j.neuropharm.2013.03.026
- Gammie, S.C., Stevenson, S.A., 2006. Intermale aggression in corticotropin-releasing factor receptor 1 deficient mice. Behav Brain Res 171, 63–69. doi:10.1016/j.bbr.2006.03.017
- Girish, C., Raj, V., Arya, J., Balakrishnan, S., 2013. Involvement of the GABAergic system in the anxiolytic-like effect of the flavonoid ellagic acid in mice. Eur J Pharmacol 710, 49– 58. doi:10.1016/j.ejphar.2013.04.003
- Gleason, G., Liu, B., Bruening, S., Zupan, B., Auerbach, A., Mark, W., Oh, J.E., Gal-Toth, J.,
 Lee, F., Toth, M., 2010. The serotonin1A receptor gene as a genetic and prenatal
 maternal environmental factor in anxiety. Proc Natl Acad Sci USA 107, 7592–7597.
 doi:10.1073/pnas.0914805107
- Gomes, P.B., Feitosa, M.L., Silva, M.I.G., Noronha, E.C., Moura, B.A., Venancio, E.T., Rios,E.R.V., de Sousa, D.P., de Vasconcelos, S.M.M., Fonteles, M.M. de F., de Sousa,F.C.F., 2010. Anxiolytic-like effect of the monoterpene 1,4-cineole in mice. Pharmacol

Biochem Behav 96, 287-293. doi:10.1016/j.pbb.2010.05.019

- Gonzalez-Trujano, Ma Eva, Martinez, A.L., Reyes-Ramirez, A., Reyes-Trejo, B., Navarrete,
 A., 2006. Palmitone isolated from Annona diversifolia induces an anxiolytic-like effect in
 mice. Planta Med 72, 703–707. doi:10.1055/s-2006-931598
- Gonzalez-Trujano, Maria Eva, Ponce-Munoz, H., Hidalgo-Figueroa, S., Navarrete-Vazquez,
 G., Estrada-Soto, S., 2015. Depressant effects of Agastache mexicana methanol
 extract and one of major metabolites tilianin. Asian Pac J Trop Med 8, 185–190.
 doi:10.1016/S1995-7645(14)60312-6
- González-Pardo, H., Conejo, N.M., Arias, J.L., 2006. Oxidative metabolism of limbic structures after acute administration of diazepam, alprazolam and zolpidem. Prog Neuropsychopharmacol Biol Psychiatry 30, 1020–1026.

doi:10.1016/j.pnpbp.2006.03.026

- Granjeiro, E.M., Gomes, F.V., Guimarães, F.S., Corrêa, F.M.A., Resstel, L.B.M., 2011.
 Effects of intracisternal administration of cannabidiol on the cardiovascular and behavioral responses to acute restraint stress. Pharmacol Biochem Behav 99, 743–748.
 doi:10.1016/j.pbb.2011.06.027
- Griebel, G., Holmes, A., 2013. 50 years of hurdles and hope in anxiolytic drug discovery. Nat Rev Drug Discov 12, 667–687. doi:10.1038/nrd4075

Griebel, G., Perrault, G., Sanger, D.J., 1998. Characterization of the behavioral profile of the non-peptide CRF receptor antagonist CP-154,526 in anxiety models in rodents. Comparison with diazepam and buspirone. Psychopharmacology (Berl) 138, 55–66.

Griebel, G., Perrault, G., Sanger, D.J., 1997. CCK receptor antagonists in animal models of anxiety: comparison between exploration tests, conflict procedures and a model based on defensive behaviours. Behav Pharmacol 8, 549–560.

Griebel, G., Perrault, G., Tan, S., Schoemaker, H., Sanger, D.J., 1999a. Comparison of the

pharmacological properties of classical and novel BZ-omega receptor ligands. Behav Pharmacol 10, 483–495.

- Griebel, G., Perrault, G., Tan, S., Schoemaker, H., Sanger, D.J., 1999b. Pharmacological studies on synthetic flavonoids: comparison with diazepam. Neuropharmacology 38, 965–977.
- Griebel, G., Simiand, J., Steinberg, R., Jung, M., Gully, D., Roger, P., Geslin, M., Scatton,
 B., Maffrand, J.-P., Soubrie, P., 2002. 4-(2-Chloro-4-methoxy-5-methylphenyl)-N-[(1S)2-cyclopropyl-1-(3-fluoro-4-methylp henyl)ethyl]5-methyl-N-(2-propynyl)-1, 3-thiazol-2amine hydrochloride (SSR125543A), a potent and selective corticotrophin-releasing
 factor(1) receptor antagonist. II. Characterization in rodent models of stress-related
 disorders. J Pharmacol Exp Ther 301, 333–345.
- Groenink, L., Pattij, T., De Jongh, R., Van der Gugten, J., Oosting, R.S., Dirks, A., Olivier, B.,
 2003. 5-HT1A receptor knockout mice and mice overexpressing corticotropin-releasing
 hormone in models of anxiety. Eur J Pharmacol 463, 185–197. doi:10.1016/S00142999(03)01281-0
- Gross, C., Zhuang, X., Stark, K., Ramboz, S., Oosting, R., Kirby, L., Santarelli, L., Beck, S., Hen, R., 2002. Serotonin1A receptor acts during development to establish normal anxiety-like behaviour in the adult. Nature 416, 396–400. doi:10.1038/416396a
- Guilloux, J.-P., Mendez-David, I., Pehrson, A., Guiard, B.P., Repérant, C., Orvoen, S.,
 Gardier, A.M., Hen, R., Ebert, B., Miller, S., Sanchez, C., David, D.J., 2013.
 Antidepressant and anxiolytic potential of the multimodal antidepressant vortioxetine
 (Lu AA21004) assessed by behavioural and neurogenesis outcomes in mice.
 Neuropharmacology 73, 147–159. doi:10.1016/j.neuropharm.2013.05.014
- Gupta, D., Radhakrishnan, M., Kurhe, Y., 2014. Anxiolytic-like effects of alverine citrate in experimental mouse models of anxiety. Eur J Pharmacol 742, 94–101.

doi:10.1016/j.ejphar.2014.08.033

- Gupta, D., Radhakrishnan, M., Thangaraj, D., Kurhe, Y., 2015. Pharmacological evaluation of novel 5-HT3 receptor antagonist, QCM-13 (N-cyclohexyl-3-methoxyquinoxalin-2carboxamide) as anti-anxiety agent in behavioral test battery. J Pharm Bioallied Sci 7, 103–108.
- Haller, J., Halász, J., 1999. Mild social stress abolishes the effects of isolation on anxiety and chlordiazepoxide reactivity. Psychopharmacology (Berl) 144, 311–315.
- Haller, J., Halász, J., Makara, G.B., 2000. Housing conditions and the anxiolytic efficacy of buspirone: the relationship between main and side effects. Behav Pharmacol 11, 403–412.
- Han, H., Ma, Y., Eun, J.-S., Li, R., Hong, J.T., Lee, M.-K., Oh, K.-W., 2009. Anxiolytic-like effects of sanjoinine A isolated from Zizyphi Spinosi Semen: possible involvement of GABAergic transmission. Pharmacol Biochem Behav 92, 206–213.
 doi:10.1016/j.pbb.2008.11.012
- Harada, K., Aota, M., Inoue, T., Matsuda, R., Mihara, T., Yamaji, T., Ishibashi, K., Matsuoka, N., 2006. Anxiolytic activity of a novel potent serotonin 5-HT2C receptor antagonist
 FR260010: a comparison with diazepam and buspirone. Eur J Pharmacol 553, 171–
 184. doi:10.1016/j.ejphar.2006.09.042
- Harris, R.B., Zhou, J., Shi, M., Redmann, S., Mynatt, R.L., Ryan, D.H., 2001. Overexpression of agouti protein and stress responsiveness in mice. Physiol Behav 73, 599–608.
- Hasenohrl, R.U., Nichau, C.H., Frisch, C.H., De Souza Silva, M.A., Huston, J.P., Mattern, C.M., Hacker, R., 1996. Anxiolytic-like effect of combined extracts of Zingiber officinale and Ginkgo biloba in the elevated plus-maze. Pharmacol Biochem Behav 53, 271–275.

Hazim, A.I., Ramanathan, S., Parthasarathy, S., Muzaimi, M., Mansor, S.M., 2014.

Anxiolytic-like effects of mitragynine in the open-field and elevated plus-maze tests in rats. J Physiol Sci 64, 161–169. doi:10.1007/s12576-014-0304-0

- Hermes, G., Li, N., Duman, C., Duman, R., 2011. Post-weaning chronic social isolation produces profound behavioral dysregulation with decreases in prefrontal cortex synaptic-associated protein expression in female rats. Physiol Behav 104, 354–359. doi:10.1016/j.physbeh.2010.12.019
- Higgins, J., Thompson, S.G., Deeks, J.J., Altman, D.G., 2003. Measuring inconsistency in meta-analyses. BMJ: British Medical Journal 327, 557–560.
- Hirani, K., Sharma, A.N., Jain, N.S., Ugale, R.R., Chopde, C.T., 2005. Evaluation of GABAergic neuroactive steroid 3alpha-hydroxy-5alpha-pregnane-20-one as a neurobiological substrate for the anti-anxiety effect of ethanol in rats.
 Psychopharmacology (Berl) 180, 267–278. doi:10.1007/s00213-005-2169-7
- Holmes, A., Lit, Q., Murphy, D.L., Gold, E., Crawley, J.N., 2003a. Abnormal anxiety-related behavior in serotonin transporter null mutant mice: the influence of genetic background. Genes Brain Behav 2, 365–380.
- Holmes, A., Yang, R.J., Lesch, K.-P., Crawley, J.N., Murphy, D.L., 2003b. Mice lacking the serotonin transporter exhibit 5-HT(1A) receptor-mediated abnormalities in tests for anxiety-like behavior. Neuropsychopharmacology 28, 2077–2088.
 doi:10.1038/sj.npp.1300266

Huerta-Reyes, M., Herrera-Ruiz, M., Gonzalez-Cortazar, M., Zamilpa, A., Leon, E., Reyes-Chilpa, R., Aguilar-Rojas, A., Tortoriello, J., 2013. Neuropharmacological in vivo effects and phytochemical profile of the extract from the aerial parts of Heteropterys brachiata (L.) DC. (Malpighiaceae). J Ethnopharmacol 146, 311–317.
doi:10.1016/j.jep.2012.12.049

Hui, K.M., Huen, M.S.Y., Wang, H.Y., Zheng, H., Sigel, E., Baur, R., Ren, H., Li, Z.W., Wong,

J.T.-F., Xue, H., 2002. Anxiolytic effect of wogonin, a benzodiazepine receptor ligand isolated from Scutellaria baicalensis Georgi. Biochem. Pharmacol. 64, 1415–1424.

- Imai, H., Tajika, A., Chen, P., Pompoli, A., Guaiana, G., Castellazzi, M., Bighelli, I., Girlanda,
 F., Barbui, C., Koesters, M., Cipriani, A., Furukawa, T.A., 2014. Azapirones versus
 placebo for panic disorder in adults. Cochrane database of systematic reviews (Online)
 9, CD010828–CD010828. doi:10.1002/14651858.CD010828.pub2
- Imanaka, A., Morinobu, S., Toki, S., Yamamoto, S., Matsuki, A., Kozuru, T., Yamawaki, S., 2008. Neonatal tactile stimulation reverses the effect of neonatal isolation on open-field and anxiety-like behavior, and pain sensitivity in male and female adult Sprague-Dawley rats. Behav Brain Res 186, 91–97. doi:10.1016/j.bbr.2007.07.039
- Imanaka, A., Morinobu, S., Toki, S., Yamawaki, S., 2006. Importance of early environment in the development of post-traumatic stress disorder-like behaviors. Behav Brain Res 173, 129–137. doi:10.1016/j.bbr.2006.06.012
- Ishaq, H., 2014. Anxiolytic effect of herbal medicine, Khamira Gaozaban Ambri Jadwar Ood Salib Wala (KGJ) in experimental rat models. Pak J Pharm Sci 27, 289–294.
- Jain, A., Dvorkin, A., Fonio, E., Golani, I., Gross, C.T., 2012. Validation of the dimensionality emergence assay for the measurement of innate anxiety in laboratory mice. Eur Neuropsychopharmacol 22, 153–163. doi:10.1016/j.euroneuro.2011.07.001
- Jain, N.S., Hirani, K., Chopde, C.T., 2005. Reversal of caffeine-induced anxiety by neurosteroid 3-alpha-hydroxy-5-alpha-pregnane-20-one in rats. Neuropharmacology 48, 627–638. doi:10.1016/j.neuropharm.2004.11.016
- Jastrzebska-Wiesek, M., Siwek, A., Partyka, A., Kubacka, M., Mogilski, S., Wasik, A., Kolaczkowski, M., Wesolowska, A., 2014. Pharmacological evaluation of the anxiolyticlike effects of EMD 386088, a partial 5-HT6 receptor agonist, in the rat elevated plusmaze and Vogel conflict tests. Neuropharmacology 85, 253–262.

doi:10.1016/j.neuropharm.2014.05.036

- Jászberényi, M., Bagosi, Z., Thurzó, B., Földesi, I., Szabó, G., Telegdy, G., 2009. Endocrine, behavioral and autonomic effects of neuropeptide AF. Horm Behav 56, 24–34. doi:10.1016/j.yhbeh.2009.02.006
- Jászberényi, M., Bagosi, Z., Thurzó, B., Földesi, I., Telegdy, G., 2007. Endocrine and behavioral effects of neuromedin S. Horm Behav 52, 631–639. doi:10.1016/j.yhbeh.2007.08.002
- Jennings, K.A., Loder, M.K., Sheward, W.J., Pei, Q., Deacon, R.M.J., Benson, M.A., Olverman, H.J., Hastie, N.D., Harmar, A.J., Shen, S., Sharp, T., 2006. Increased expression of the 5-HT transporter confers a low-anxiety phenotype linked to decreased 5-HT transmission. J Neurosci 26, 8955–8964. doi:10.1523/JNEUROSCI.5356-05.2006
- Jessa, M., Nazar, M., Bidzinski, A., Plaznik, A., 1996. The effects of repeated administration of diazepam, MK-801 and CGP 37849 on rat behavior in two models of anxiety. European Neuropsychopharmacology 6, 55–61.
- Jones, G.H., Schneider, C., Schneider, H.H., Seidler, J., Cole, B.J., Stephens, D.N., 1994. Comparison of several benzodiazepine receptor ligands in two models of anxiolytic activity in the mouse: an analysis based on fractional receptor occupancies. Psychopharmacology (Berl) 114, 191–199.
- Joshi, J.C., Ray, A., Gulati, K., 2014. Differential modulatory effects of morphine on acute and chronic stress induced neurobehavioral and cellular markers in rats. Eur J Pharmacol 729, 17–21. doi:10.1016/j.ejphar.2014.01.058
- Kalouda, T., Pitsikas, N., 2015. The nitric oxide donor molsidomine induces anxiolytic-like behaviour in two different rat models of anxiety. Pharmacol Biochem Behav 138, 111– 116. doi:10.1016/j.pbb.2015.09.004

- Kalueff, A.V., Fox, M.A., Gallagher, P.S., Murphy, D.L., 2007a. Hypolocomotion, anxiety and serotonin syndrome-like behavior contribute to the complex phenotype of serotonin transporter knockout mice. Genes Brain Behav 6, 389–400. doi:10.1111/j.1601-183X.2006.00270.x
- Kalueff, A.V., Jensen, C.L., Murphy, D.L., 2007b. Locomotory patterns, spatiotemporal organization of exploration and spatial memory in serotonin transporter knockout mice. Brain Res 1169, 87–97. doi:10.1016/j.brainres.2007.07.009
- Karakas, A., Coskun, H., Kaya, A., Kucuk, A., Gunduz, B., 2011. The effects of the intraamygdalar melatonin injections on the anxiety like behavior and the spatial memory performance in male Wistar rats. Behav Brain Res 222, 141–150. doi:10.1016/j.bbr.2011.03.029
- Karim, N., Gavande, N., Wellendorph, P., Johnston, G.A.R., Hanrahan, J.R., Chebib, M.,
 2011. 3-Hydroxy-2'-methoxy-6-methylflavone: a potent anxiolytic with a unique selectivity profile at GABA(A) receptor subtypes. Biochem. Pharmacol. 82, 1971–1983.
 doi:10.1016/j.bcp.2011.09.002
- Kebebew, Z., Shibeshi, W., 2013. Evaluation of anxiolytic and sedative effects of 80%
 ethanolic Carica papaya L. (Caricaceae) pulp extract in mice. J Ethnopharmacol 150,
 665–671. doi:10.1016/j.jep.2013.09.023
- Kirsch, I., Deacon, B.J., Huedo-Medina, T.B., Scoboria, A., Moore, T.J., Johnson, B.T.,
 2008. Initial severity and antidepressant benefits: a meta-analysis of data submitted to the Food and Drug Administration. PLoS Med 5, e45.
 doi:10.1371/journal.pmed.0050045
- Klemenhagen, K.C., Gordon, J.A., David, D.J., Hen, R., Gross, C.T., 2006. Increased fear response to contextual cues in mice lacking the 5-HT1A receptor.
 Neuropsychopharmacology 31, 101–111. doi:10.1038/sj.npp.1300774

- Klodzinska, A., Tatarczynska, E., Chojnacka-Wojcik, E., Nowak, G., Cosford, N.D.P., Pilc, A., 2004a. Anxiolytic-like effects of MTEP, a potent and selective mGlu5 receptor agonist does not involve GABA(A) signaling. Neuropharmacology 47, 342–350.
- Klodzinska, A., Tatarczynska, E., Stachowicz, K., Chojnacka-Wojcik, E., 2004b. The anxiolytic-like activity of AIDA (1-aminoindan-1,5-dicarboxylic acid), an mGLu 1 receptor antagonist. J Physiol Pharmacol 55, 113–126.
- Knuth, E.D., Etgen, A.M., 2007. Long-term behavioral consequences of brief, repeated neonatal isolation. Brain Res 1128, 139–147. doi:10.1016/j.brainres.2006.10.054
- Koike, H., Ibi, D., Mizoguchi, H., Nagai, T., Nitta, A., Takuma, K., Nabeshima, T., Yoneda, Y., Yamada, K., 2009. Behavioral abnormality and pharmacologic response in social isolation-reared mice. Behav Brain Res 202, 114–121. doi:10.1016/j.bbr.2009.03.028
- Kokare, D.M., Dandekar, M.P., Singru, P.S., Gupta, G.L., Subhedar, N.K., 2010.
 Involvement of alpha-MSH in the social isolation induced anxiety- and depression-like behaviors in rat. Neuropharmacology 58, 1009–1018.
 doi:10.1016/j.neuropharm.2010.01.006
- Kong, W.X., Chen, S.W., Li, Y.L., Zhang, Y.J., Wang, R., Min, L., Mi, X., 2006. Effects of taurine on rat behaviors in three anxiety models. Pharmacol Biochem Behav 83, 271–276. doi:10.1016/j.pbb.2006.02.007
- Kormos, V., Gaszner, B., 2013. Role of neuropeptides in anxiety, stress, and depression: from animals to humans. Neuropeptides 47, 401–419. doi:10.1016/j.npep.2013.10.014

Kumar, D., Bhat, Z.A., 2014. Apigenin 7-glucoside from Stachys tibetica Vatke and its anxiolytic effect in rats. Phytomedicine 21, 1010–1014. doi:10.1016/j.phymed.2013.12.001

Kurhe, Y.V., Radhakrishnan, M., Thangaraj, D., Gupta, D., 2014. Anti-anxiety effect of a novel 5-HT(3) receptor antagonist N-(benzo[d]thiazol-2-yl)-3-ethoxyquinoxalin-2-

carboxamide (6k) using battery tests for anxiety in mice. Indian J Pharmacol 46, 100– 104.

- Kuribara, H., Kishi, E., Kimura, M., Weintraub, S.T., Maruyama, Y., 2000. Comparative assessment of the anxiolytic-like activities of honokiol and derivatives. Pharmacol Biochem Behav 67, 597–601.
- Kusserow, H., Davies, B., Hörtnagl, H., Voigt, I., Stroh, T., Bert, B., Deng, D.R., Fink, H.,
 Veh, R.W., Theuring, F., 2004. Reduced anxiety-related behaviour in transgenic mice overexpressing serotonin 1A receptors. Brain Res Mol Brain Res 129, 104–116.
 doi:10.1016/j.molbrainres.2004.06.028
- Ia Pena, de, J.B.I., Lee, H.L., Yoon, S.Y., Kim, G.H., Lee, Y.S., Cheong, J.H., 2013. The involvement of magnoflorine in the sedative and anxiolytic effects of Sinomeni Caulis et Rhizoma in mice. J Nat Med 67, 814–821. doi:10.1007/s11418-013-0754-3
- LaBuda, C.J., Fuchs, P.N., 2001. The anxiolytic effect of acute ethanol or diazepam exposure is unaltered in mu-opioid receptor knockout mice. Brain Res Bull 55, 755–760.
- Langen, B., Egerland, U., Bernoster, K., Dost, R., Unverferth, K., Rundfeldt, C., 2005. Characterization in rats of the anxiolytic potential of ELB139 [1-(4-chlorophenyl)-4piperidin-1-yl-1,5-dihydro-imidazol-2-on], a new agonist at the benzodiazepine binding site of the GABAA receptor. J Pharmacol Exp Ther 314, 717–724. doi:10.1124/jpet.105.084681
- Lapiz, M.D., Mateo, Y., Durkin, S., Parker, T., Marsden, C.A., 2001. Effects of central noradrenaline depletion by the selective neurotoxin DSP-4 on the behaviour of the isolated rat in the elevated plus maze and water maze. Psychopharmacology (Berl) 155, 251–259.

Leggio, G.M., Micale, V., Le Foll, B., Mazzola, C., Nobrega, J.N., Drago, F., 2011. Dopamine

D3 receptor knock-out mice exhibit increased behavioral sensitivity to the anxiolytic drug diazepam. European Neuropsychopharmacology 21, 325–332. doi:10.1016/j.euroneuro.2010.05.006

- Leite-Almeida, H., Cerqueira, J.J., Wei, H., Ribeiro-Costa, N., Anjos-Martins, H., Sousa, N., Pertovaara, A., Almeida, A., 2012. Differential effects of left/right neuropathy on rats' anxiety and cognitive behavior. Pain 153, 2218–2225. doi:10.1016/j.pain.2012.07.007
- Lepicard, E.M., Joubert, C., Hagneau, I., Perez-Diaz, F., Chapouthier, G., 2000. Differences in anxiety-related behavior and response to diazepam in BALB/cByJ and C57BL/6J strains of mice. Pharmacol Biochem Behav 67, 739–748.
- Leussis, M.P., Andersen, S.L., 2008. Is adolescence a sensitive period for depression? Behavioral and neuroanatomical findings from a social stress model. Synapse 62, 22– 30. doi:10.1002/syn.20462
- Li, Q., Holmes, A., Ma, L., Van de Kar, L.D., Garcia, F., Murphy, D.L., 2004. Medial hypothalamic 5-hydroxytryptamine (5-HT)1A receptors regulate neuroendocrine responses to stress and exploratory locomotor activity: application of recombinant adenovirus containing 5-HT1A sequences. J Neurosci 24, 10868–10877. doi:10.1523/JNEUROSCI.3223-04.2004
- Liebsch, G., Landgraf, R., Engelmann, M., Lörscher, P., Holsboer, F., 1999. Differential behavioural effects of chronic infusion of CRH 1 and CRH 2 receptor antisense oligonucleotides into the rat brain. J Psychiatr Res 33, 153–163.
- Liebsch, G., Landgraf, R., Gerstberger, R., Probst, J.C., Wotjak, C.T., Engelmann, M.,
 Holsboer, F., Montkowski, A., 1995. Chronic infusion of a CRH1 receptor antisense
 oligodeoxynucleotide into the central nucleus of the amygdala reduced anxiety-related
 behavior in socially defeated rats. Regul. Pept. 59, 229–239.

Line, S.J., Barkus, C., Coyle, C., Jennings, K.A., Deacon, R.M., Lesch, K.P., Sharp, T.,

Bannerman, D.M., 2011. Opposing alterations in anxiety and species-typical behaviours in serotonin transporter overexpressor and knockout mice. Eur Neuropsychopharmacol 21, 108–116. doi:10.1016/j.euroneuro.2010.08.005

- Linge, R., Pazos, A., Diaz, A., 2013. Social isolation differentially affects anxiety and depressive-like responses of bulbectomized mice. Behav Brain Res 245, 1–6. doi:10.1016/j.bbr.2013.01.041
- Lira, A., Zhou, M., Castanon, N., Ansorge, M.S., Gordon, J.A., Francis, J.H., Bradley-Moore,
 M., Lira, J., Underwood, M.D., Arango, V., Kung, H.F., Hofer, M.A., Hen, R., Gingrich,
 J.A., 2003. Altered depression-related behaviors and functional changes in the dorsal
 raphe nucleus of serotonin transporter-deficient mice. Biol Psychiatry 54, 960–971.
- Liu, Chen, Maejima, T., Wyler, S.C., Casadesus, G., Herlitze, S., Deneris, E.S., 2010. Pet-1 is required across different stages of life to regulate serotonergic function. Nat Neurosci 13, 1190–1198. doi:10.1038/nn.2623
- Liu, Jie, Zhai, W.-M., Yang, Y.-X., Shi, J.-L., Liu, Q.-T., Liu, G.-L., Fang, N., Li, J., Guo, J.-Y., 2015. GABA and 5-HT systems are implicated in the anxiolytic-like effect of spinosin in mice. Pharmacol Biochem Behav 128, 41–49. doi:10.1016/j.pbb.2014.11.003
- Liu, Jing, Garza, J.C., Li, W., Lu, X.-Y., 2011. Melanocortin-4 receptor in the medial amygdala regulates emotional stress-induced anxiety-like behaviour, anorexia and corticosterone secretion. Int J Neuropsychopharmacol 16, 105–120. doi:10.1017/S146114571100174X
- Liu, Xiao, Wu, R., Tai, F., Ma, L., Wei, B., Yang, X., Zhang, X., Jia, R., 2013. Effects of group housing on stress induced emotional and neuroendocrine alterations. Brain Res 1502, 71–80. doi:10.1016/j.brainres.2013.01.044
- Liu, Yan, Yang, L., Yu, J., Zhang, Y.-Q., 2015. Persistent, comorbid pain and anxiety can be uncoupled in a mouse model. Physiol Behav 151, 55–63.

doi:10.1016/j.physbeh.2015.07.004

- Locchi, F., Dall'olio, R., Gandolfi, O., Rimondini, R., 2008. Olanzapine counteracts stressinduced anxiety-like behavior in rats. Neurosci Lett 438, 146–149. doi:10.1016/j.neulet.2008.04.017
- Lolli, L.F., Sato, C.M., Romanini, C.V., Villas-Boas, L.D.B., Santos, C.A.M., de Oliveira, R.M.W., 2007. Possible involvement of GABA A-benzodiazepine receptor in the anxiolytic-like effect induced by Passiflora actinia extracts in mice. J Ethnopharmacol 111, 308–314. doi:10.1016/j.jep.2006.11.021
- Lukkes, J.L., Mokin, M.V., Scholl, J.L., Forster, G.L., 2009. Adult rats exposed to early-life social isolation exhibit increased anxiety and conditioned fear behavior, and altered hormonal stress responses. Horm Behav 55, 248–256. doi:10.1016/j.yhbeh.2008.10.014

- Lunga, P., Herbert, J., 2004. 17Beta-oestradiol modulates glucocorticoid, neural and behavioural adaptations to repeated restraint stress in female rats. Journal of Neuroendocrinology 16, 776–785. doi:10.1111/j.1365-2826.2004.01234.x
- Mahendra, P., Bisht, S., 2011. Anti-anxiety activity of Coriandrum sativum assessed using different experimental anxiety models. Indian J Pharmacol 43, 574–577.
- Majercsik, E., Haller, J., Leveleki, C., Baranyi, J., Halász, J., Rodgers, R.J., 2003. The effect of social factors on the anxiolytic efficacy of buspirone in male rats, male mice, and men. Prog Neuropsychopharmacol Biol Psychiatry 27, 1187–1199. doi:10.1016/j.pnpbp.2003.09.013
- Mansouri, M.T., Soltani, M., Naghizadeh, B., Farbood, Y., Mashak, A., Sarkaki, A., 2014. A possible mechanism for the anxiolytic-like effect of gallic acid in the rat elevated plus maze. Pharmacol Biochem Behav 117, 40–46. doi:10.1016/j.pbb.2013.12.011

Martinez, A.L., Dominguez, F., Orozco, S., Chavez, M., Salgado, H., González, M.,

Gonzalez-Trujano, M.E., 2006. Neuropharmacological effects of an ethanol extract of the Magnolia dealbata Zucc. leaves in mice. J Ethnopharmacol 106, 250–255. doi:10.1016/j.jep.2006.01.003

- Matsuzawa-Yanagida, K., Narita, M., Nakajima, M., Kuzumaki, N., Niikura, K., Nozaki, H., Takagi, T., Tamai, E., Hareyama, N., Terada, M., Yamazaki, M., Suzuki, T., 2008.
 Usefulness of antidepressants for improving the neuropathic pain-like state and paininduced anxiety through actions at different brain sites. Neuropsychopharmacology 33, 1952–1965. doi:10.1038/sj.npp.1301590
- McCool, B.A., Chappell, A.M., 2009. Early social isolation in male Long-Evans rats alters both appetitive and consummatory behaviors expressed during operant ethanol selfadministration. Alcohol Clin Exp Res 33, 273–282. doi:10.1111/j.1530-0277.2008.00830.x
- Mechan, A.O., Moran, P.M., Elliott, M., Young, A.J., Joseph, M.H., Green, R., 2002. A comparison between Dark Agouti and Sprague-Dawley rats in their behaviour on the elevated plus-maze, open-field apparatus and activity meters, and their response to diazepam. Psychopharmacology (Berl) 159, 188–195. doi:10.1007/s002130100902
- Melo, F.H.C., Venancio, E.T., de Sousa, D.P., de Franca Fonteles, M.M., de Vasconcelos,
 S.M.M., Viana, G.S.B., de Sousa, F.C.F., 2010. Anxiolytic-like effect of Carvacrol (5isopropyl-2-methylphenol) in mice: involvement with GABAergic transmission. Fundam Clin Pharmacol 24, 437–443. doi:10.1111/j.1472-8206.2009.00788.x
- Mesfin, M., Asres, K., Shibeshi, W., 2014. Evaluation of anxiolytic activity of the essential oil of the aerial part of Foeniculum vulgare Miller in mice. BMC Complement Altern Med 14, 310. doi:10.1186/1472-6882-14-310
- Meyer, L., Boujedaini, N., Patte-Mensah, C., Mensah-Nyagan, A.G., 2013. Pharmacological effect of gelsemine on anxiety-like behavior in rat. Behav Brain Res 253, 90–94.

doi:10.1016/j.bbr.2013.07.010

- Mi, X.J., Chen, S.W., Wang, W.J., Wang, R., Zhang, Y.J., Li, W.J., Li, Y.L., 2005. Anxiolytic-like effect of paeonol in mice. Pharmacol Biochem Behav 81, 683–687.
 doi:10.1016/j.pbb.2005.04.016
- Micale, V., Cristino, L., Tamburella, A., Petrosino, S., Leggio, G.M., Drago, F., Di Marzo, V.,
 2009. Anxiolytic effects in mice of a dual blocker of fatty acid amide hydrolase and
 transient receptor potential vanilloid type-1 channels. Neuropsychopharmacology 34,
 593–606. doi:10.1038/npp.2008.98
- Micale, V., Tamburella, A., Leggio, G.M., Mazzola, C., Li Volsi, V., Drago, F., 2008.
 Behavioral effects of saredutant, a tachykinin NK2 receptor antagonist, in experimental models of mood disorders under basal and stress-related conditions. Pharmacol Biochem Behav 90, 463–469. doi:10.1016/j.pbb.2008.04.003
- Molander, A.C., Mar, A., Norbury, A., Steventon, S., Moreno, M., Caprioli, D., Theobald, D.E.H., Belin, D., Everitt, B.J., Robbins, T.W., Dalley, J.W., 2011. High impulsivity predicting vulnerability to cocaine addiction in rats: some relationship with novelty preference but not novelty reactivity, anxiety or stress. Psychopharmacology (Berl) 215, 721–731. doi:10.1007/s00213-011-2167-x
- Molina-Hernandez, M., Tellez-Alcantara, N.P., Garcia, J.P., Lopez, J.I.O., Jaramillo, M.T., 2004. Anxiolytic-like actions of leaves of Casimiroa edulis (Rutaceae) in male Wistar rats. J Ethnopharmacol 93, 93–98.
- Mora, S., Díaz-Véliz, G., Lungenstrass, H., Garcia-Gonzalez, M., Coto-Morales, T., Poletti,
 C., De Lima, T.C.M., Herrera-Ruiz, M., Tortoriello, J., 2005. Central nervous system
 activity of the hydroalcoholic extract of Casimiroa edulis in rats and mice. J
 Ethnopharmacol 97, 191–197. doi:10.1016/j.jep.2004.10.028

Moragrega, I., Carrasco, M.C., Vicens, P., Redolat, R., 2003. Spatial learning in male mice

Rodent anxiety review 48

with different levels of aggressiveness: effects of housing conditions and nicotine administration. Behav Brain Res 147, 1–8.

- Moreira, M.R.C., Salvadori, M.G.D.S.S., de Almeida, A.A.C., de Sousa, D.P., Jordan, J., Satyal, P., de Freitas, R.M., de Almeida, R.N., 2014. Anxiolytic-like effects and mechanism of (-)-myrtenol: a monoterpene alcohol. Neurosci Lett 579, 119–124. doi:10.1016/j.neulet.2014.07.007
- Moya, P.R., Fox, M.A., Jensen, C.L., LaPorte, J.L., French, H.T., Wendland, J.R., Murphy,
 D.L., 2011. Altered 5-HT2C receptor agonist-induced responses and 5-HT2C receptor
 RNA editing in the amygdala of serotonin transporter knockout mice. BMC Pharmacol.
 11, 3. doi:10.1186/1471-2210-11-3
- Müller, M.B., Zimmermann, S., Sillaber, I., Hagemeyer, T.P., Deussing, J.M., Timpl, P., Kormann, M.S.D., Droste, S.K., Kühn, R., Reul, J.M.H.M., Holsboer, F., Wurst, W., 2003. Limbic corticotropin-releasing hormone receptor 1 mediates anxiety-related behavior and hormonal adaptation to stress. Nat Neurosci 6, 1100–1107. doi:10.1038/nn1123
- Nagaraja, T.S., Mahmood, R., Krishna, V., Thippeswamy, B.S., Veerapur, V.P., 2012. Evaluation of anxiolytic effect of Erythrina mysorensis Gamb. in mice. Indian J Pharmacol 44, 489–492.
- Nosek, K., Dennis, K., Andrus, B.M., Ahmadiyeh, N., Baum, A.E., Solberg Woods, L.C., Redei, E.E., 2008. Context and strain-dependent behavioral response to stress. Behav Brain Funct 4, 23. doi:10.1186/1744-9081-4-23
- Ochoa-Sanchez, R., Rainer, Q., Comai, S., Spadoni, G., Bedini, A., Rivara, S., Fraschini, F., Mor, M., Tarzia, G., Gobbi, G., 2012. Anxiolytic effects of the melatonin MT(2) receptor partial agonist UCM765: comparison with melatonin and diazepam. Prog Neuropsychopharmacol Biol Psychiatry 39, 318–325. doi:10.1016/j.pnpbp.2012.07.003

- Ognibene, E., Bovicelli, P., Adriani, W., Saso, L., Laviola, G., 2008. Behavioral effects of 6bromoflavanone and 5-methoxy-6,8-dibromoflavanone as anxiolytic compounds. Prog Neuropsychopharmacol Biol Psychiatry 32, 128–134. doi:10.1016/j.pnpbp.2007.07.023
- Okuyama, S., Chaki, S., Yoshikawa, R., Ogawa, S., Suzuki, Y., Okubo, T., Nakazato, A., Nagamine, M., Tomisawa, K., 1999. Neuropharmacological profile of peripheral benzodiazepine receptor agonists, DAA1097 and DAA1106. Life Sci. 64, 1455–1464.
- Olivier, J.D.A., Van Der Hart, M.G.C., Van Swelm, R.P.L., Dederen, P.J., Homberg, J.R., Cremers, T., Deen, P.M.T., Cuppen, E., Cools, A.R., Ellenbroek, B.A., 2008. A study in male and female 5-HT transporter knockout rats: an animal model for anxiety and depression disorders. Neuroscience 152, 573–584. doi:10.1016/j.neuroscience.2007.12.032
- Onusic, G.M., Nogueira, R.L., Pereira, A.M.S., Viana, M.B., 2002. Effect of acute treatment with a water-alcohol extract of Erythrina mulungu on anxiety-related responses in rats. Braz J Med Biol Res 35, 473–477.
- Ouagazzal, A.-M., Moreau, J.-L., Pauly-Evers, M., Jenck, F., 2003. Impact of environmental housing conditions on the emotional responses of mice deficient for nociceptin/orphanin FQ peptide precursor gene. Behav Brain Res 144, 111–117.
- Owens, M.J., Nemeroff, C.B., 1991. Physiology and pharmacology of corticotropinreleasing factor. Pharmacol Rev 43, 425–473.
- Paez-Pereda, M., Hausch, F., Holsboer, F., 2011. Corticotropin releasing factor receptor antagonists for major depressive disorder. Expert Opin Investig Drugs 20, 519–535.
 doi:10.1517/13543784.2011.565330
- Pain, L., Oberling, P., Launoy, A., Di Scala, G., 1999. Effect of nonsedative doses of propofol on an innate anxiogenic situation in rats. Anesthesiology 90, 191–196.
- Paine, T.A., Jackman, S.L., Olmstead, M.C., 2002. Cocaine-induced anxiety: alleviation by

diazepam, but not buspirone, dimenhydrinate or diphenhydramine. Behav Pharmacol 13, 511–523.

- Pang, R.D., Wang, Z., Klosinski, L.P., Guo, Y., Herman, D.H., Celikel, T., Dong, H.-W.,
 Holschneider, D.P., 2011. Mapping functional brain activation using [14C]iodoantipyrine in male serotonin transporter knockout mice. PLoS ONE 6, e23869.
 doi:10.1371/journal.pone.0023869
- Parent, A.J., Beaudet, N., Beaudry, H., Bergeron, J., Bérubé, P., Drolet, G., Sarret, P., Gendron, L., 2012. Increased anxiety-like behaviors in rats experiencing chronic inflammatory pain. Behav Brain Res 229, 160–167. doi:10.1016/j.bbr.2012.01.001
- Parks, C.L., Robinson, P.S., Sibille, E., Shenk, T., Toth, M., 1998. Increased anxiety of mice lacking the serotonin1A receptor. Proc Natl Acad Sci USA 95, 10734–10739.
- Pellow, S., Chopin, P., File, S.E., Briley, M., 1985. Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods 14, 149– 167.
- Peng, W.-H., Wu, C.-R., Chen, C.-S., Chen, C.-F., Leu, Z.-C., Hsieh, M.-T., 2004. Anxiolytic effect of berberine on exploratory activity of the mouse in two experimental anxiety models: interaction with drugs acting at 5-HT receptors. Life Sci. 75, 2451–2462.
- Perez-Caballero, L., Torres-Sanchez, S., Bravo, L., Mico, J.A., Berrocoso, E., 2014.
 Fluoxetine: a case history of its discovery and preclinical development. Expert Opin
 Drug Discov 9, 567–578. doi:10.1517/17460441.2014.907790
- Pires, L.F., Costa, L.M., Silva, O.A., de Almeida, A.A.C., Cerqueira, G.S., de Sousa, D.P., de Freitas, R.M., 2013. Anxiolytic-like effects of carvacryl acetate, a derivative of carvacrol, in mice. Pharmacol Biochem Behav 112, 42–48. doi:10.1016/j.pbb.2013.09.001
- Pisu, M.G., Garau, A., Olla, P., Biggio, F., Utzeri, C., Dore, R., Serra, M., 2013. Altered stress responsiveness and hypothalamic-pituitary-adrenal axis function in male rat

offspring of socially isolated parents. J Neurochem 126, 493–502.

doi:10.1111/jnc.12273

- Pisu, M.G., Mostallino, M.C., Dore, R., Maciocco, E., Secci, P.P., Serra, M., 2011. Effects of voluntary ethanol consumption on emotional state and stress responsiveness in socially isolated rats. European Neuropsychopharmacology 21, 414–425. doi:10.1016/j.euroneuro.2010.07.006
- Piszczek, L., Schlax, K., Wyrzykowska, A., Piszczek, A., Audero, E., Thilo Gross, C., 2013. Serotonin 1A auto-receptors are not sufficient to modulate anxiety in mice. Eur J Neurosci 38, 2621–2627. doi:10.1111/ejn.12260
- Plaznik, A., Palejko, W., Nazar, M., Jessa, M., 1994. Effects of antagonists at the NMDA receptor complex in two models of anxiety. European Neuropsychopharmacology 4, 503–512.
- Ponten, E., Fredriksson, A., Gordh, T., Eriksson, P., Viberg, H., 2011. Neonatal exposure to propofol affects BDNF but not CaMKII, GAP-43, synaptophysin and tau in the neonatal brain and causes an altered behavioural response to diazepam in the adult mouse brain. Behav Brain Res 223, 75–80. doi:10.1016/j.bbr.2011.04.019
- Popik, P., Kostakis, E., Krawczyk, M., Nowak, G., Szewczyk, B., Krieter, P., Chen, Z., Russek, S.J., Gibbs, T.T., Farb, D.H., Skolnick, P., Lippa, A.S., Basile, A.S., 2006. The anxioselective agent 7-(2-chloropyridin-4-yl)pyrazolo-[1,5-a]-pyrimidin-3-yl](pyridin-2yl)methanone (DOV 51892) is more efficacious than diazepam at enhancing GABAgated currents at alpha1 subunit-containing GABAA receptors. J Pharmacol Exp Ther 319, 1244–1252. doi:10.1124/jpet.106.107201
- Pritchard, L.M., Van Kempen, T.A., Williams, H., Zimmerberg, B., 2008. A laboratory exercise for a college-level, introductory neuroscience course demonstrating effects of housing environment on anxiety and psychostimulant sensitivity. J Undergrad Neurosci

Educ 7, A26-32.

- Prut, L., Belzung, C., 2003. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. Eur J Pharmacol 463, 3–33. doi:10.1016/S0014-2999(03)01272-X
- Quintino-dos-Santos, J.W., Muller, C.J.T., Bernabe, C.S., Rosa, C.A., Tufik, S., Schenberg, L.C., 2014. Evidence that the periaqueductal gray matter mediates the facilitation of panic-like reactions in neonatally-isolated adult rats. PLoS ONE 9, e90726.
- Radulovic, N.S., Miltojevic, A.B., Randjelovic, P.J., Stojanovic, N.M., Boylan, F., 2013.
 Effects of methyl and isopropyl N-methylanthranilates from Choisya ternata Kunth (Rutaceae) on experimental anxiety and depression in mice. Phytother Res 27, 1334– 1338. doi:10.1002/ptr.4877
- Rago, L., Kiivet, R.A., Harro, J., Pold, M., 1988. Behavioral differences in an elevated plusmaze: correlation between anxiety and decreased number of GABA and benzodiazepine receptors in mouse cerebral cortex. Naunyn Schmiedebergs Arch Pharmacol 337, 675–678.
- Ramanathan, M., Jaiswal, A.K., Bhattacharya, S.K., 1998. Differential effects of diazepam on anxiety in streptozotocin induced diabetic and non-diabetic rats.
 Psychopharmacology (Berl) 135, 361–367.
- Ramboz, S., Oosting, R., Amara, D.A., Kung, H.F., Blier, P., Mendelsohn, M., Mann, J.J., Brunner, D., Hen, R., 1998. Serotonin receptor 1A knockout: an animal model of anxiety-related disorder. Proc Natl Acad Sci USA 95, 14476–14481.
- Raquibul Hasan, S.M., Hossain, M.M., Akter, R., Jamila, M., Mazumder, E.H., Rahman, S.,
 2009. Sedative and anxiolytic effects of different fractions of the Commelina
 benghalensis Linn. Drug Discov Ther 3, 221–227.
- Reis, D.G., Scopinho, A.A., Guimarães, F.S., Corrêa, F.M.A., Resstel, L.B.M., 2011.

Behavioral and autonomic responses to acute restraint stress are segregated within the lateral septal area of rats. PLoS ONE 6, e23171. doi:10.1371/journal.pone.0023171

- Reis, F.M.C.V., Albrechet-Souza, L., Franci, C.R., Brandao, M.L., 2012. Risk assessment behaviors associated with corticosterone trigger the defense reaction to social isolation in rats: role of the anterior cingulate cortex. Stress 15, 318–328. doi:10.3109/10253890.2011.623740
- Rejon-Orantes, J.C., Suarez, D.P.P., Rejon-Rodriguez, A., Hernandez, S.H., Lievano,
 O.E.G., Rodriguez, D.L., la Mora, de, M.P., 2013. Aqueous root extracts from Mimosa albida Humb. & Bonpl. ex Willd display antinociceptive activity in mice. J
 Ethnopharmacol 149, 522–526. doi:10.1016/j.jep.2013.07.010
- Rex, A., Morgenstern, E., Fink, H., 2002. Anxiolytic-like effects of kava-kava in the elevated plus maze test--a comparison with diazepam. Prog Neuropsychopharmacol Biol Psychiatry 26, 855–860.
- Rochford, J., Beaulieu, S., Rousse, I., Glowa, J.R., Barden, N., 1997. Behavioral reactivity to aversive stimuli in a transgenic mouse model of impaired glucocorticoid (type II) receptor function: effects of diazepam and FG-7142. Psychopharmacology (Berl) 132, 145–152.
- Rodgers, R.J., Cole, J.C., 1993. Influence of social isolation, gender, strain, and prior novelty on plus-maze behaviour in mice. Physiol Behav 54, 729–736.
- Rylkova, D., Shah, H.P., Small, E., Bruijnzeel, A.W., 2009. Deficit in brain reward function and acute and protracted anxiety-like behavior after discontinuation of a chronic alcohol liquid diet in rats. Psychopharmacology (Berl) 203, 629–640. doi:10.1007/s00213-008-1409-z
- Ryu, V., Yoo, S.B., Kang, D.-W., Lee, J.-H., Jahng, J.W., 2009. Post-weaning isolation promotes food intake and body weight gain in rats that experienced neonatal maternal

separation. Brain Res 1295, 127–134. doi:10.1016/j.brainres.2009.08.006

- Saiyudthong, S., Marsden, C.A., 2011. Acute effects of bergamot oil on anxiety-related behaviour and corticosterone level in rats. Phytother Res 25, 858–862. doi:10.1002/ptr.3325
- Sakaue, M., Ago, Y., Sowa, C., Koyama, Y., Baba, A., Matsuda, T., 2003. The 5-HT1A receptor agonist MKC-242 increases the exploratory activity of mice in the elevated plus-maze. Eur J Pharmacol 458, 141–144.
- Samuels, B.A., Mendez-David, I., Faye, C., David, S.A., Pierz, K.A., Gardier, A.M., Hen, R., David, D.J., 2014. Serotonin 1A and Serotonin 4 Receptors: Essential Mediators of the Neurogenic and Behavioral Actions of Antidepressants. Neuroscientist. doi:10.1177/1073858414561303
- Santos Rosa, D., Faggion, S.A., Gavin, A.S., Anderson de Souza, M., Fachim, H.A., Ferreira dos Santos, W., Soares Pereira, A.M., Cunha, A.O.S., Beleboni, R.O., 2012.
 Erysothrine, an alkaloid extracted from flowers of Erythrina mulungu Mart. ex Benth: evaluating its anticonvulsant and anxiolytic potential. Epilepsy Behav 23, 205–212. doi:10.1016/j.yebeh.2012.01.003
- Santos, dos, L., de Andrade, T.G.C.S., Graeff, F.G., 2010. Social separation and diazepam withdrawal increase anxiety in the elevated plus-maze and serotonin turnover in the median raphe and hippocampus. J Psychopharmacol (Oxford) 24, 725–731. doi:10.1177/0269881109106954
- Satyan, K.S., Jaiswal, A.K., Ghosal, S., Bhattacharya, S.K., 1998. Anxiolytic activity of ginkgolic acid conjugates from Indian Ginkgo biloba. Psychopharmacology (Berl) 136, 148–152.
- Schaefer, A.T., Claridge-Chang, A., 2012. The surveillance state of behavioral automation. Curr Opin Neurobiol 22, 170–176. doi:10.1016/j.conb.2011.11.004

- Schellinck, H.M., Stanford, L., Darrah, M., 2003. Repetitive acute pain in infancy increases anxiety but does not alter spatial learning ability in juvenile mice. Behav Brain Res 142, 157–165. doi:10.1016/S0166-4328(02)00406-0
- Schipper, P., Nonkes, L.J.P., Karel, P., Kiliaan, A.J., Homberg, J.R., 2011. Serotonin transporter genotype x construction stress interaction in rats. Behav Brain Res 223, 169–175. doi:10.1016/j.bbr.2011.04.037
- Schmitt, U., Luddens, H., Hiemke, C., 2002. Anxiolytic-like effects of acute and chronic GABA transporter inhibition in rats. J Neural Transm 109, 871–880. doi:10.1007/s007020200071
- Schmitt, U., Luddens, H., Hiemke, C., 2001. Behavioral analysis indicates benzodiazepinetolerance mediated by the benzodiazepine binding-site at the GABA(A)-receptor. Prog Neuropsychopharmacol Biol Psychiatry 25, 1145–1160.
- Sena, E.S., van der Worp, H.B., Bath, P.M.W., Howells, D.W., Macleod, M.R., 2010.
 Publication bias in reports of animal stroke studies leads to major overstatement of efficacy. PLoS Biol 8, e1000344. doi:10.1371/journal.pbio.1000344
- Serra, M., Pisu, M.G., Littera, M., Papi, G., Sanna, E., Tuveri, F., Usala, L., Purdy, R.H., Biggio, G., 2000. Social isolation-induced decreases in both the abundance of neuroactive steroids and GABA(A) receptor function in rat brain. J Neurochem 75, 732– 740.
- Shang, L., Xu, T.-L., Li, F., Su, J., Li, W.-G., 2014. Temporal dynamics of anxiety phenotypes in a dental pulp injury model. Mol Pain 11, 40–40. doi:10.1186/s12990-015-0040-3
- Sherif, F., Harro, J., el-Hwuegi, A., Oreland, L., 1994. Anxiolytic-like effect of the GABAtransaminase inhibitor vigabatrin (gamma-vinyl GABA) on rat exploratory activity. Pharmacol Biochem Behav 49, 801–805.

Sherif, F., Oreland, L., 1995. Effect of the GABA-transaminase inhibitor vigabatrin on exploratory behaviour in socially isolated rats. Behav Brain Res 72, 135–140.

- Silva, M.I.G., de Aquino Neto, M.R., Teixeira Neto, P.F., Moura, B.A., do Amaral, J.F., de Sousa, D.P., Vasconcelos, S.M.M., de Sousa, F.C.F., 2007. Central nervous system activity of acute administration of isopulegol in mice. Pharmacol Biochem Behav 88, 141–147. doi:10.1016/j.pbb.2007.07.015
- Simon, P., Dupuis, R., Costentin, J., 1994. Thigmotaxis as an index of anxiety in mice. Influence of dopaminergic transmissions. Behav Brain Res 61, 59–64.
- Simpson, J., Kelly, J.P., 2012. The effects of isolated and enriched housing conditions on baseline and drug-induced behavioural responses in the male rat. Behav Brain Res 234, 175–183. doi:10.1016/j.bbr.2012.06.015
- Smith, G.W., Aubry, J.M., Dellu, F., Contarino, A., Bilezikjian, L.M., Gold, L.H., Chen, R.,
 Marchuk, Y., Hauser, C., Bentley, C.A., Sawchenko, P.E., Koob, G.F., Vale, W., Lee,
 K.F., 1998. Corticotropin releasing factor receptor 1-deficient mice display decreased
 anxiety, impaired stress response, and aberrant neuroendocrine development. Neuron
 20, 1093–1102.
- Sorra, K., Chen, C.-S., Chang, C.-F., Pusuluri, S., Mukkanti, K., Wu, C.-R., Chuang, T.-H., 2014. Synthesis, anticonvulsant, sedative and anxiolytic activities of novel annulated pyrrolo[1,4]benzodiazepines. Int J Mol Sci 15, 16500–16510. doi:10.3390/ijms150916500
- Srinivasan, J., Suresh, B., Ramanathan, M., 2003. Differential anxiolytic effect of enalapril and losartan in normotensive and renal hypertensive rats. Physiol Behav 78, 585–591.
- Stankevicius, D., Rodrigues-Costa, E.C., Camilo Florio, J., Palermo-Neto, J., 2008. Neuroendocrine, behavioral and macrophage activity changes induced by picrotoxin effects in mice. Neuropharmacology 54, 300–308.

doi:10.1016/j.neuropharm.2007.09.011

- Stefanski, R., Palejko, W., Kostowski, W., Plaznik, A., 1992. The comparison of benzodiazepine derivatives and serotonergic agonists and antagonists in two animal models of anxiety. Neuropharmacology 31, 1251–1258.
- Steiner, M.A., Lecourt, H., Jenck, F., 2012. The brain orexin system and almorexant in fearconditioned startle reactions in the rat. Psychopharmacology (Berl) 223, 465–475. doi:10.1007/s00213-012-2736-7
- Stemmelin, J., Cohen, C., Terranova, J.-P., Lopez-Grancha, M., Pichat, P., Bergis, O.,
 Decobert, M., Santucci, V., Francon, D., Alonso, R., Stahl, S.M., Keane, P., Avenet, P.,
 Scatton, B., le Fur, G., Griebel, G., 2008. Stimulation of the beta3-Adrenoceptor as a
 novel treatment strategy for anxiety and depressive disorders.
 Neuropsychopharmacology 33, 574–587. doi:10.1038/sj.npp.1301424
- Sterne, J.A.C., Sutton, A.J., Ioannidis, J.P.A., Terrin, N., Jones, D.R., Lau, J., Carpenter, J.,
 Rücker, G., Harbord, R.M., Schmid, C.H., Tetzlaff, J., Deeks, J.J., Peters, J., Macaskill,
 P., Schwarzer, G., Duval, S., Altman, D.G., Moher, D., Higgins, J.P.T., 2011.
 Recommendations for examining and interpreting funnel plot asymmetry in metaanalyses of randomised controlled trials. BMJ 343, d4002.
- Sugiyama, A., Saitoh, A., Iwai, T., Takahashi, K., Yamada, M., Sasaki-Hamada, S., Oka, J.I., Inagaki, M., Yamada, M., 2012. Riluzole produces distinct anxiolytic-like effects in rats without the adverse effects associated with benzodiazepines. Neuropharmacology 62, 2489–2498. doi:10.1016/j.neuropharm.2012.02.012
- Swami, U.S., Lande, A.A., Ghadge, P.M., Adkar, P.P., Ambavade, S.D., 2014.
 Pharmacological evaluation of Chlorophytum borivilianum Sant. & Fern. for anxiolytic activity and effect on brain GABA level. Orient Pharm Exp Med 14, 169–180.
 doi:10.1007/s13596-013-0145-z

- Taiwo, A.E., Leite, F.B., Lucena, G.M., Barros, M., Silveira, D., Silva, M.V., Ferreira, V.M.,
 2012. Anxiolytic and antidepressant-like effects of Melissa officinalis (lemon balm)
 extract in rats: Influence of administration and gender. Indian J Pharmacol 44, 189–192.
 doi:10.4103/0253-7613.93846
- Tanaka, M., Satou, T., Koike, K., 2013. Anxiolytic-like effect of Shigyakusan extract with low side effects in mice. J Nat Med 67, 862–866. doi:10.1007/s11418-013-0746-3
- Tatarczynska, E., Klodzinska, A., Stachowicz, K., Chojnacka-Wojcik, E., 2004. Effects of a selective 5-HT1B receptor agonist and antagonists in animal models of anxiety and depression. Behav Pharmacol 15, 523–534.
- Thakker, D.R., Natt, F., Hüsken, D., van der Putten, H., Maier, R., Hoyer, D., Cryan, J.F., 2005. siRNA-mediated knockdown of the serotonin transporter in the adult mouse brain. Mol Psychiatry 10, 782–9–714. doi:10.1038/sj.mp.4001687
- Thippeswamy, B.S., Mishra, B., Veerapur, V.P., Gupta, G., 2011. Anxiolytic activity of
 Nymphaea alba Linn. in mice as experimental models of anxiety. Indian J Pharmacol
 43, 50–55. doi:10.4103/0253-7613.75670
- Thompson, T., Grabowski-Boase, L., Tarantino, L.M., 2015. Prototypical anxiolytics do not reduce anxiety-like behavior in the open field in C57BL/6J mice. Pharmacol Biochem Behav 133, 7–17. doi:10.1016/j.pbb.2015.03.011
- Thongsaard, W., Deachapunya, C., Pongsakorn, S., Boyd, E.A., Bennett, G.W., Marsden, C.A., 1996. Barakol: a potential anxiolytic extracted from Cassia siamea. Pharmacol Biochem Behav 53, 753–758.
- Thorsell, A., Slawecki, C.J., Khoury, El, A., Mathe, A.A., Ehlers, C.L., 2006. The effects of social isolation on neuropeptide Y levels, exploratory and anxiety-related behaviors in rats. Pharmacol Biochem Behav 83, 28–34. doi:10.1016/j.pbb.2005.12.005

Tolardo, R., Zetterman, L., Bitencourtt, D.R., Mora, T.C., de Oliveira, F.L., Biavatti, M.W.,

Amoah, S.K.S., Burger, C., de Souza, M.M., 2010. Evaluation of behavioral and pharmacological effects of Hedyosmum brasiliense and isolated sesquiterpene lactones in rodents. J Ethnopharmacol 128, 63–70. doi:10.1016/j.jep.2009.12.026

Tone, A., 2009. The Age of Anxiety: A History of America's Turbulent Affair with Tranquilizers. Basic Books, New York.

Trimble, N., Johnson, A.C., Foster, A., Greenwood-van Meerveld, B., 2007. Corticotropinreleasing factor receptor 1-deficient mice show decreased anxiety and colonic sensitivity. Neurogastroenterol Motil 19, 754–760. doi:10.1111/j.1365-2982.2007.00951.x

- van Praag, H.M., 2003. Can stress cause depression? Prog Neuropsychopharmacol Biol Psychiatry 28, 891–907. doi:10.1016/j.pnpbp.2004.05.031
- Varty, G.B., Morgan, C.A., Cohen-Williams, M.E., Coffin, V.L., Carey, G.J., 2002. The gerbil elevated plus-maze I: behavioral characterization and pharmacological validation. Neuropsychopharmacology 27, 357–370. doi:10.1016/S0893-133X(02)00312-3
- Venancio, E.T., Rocha, N.F.M., Rios, E.R.V., Feitosa, M.L., Linhares, M.I., Melo, F.H.C., Matias, M.S., Fonseca, F.N., Sousa, F.C.F., Leal, L.K.A.M., Fonteles, M.M.F., 2011.
 Anxiolytic-like effects of standardized extract of Justicia pectoralis (SEJP) in mice: Involvement of GABA/benzodiazepine in receptor. Phytother Res 25, 444–450. doi:10.1002/ptr.3274
- Vesterinen, H.M., Sena, E.S., Egan, K.J., Hirst, T.C., Churolov, L., Currie, G.L., Antonic, A., Howells, D.W., Macleod, M.R., 2014. Meta-analysis of data from animal studies: a practical guide. J Neurosci Methods 221, 92–102. doi:10.1016/j.jneumeth.2013.09.010
- Viechtbauer, W., 2010. Conducting meta-analyses in R with the metafor package. Journal of Statistical Software 36, 1–48.

Vinkers, C.H., Oosting, R.S., van Bogaert, M.J.V., Olivier, B., Groenink, L., 2010. Early-life

Rodent anxiety review 60

blockade of 5-HT(1A) receptors alters adult anxiety behavior and benzodiazepine sensitivity. Biol Psychiatry 67, 309–316. doi:10.1016/j.biopsych.2009.08.013

- Voikar, V., Polus, A., Vasar, E., Rauvala, H., 2005. Long-term individual housing in C57BL/6J and DBA/2 mice: assessment of behavioral consequences. Genes Brain Behav 4, 240–252. doi:10.1111/j.1601-183X.2004.00106.x
- Volke, V., Soosaar, A., Koks, S., Vasar, E., Mannisto, P.T., 1998. L-Arginine abolishes the anxiolytic-like effect of diazepam in the elevated plus-maze test in rats. Eur J Pharmacol 351, 287–290.
- Wada, T., Fukuda, N., 1991. Effects of DN-2327, a new anxiolytic, diazepam and buspirone on exploratory activity of the rat in an elevated plus-maze. Psychopharmacology (Berl) 104, 444–450.
- Walf, A.A., Frye, C.A., 2012. Gestational or acute restraint in adulthood reduces levels of 5alpha-reduced testosterone metabolites in the hippocampus and produces behavioral inhibition of adult male rats. Front Cell Neurosci 6, 1–11. doi:10.3389/fncel.2012.00040/abstract
- Wanasuntronwong, A., Tantisira, M.H., Tantisira, B., Watanabe, H., 2012. Anxiolytic effects of standardized extract of Centella asiatica (ECa 233) after chronic immobilization stress in mice. J Ethnopharmacol 143, 579–585. doi:10.1016/j.jep.2012.07.010
- Wang, X., Li, G., Li, P., Huang, L., Huang, J., Zhai, H., 2015. Anxiolytic effects of orcinol glucoside and orcinol monohydrate in mice. Pharm Biol 53, 876–881.doi:10.3109/13880209.2014.946060
- Wei, X.Y., Yang, J.Y., Dong, Y.X., Wu, C.F., 2007. Anxiolytic-like effects of oleamide in group-housed and socially isolated mice. Prog Neuropsychopharmacol Biol Psychiatry 31, 1189–1195. doi:10.1016/j.pnpbp.2007.04.008

Weninger, S.C., Dunn, A.J., Muglia, L.J., Dikkes, P., Miczek, K.A., Swiergiel, A.H., Berridge,

C.W., Majzoub, J.A., 1999. Stress-induced behaviors require the corticotropin-releasing hormone (CRH) receptor, but not CRH. Proc Natl Acad Sci USA 96, 8283–8288.

- Wesolowska, A., Nikiforuk, A., 2007. Effects of the brain-penetrant and selective 5-HT6 receptor antagonist SB-399885 in animal models of anxiety and depression. Neuropharmacology 52, 1274–1283. doi:10.1016/j.neuropharm.2007.01.007
- Wikinski, S.I., Acosta, G.B., Gravielle, M.C., Bonavita, C.D., Bisagno, V., Fiszer de Plazas,S., Rubio, M.C., 2001. Diazepam fails to potentiate GABA-induced chloride uptake andto produce anxiolytic-like action in aged rats. Pharmacol Biochem Behav 68, 721–727.
- Wolfman, C., Viola, H., Paladini, A., Dajas, F., Medina, J.H., 1994. Possible anxiolytic effects of chrysin, a central benzodiazepine receptor ligand isolated from Passiflora coerulea. Pharmacol Biochem Behav 47, 1–4.
- Workman, J.L., Fonken, L.K., Gusfa, J., Kassouf, K.M., Nelson, R.J., 2011. Post-weaning environmental enrichment alters affective responses and interacts with behavioral testing to alter nNOS immunoreactivity. Pharmacol Biochem Behav 100, 25–32. doi:10.1016/j.pbb.2011.07.008
- Wright, R.C., Ingenito, A.J., 2001. Prevention of isolation-induced hypertension by intrahippocampal administration of a nonpeptide kappa-opioid receptor agonist.
 Hippocampus 11, 445–451. doi:10.1002/hipo.1059
- Yadav, A.V., Kawale, L.A., Nade, V.S., 2008. Effect of Morus alba L. (mulberry) leaves on anxiety in mice. Indian J Pharmacol 40, 32–36. doi:10.4103/0253-7613.40487
- Yamada, K., Iida, R., Miyamoto, Y., Saito, K., Sekikawa, K., Seishima, M., Nabeshima, T.,
 2000. Neurobehavioral alterations in mice with a targeted deletion of the tumor necrosis
 factor-alpha gene: implications for emotional behavior. J Neuroimmunol 111, 131–138.
- Yao, Y., Jia, M., Wu, J.-G., Zhang, H., Sun, L.-N., Chen, W.-S., Rahman, K., 2010. Anxiolytic and sedative-hypnotic activities of polygalasaponins from Polygala tenuifolia

in mice. Pharm Biol 48, 801-807. doi:10.3109/13880200903280042

- Yasumatsu, H., Morimoto, Y., Yamamoto, Y., Takehara, S., Fukuda, T., Nakao, T., Setoguchi, M., 1994. The pharmacological properties of Y-23684, a benzodiazepine receptor partial agonist. Br J Pharmacol 111, 1170–1178.
- Yildirim, E., Erol, K., Ulupinar, E., 2012. Effects of sertraline on behavioral alterations caused by environmental enrichment and social isolation. Pharmacol Biochem Behav 101, 278–287. doi:10.1016/j.pbb.2011.12.017
- Yildizoglu, T., Weislogel, J.-M., Mohammad, F., Chan, E.S.Y., Assam, P.N., Claridge-Chang, A., 2015. Estimating Information Processing in a Memory System: The Utility of Meta-analytic Methods for Genetics. PLoS Genet 11, e1005718. doi:10.1371/journal.pgen.1005718
- Yorgason, J.T., Espana, R.A., Konstantopoulos, J.K., Weiner, J.L., Jones, S.R., 2013. Enduring increases in anxiety-like behavior and rapid nucleus accumbens dopamine signaling in socially isolated rats. Eur J Neurosci 37, 1022–1031. doi:10.1111/ejn.12113
- Zanoli, P., Rivasi, M., Baraldi, C., Baraldi, M., 2002. Pharmacological activity of hyperform acetate in rats. Behav Pharmacol 13, 645–651.

doi:10.1097/01.fbp.0000047149.28986.2e

- Zhang, L.-M., Zhao, N., Guo, W.-Z., Jin, Z.-L., Qiu, Z.-K., Chen, H.-X., Xue, R., Zhang, Y.-Z.,
 Yang, R.-F., Li, Y.-F., 2014. Antidepressant-like and anxiolytic-like effects of YL-IPA08,
 a potent ligand for the translocator protein (18 kDa). Neuropharmacology 81, 116–125.
 doi:10.1016/j.neuropharm.2013.09.016
- Zhang, Y., Zu, X., Luo, W., Yang, H., Luo, G., Zhang, M., Tang, S., 2012. Social isolation produces anxiety-like behaviors and changes PSD-95 levels in the forebrain. Neurosci Lett 514, 27–30. doi:10.1016/j.neulet.2012.02.043

Zhao, S., Edwards, J., Carroll, J., Wiedholz, L., Millstein, R.A., Jaing, C., Murphy, D.L.,

Lanthorn, T.H., Holmes, A., 2006. Insertion mutation at the C-terminus of the serotonin transporter disrupts brain serotonin function and emotion-related behaviors in mice. Neuroscience 140, 321–334. doi:10.1016/j.neuroscience.2006.01.049

Zheng, F., Adelsberger, H., Muller, M.R., Fritschy, J.M., Werner, S., Alzheimer, C., 2009. Activin tunes GABAergic neurotransmission and modulates anxiety-like behavior. Mol Psychiatry 14, 332–346. doi:10.1038/sj.mp.4002131

FIGURES AND TABLES

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.

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.

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.

Figure 4. Meta-analyses of serotonin receptor 1A interventions on rodent anxietyrelated 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.

Figure 5. Meta-analyses of serotonin transporter interventions on rodent anxietyrelated 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.

Figure 6. Meta-analyses of the effects of stress signaling genes on anxietyrelated 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.

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.

Figure 8. Summary effect sizes of all meta-analyses.

The weighted mean effect sizes of all 10 interventions are shown here. 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. 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.

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.

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, the corresponding 95% confidence interval for the intercept, and the P-values of Egger's test are listed.

Table 3. 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 Legends

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

1169 articles identified in PubMed and Embase searches for 10 anxiety related interventions using the search phrases given in Table1

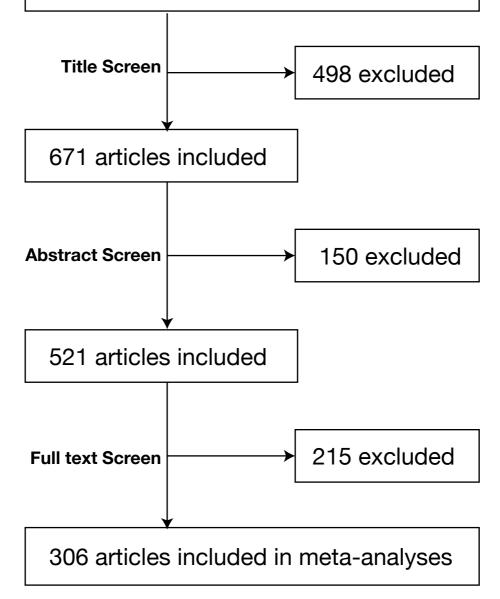


Figure 1. Flow chart of the systematic literature review of 10 anxiotropic interventions. The literature was reviewed in a four-stage process, starting with searches of the Pubmed and EMBASE databases that yielded 1169 articles, followed by three screens of increasing detail, reviewing the article title, 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.

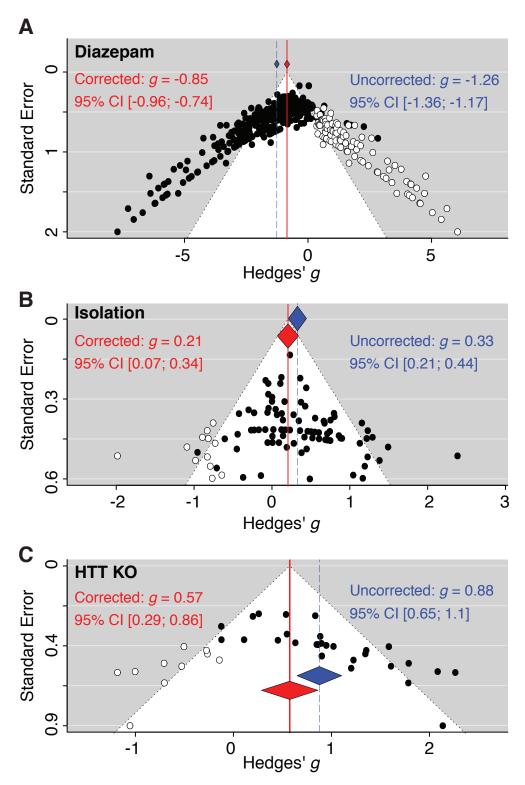


Figure 2. Funnel plots of three meta-analyses with evidence for publication bias. Where at least ten experiments were available for meta-analysis, the effect sizes (Hedges'g) of the experiments are plotted against their respective standard errors. Points on each plot represent individual experiments. 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.

Assay = EPM Gonzalez-Trujano ME 2012 Mice 0.01mg/kg Male Gonzalez-Trujano ME 2012 Mice 0.03mg/kg Male Gonzalez-Trujano ME 2015 Mice 0.1mg/kg Male Leggio 2011 Mice 0.1mg/kg Male Gonzalez-Trujano ME 2012 Mice 0.1mg/kg Male	Nem SD 6 25.05 9.349 6 25.05 9.349 6 15.68 2.402 7 0.61 0.108 6 25.05 9.349	N ₇ Mean SD 6 36.72 6.15 6 31.72 14.18 6 31.84 9.97 7 0.94 0.22 6 40.89 10.66 10.61	mean difference	-1.36 [-2.67; - -0.51 [-1.67; -2.06 [-3.56; - -1.79 [-3.09; - -1.46 [-2.79; -	0.646] 0. 0.552] 0. 0.484] 0. 0.126] 0.
Harada K 2006 Rats 0.1mg/kg Male Griebel G 1999 Rats 0.1mg/kg Male Ponten 2011 Mice 0.1mg/kg Male Leggio 2011 Mice 0.1mg/kg Male Leggio 2011 Mice 0.1mg/kg Male	16 9.11 4.224 11 15.20 16.915 7 17.29 1.608 7 1.96 0.217 7 1.27 0.541 7 0.61 0.108	16 11.88 7.39 11 20.10 23.55 7 15.46 4.54 7 1.27 1.73 7 0.61 0.11 7 0.28 0.11		-0.45 [-1.15; -0.23 [-1.07; 0.50 [-0.57; 0.53 [-0.54; 1.57 [0.32; 2.83 [1.22;	0.609] 0 1.572] 0 1.600] 0 2.818] 0 4.440] 0
Jones GH 1994 Mice 0.2mg/kg Male Satyan 1998 Rats 0.25mg/kg Female and Male Ene HM 2015 Mice 0.25mg/kg Male Ramanathan M 1998 Rats 0.25mg/kg Female and Male Griebel G 1998 Rats 0.25mg/kg Male Yasumatsu H 1994 Rats 0.25mg/kg Male	11 14.65 18.511 20 6.07 3.737	8 23.43 8.58 5 14.13 9.49 7 14.83 7.58 7 5.97 2.33 11 25.81 15.62 20 8.40 5.11		-0.41 [-1.41; -1.97 [-3.32;- -1.85 [-3.31;- -1.05 [-2.19; -0.63 [-1.49; -0.51 [-1.14;	0.626] 0 0.399] 0 0.090] 0 0.233] 0 0.121] 0
Gonzalez-Trujano ME 2012 Mice 0.3mg/kg Male Nolfman C 1994 Mice 0.3mg/kg Male Assi MB 1993 Mice 0.3mg/kg Male Griebel G 1999 Rats 0.3mg/kg Male Junn 1998 Rats 0.3mg/kg Male Harada K 2006 Rats 0.32mg/kg Male	6 25.05 9.349 51 5.21 11.102 10 22.80 4.322 11 15.20 16.915 6 8.21 4.012 16 9.11 4.224	6 55.89 12.78 21 20.95 15.62 10 31.47 8.64 11 17.90 25.87 6 7.46 7.86 16 11.75 5.81		-2.54 [-4.21; - -1.24 [-1.79; - -1.21 [-2.19; - -0.12 [-0.96; 0.11 [-1.02; -0.51 [-1.21;	0.691] 0 0.244] 0 0.718] 0 1.243] 0
Jones GH 1994 Mice 0.39mg/kg Male Brioni 1994 Rats 0.484mg/kg Male Mahendra P 2011 Mice 0.5mg/kg Male Leggio 2011 Mice 0.5mg/kg Male Leggio 2011 Mice 0.5mg/kg Male Leggio 2011 Mice 0.5mg/kg Male	8 20.17 6.223 8 7.00 3.488 6 15.61 3.731 7 1.39 0.217 7 1.72 0.210 7 0.70 0.109	7 29.33 9.43 6 13.50 27.48 6 46.17 4.25 7 3.72 0.43 7 4.38 0.54 7 1.06 0.11	+0 +0+ +0+ +0+ +0+	-1.10 [-2.21; -0.34 [-1.41; -7.05 [-10.64; - -6.38 [-9.33; - -6.06 [-8.88; - -3.18 [-4.91; -	0.014] 0 0.729] 0 3.472] 0 3.426] 0 3.236] 0
Ramanathan M 1998 Rats 0.5mg/kg Female and Male Ognibene E 2008 Mice 0.5mg/kg Male Ene HM 2015 Mice 0.5mg/kg Male Srinivasan J 2003 Rats 0.5mg/kg Male Ponten 2011 Mice 0.5mg/kg Male	7 3.34 2.333 8 9.80 3.576 5 2.97 1.056 6 4.07 1.306 7 17.29 1.608	7 10.70 2.33 8 20.02 4.47 6 10.03 4.60 6 5.77 0.33 7 30.36 15.48	+0+++-+0++0+++0++0+0	-2.95 [-4.60; - -2.39 [-3.75; - -1.84 [-3.36; - -1.65 [-3.03; - -1.11 [-2.26;	1.300] 0 1.026] 0 0.325] 0 0.265] 0 0.041] 0
Peng WH 2004 Mice 0.5mg/kg Male fasumatsu H 1994 Rats 0.5mg/kg Male famada K 2000 Mice 0.5mg/kg Not specified Orapier D 2007 Rats 0.5mg/kg Male Mesfin M 2014 Mice 0.5mg/kg Male Lepicard EM 2000 Mice 0.5mg/kg Male	12 2.39 1.409 20 6.07 3.737 6 18.69 3.910 11 29.45 11.209 6 14.72 16.485 18 17.54 9.498	$\begin{array}{cccccccccccccccccccccccccccccccccccc$		-1.09 [-1.96; - -1.04 [-1.70; - -0.94 [-2.16; -0.87 [-1.73; - -0.78 [-1.97; -0.77 [-1.45; -	0.371] 0 0.280] 0 0.005] 0 0.415] 0
Lepicard EM 2000 Mice 0.5mg/kg Male Griebel G 1999 Rats 0.5mg/kg Male Rochford J 1997 Mice 0.5mg/kg Male Fraser LM 2010 Mice 0.5mg/kg Female and Male Sakaue M1 2003 Mice 0.5mg/kg Male Jain 2005 Rats 0.5mg/kg Male	15 16.42 26.013 10 18.18 15.245 8 3.27 3.081 15 17.19 14.012 17 9.60 13.606 6 21 55 4 432	18 36.94 28.90 10 27.13 19.17 8 5.01 3.70 15 21.26 16.35 17 12.60 10.31 6 21.87 7.89		-0.72 [-1.43; - -0.50 [-1.39; -0.48 [-1.48; -0.26 [-0.98; -0.24 [-0.92; -0.05 [-1.18;	0.398] 0 0.514] 0 0.459] 0 0.432] 0
Traser LM 2010 Mice 0.5mg/kg Female and Male Sakaue M1 2003 Mice 0.5mg/kg Male Griebel G 1998 Rats 0.5mg/kg Male Griebel G 1998 Rats 0.5mg/kg Male eggio 2011 Mice 0.5mg/kg Male Volfman C 1994 Mice 0.6mg/kg Male Da Silva NL 1996 Rats 0.75mg/kg Male Brioni 1994 Mice 0.97mg/kg Male Brioni 1994 Mice 0.997mg/kg Male Brioni 1994 Mice 1mg/kg Male Brioni 2011 Mice 1mg/kg Male Brioni 2011 Mice 1mg/kg Male Brioni 2014 Rats 1mg/kg Male Saitoh A 2013 Rats 1mg/kg Male Silva 2007 Mice 1mg/kg Male Male Male Manda K 2000 Mice 1mg/kg Male Male Mice 100 Mice 1mg/kg Male Micale 2008 Mice 1mg/kg Male Di L 2007 Mice 1mg/kg Male Di L 2007 Mice 1mg/kg Male Brineh C 2013 Mice 1mg/kg Male Brineh C 2013 Mice 1mg/kg Male Brineh C 2013 Mice 1mg/kg Male Di L 2007 Mice 1mg/kg Male Di L 2007 Mice 1mg/kg Male Di L 2007 Mice 1mg/kg Male Brineh C 2013 Mice 1mg/kg Male Di Brion C 2013 Mice 1mg/kg Male Di Di D 2007 Mice 1mg/kg Male Di D 2007	11 14.65 18.511 7 1.39 0.217 51 5.21 11.102 10 7.58 5.124 9 48.42 11.513	11 14.30 11.57 7 1.06 0.13 22 16.45 27.91 10 40.99 22.28 9 68.80 8.86	+0+ +0+ +0+ +0+ +0+	0.02 [-0.81; 1.73 [0.44; -0.62 [-1.14; - -1.98 [-3.09; - -1.89 [-3.04; -	0.858] 0 3.016] 0 0.114] 0 0.870] 0 0.734] 0
Ja Silva NL 1996 Hats 0.75mg/kg Male Rgo L 1988 Mice 0.75mg/kg Male Da Silva NL 1996 Rats 0.75mg/kg Female Jones GH 1994 Mice 0.78mg/kg Male 3rioni 1994 Rats 0.997mg/kg Male 3rioni 1994 Mice 0.997mg/kg Male	12 39.61 27.444 18 22.50 12.374 12 44.50 10.978 8 20.17 6.223 8 7.00 3.488 8 11.60 6.788	12 36.84 16.92 8 20.00 14.14 12 37.63 9.15 8 36.53 8.30 8 17.03 29.60 8 24.40 40.80		0.12 [-0.68; 0.19 [-0.65; 0.66 [-0.17; -2.11 [-3.40; - -0.45 [-1.45; -0.41 [-1.41;	1.022] 0 1.481] 0 0.822] 0 0.546] 0
Ramanathan M 1998 Rats 1mg/kg Female and Male Wicale 2009 Mice 1mg/kg Male Martinez AL 2006 Mice 1mg/kg Male .eggio 2011 Mice 1mg/kg Male .eggio 2011 Mice 1mg/kg Male Suriyama A 2012 Bats 1mg/kg Male	7 3.34 2.333 9 9.29 3.182 6 14.22 6.834 7 1.96 0.216 7 2.29 0.324 6 13.06 12.711	7 21.50 2.33 9 32.32 3.64 6 66.83 8.74 7 9.63 1.73 7 13.27 2.48 6 89.29 11.72	+ 0 + + 0 + 10 + + 0 + 0	-7.28 [-10.60; - -6.42 [-8.95; - -6.19 [-9.37; - -5.83 [-8.57; - -5.80 [-8.52; - -5.75 [-8.75; -	3.889] 0 2.998] 0 3.102] 0 3.083] 0
Saitoh A 2013 Rats 1mg/kg Male Kumar D 2014 Rats 1mg/kg Female and Male Gonzalez-Trujano ME 2012 Mice 1mg/kg Male Karim N 2011 Mice 1mg/kg Male Carro-Juarez M 2012 Rats 1mg/kg Male	5 27.38 14.456 6 14.41 4.190 6 25.05 9.349 8 16.84 5.380 7 11.49 5.242	6 93.73 8.52 6 40.51 5.28 6 69.94 8.18 8 38.39 5.38 7 29.88 4.19		-5.25 [-8.20; - -5.06 [-7.74; - -4.72 [-7.25; - -3.79 [-5.58; - -3.63 [-5.51; -	2.305] 0 2.371] 0 2.177] 0 1.996] 0 1.740] 0
Silva 2007 Mice 1mg/kg Male Velo FH 2010 Mice 1mg/kg Male Yamada K 2000 Mice 1mg/kg Not specified /enncio ET 2011 Rats 1mg/kg Male Yadav AV 2008 Mice 1mg/kg Male Sumar D 2014 Rats 1mg/kg Female and Male	13 21.63 8.312 12 23.78 10.917 6 18.69 3.910 9 24.27 8.800 6 10.27 5.226 6 13.96 5 155	13 65.85 14.89 12 61.41 11.11 6 32.49 3.91 9 60.75 13.20 6 35.67 9.80 6 38.93 9.96		-3.55 [-4.85; - -3.30 [-4.59; - -3.26 [-5.19; - -3.10 [-4.56; - -2.98 [-4.81; - -2.90 [-4.70; -	2.006] 0 1.322] 0 1.632] 0 1.155] 0
Dchoa-Sanchez R 2012 Rats 1mg/kg Male Thippeswamy BS 2011 Mice 1mg/kg Male Lolli LF 2007 Mice 1mg/kg Male Lolli LF 2007 Mice 1mg/kg Male Peng WH 2004 Mice 1mg/kg Male	12 11.62 6.396 6 14.27 7.887 10 15.46 9.781 10 7.42 5.866 15 2.40 2.262	7 62.85 27.94 6 37.73 7.67 10 50.08 13.75 10 47.01 19.50 15 8.42 2.26 9 51.99 13.81	+ O + + O + + O + + O + + O + + O + + O +	-2.82 [-4.18; - -2.78 [-4.54; - -2.78 [-4.54; - -2.78 [-4.07; - -2.63 [-3.89; - -2.63 [-3.89; - -2.58 [-3.59; - -2.45 [-3.74; -	1.450] 0 1.029] 0 1.482] 0 1.373] 0 1.584] 0
Girish C 2013 Mice 1mg/kg Male Girish C 2013 Mice 1mg/kg Male Micale 2008 Mice 1mg/kg Male Saiyudthong 2011 Rats 1mg/kg Male Consoli D 2007 Mice 1mg/kg Male	6 25.66 6.699 6 25.66 6.700 10 12.05 3.536 10 22.48 11.439 10 7.60 5.481	6 57.31 15.80 6 57.31 15.80 10 27.05 7.79 10 53.29 13.40 10 18.87 3.79		-2.41 [-4.03; - -2.41 [-4.03; - -2.38 [-3.57; - -2.37 [-3.56; - -2.29 [-3.47; -	0.787] 0 0.787] 0 1.178] 0 1.172] 0 1.111] 0
Nagaraja TS 2012 Mice 1mg/kg Male Girish C 2013 Mice 1mg/kg Male Iaiwo AE 2012 Rats 1mg/kg Female Micale 2009 Mice 1mg/kg Male Je Sousa FC 2007 Mice 1mg/kg Male Jomes PB 2010 Mice 1mg/kg Male	6 11.50 3.919 6 26.93 7.321 10 4.41 4.912 10 15.72 3.373 12 33.29 10.049 12 36.90 10.288	6 21.70 4.41 6 57.24 15.96 10 25.00 11.39 10 45.27 17.69 12 59.58 13.31 12 59.75 10.29	+ 0 + + 0 + + 0 + + 0 + + 0 + + 0 + + 0 +	-2.26 [-3.83; - -2.25 [-3.82; - -2.25 [-3.42; - -2.22 [-3.39; - -2.15 [-3.19; - -2.14 [-3.18; -	0.685] 0 1.079] 0 1.060] 0 1.114] 0
Micale 2009 Mice 1mg/kg Male de Sousa FC 2007 Mice 1mg/kg Male Gomes PB 2010 Mice 1mg/kg Male Bahi A 2014 Mice 1mg/kg Male Harada K 2006 Rats 1mg/kg Male Barbosa PR 2008 Rats 1mg/kg Male Mansouri MT 2014 Rats 1mg/kg Male Blainski A 2010 Mice 1mg/kg Male	9 27.69 14.703 16 9.11 4.224 15 19.29 14.942 10 12.96 6.633	8 65.79 20.21 16 22.18 7.92 15 58.38 22.91 10 35.37 13.94 9 40.71 13.93	+0+ +0+ +0+ +0+ +0+	-2.07 [-3.30; - -2.01 [-2.88; - -1.97 [-2.86; - -1.97 [-3.07; - -1.91 [-3.07; -	0.832] 0 1.139] 0 1.075] 0 0.859] 0 0.748] 0
Blainski A 2010 Mice 1mg/kg Male Wang X 2015 Mice 1mg/kg Male Mansouri MT 2014 Rats 1mg/kg Male Je-Paris F 2000 Rats 1mg/kg Male Nagaraja TS 2012 Mice 1mg/kg Male Rejon-Orantes JC 2013 Mice 1mg/kg Male Je Sousa FC 2007 Mice 1mg/kg Male	10 8.02 7.485 10 13.46 5.788 15 21.38 13.052 6 7.90 2.939 5 4.03 2.907 12 36.29 14.063	10 20.38 4.76 10 35.17 14.56 10 61.30 28.93 6 15.30 4.41 5 12.63 5.44 12 59.02 10.47		-1.89 [-2.98; - -1.88 [-2.96; - -1.86 [-2.84; - -1.82 [-3.25; - -1.78 [-3.36; - -1.77 [-2.74; -	0.788] 0 0.883] 0 0.390] 0 0.197] 0
Je Sousa FC 2007 Mice 1mg/kg Male Kebebew Z 2013 Mice 1mg/kg Male Ponten 2011 Mice 1mg/kg Male Le Melo CT 2006 Mice 1mg/kg Male Cechin EM 2003 Rats 1mg/kg Male Sorra K 2014 Mice 1mg/kg Male Faiwo AE 2012 Rats 1mg/kg Male Acchford J 1997 Mice 1mg/kg Male Sugiyama A 2012 Rats 1mg/kg Male Dunn 1998 Rats 1mg/kg Male Lepicard EM 2000 Mice 1mg/kg Male Acuribara 2000 Mice 1mg/kg Male Huerta-Reves M 2013 Mice 1mg/kg Female and Male	8 14.67 18.051 7 17.29 1.608 13 38.44 11.468 13 23.87 18.148 4 31.44 7.235	8 46.92 17.27 7 32.31 11.73 13 64.55 18.38 13 61.30 26.92 4 44.67 7.98	+0+ +0+ +0+ +0+ +0+ +0+	-1.73 [-2.92; - -1.68 [-2.95; - -1.65 [-2.56; - -1.58 [-2.48; - -1.51 [-3.22;	0.531] 0 0.404] 0 0.741] 0 0.681] 0 0.205] 0
lawo AE 2012 Hats 1mg/kg Male Rochford J 1997 Mice 1mg/kg Male Sugiyama A 2012 Rats 1mg/kg Male Dunn 1998 Rats 1mg/kg Male Lepicard EM 2000 Mice 1mg/kg Male Kuribara 2000 Mice 1mg/kg Male	10 8.66 7.751 8 3.27 3.081 5 28.72 20.342 6 8.21 4.012 15 16.42 26.013 10 1.07 1.370	10 28.91 17.01 8 11.11 6.47 5 69.02 28.84 6 23.98 13.63 19 50.75 23.12 10 7.37 6.11		-1.47 [-2.48; - -1.46 [-2.60; - -1.46 [-2.94; -1.45 [-2.78; - -1.37 [-2.13; - -1.36 [-2.36; -	0.325] 0 0.021] 0 0.119] 0 0.612] 0
				-1.36 [-2.37, - -1.35 [-2.47; - -1.28 [-2.27; - -1.24 [-2.22; - -1.24 [-2.21; - -1.18 [-2.14; - -1.14 [-1.81; -	0.233] 0 0.303] 0 0.268] 0 0.264] 0 0.213] 0
Raquibul Hasan SM 2009 Mice 1mg/kg Male Raquibul Hasan SM 2009 Mice 1mg/kg Male Bhattacharya 1996 Rats 1mg/kg Male Mechan AO 2002 Rats 1mg/kg Male Pellow S 1985 Rats 1mg/kg Male	5 27.47 30.320 9 16.25 24.795 24 14.27 15.350 9 20.38 13.506 7 18.88 4.371	20 10.77 4.33 5 66.05 31.61 10 49.04 30.58 10 33.27 20.58 9 38.13 17.44 7 24.14 5.48 11 20 46 10 35		-1.12 [-2.51; -1.12 [-2.10; - -1.09 [-1.88; - -1.08 [-2.09; - -0.99 [-2.13;	0.262] 0 0.134] 0 0.305] 0 0.077] 0 0.138] 0
rarent AJ 2012 Rats 1mg/kg Male Thongsaard W 1996 Rats 1mg/kg Male Aora S 2005 Rats 1mg/kg Female and Male Griebel G 1999 Rats 1mg/kg Male Lepicard EM 2000 Mice 1mg/kg Male	11 23.68 11.378 10 0.87 1.095 16 11.11 10.230 11 15.20 16.915 19 12.00 12.641 18 17.54 0 400	11 39.46 19.35 10 16.28 21.90 16 32.07 30.30 11 33.20 25.21 19 23.80 17.44 17 29.85 20.58	0 0 0 0	-0.96 [-1.85; - -0.95 [-1.89; - -0.90 [-1.64; - -0.81 [-1.68; -0.76 [-1.42; - -0.76 [-1.45; -	0.065] 0. 0.016] 0. 0.171] 0. 0.069] 0. 0.098] 0.
Faiwo AE 2012 Rats 1mg/kg Male Gaiwo AE 2012 Rats 1mg/kg Male Faiwo 2012 Rats 1mg/kg Male Faiwo 2012 Rats 1mg/kg Male Aguibul Hasan SM 2009 Mice 1mg/kg Male Aquibul Hasan SM 2009 Mice 1mg/kg Male Battacharya 1991 Mice 1mg/kg Male Wechan AO 2002 Rats 1mg/kg Male Pellow S 1985 Rats 1mg/kg Male Pellow S 1000 Mice 1mg/kg Male Pellow S 1000 Mice 1mg/kg Male Pellow C 1002 Rats 1mg/kg Male Pelice T 2002 Rats 1mg/kg Male Paine TA 2002 Rats 1mg/kg Male Paine TA 2002 Rats 1mg/kg Male Paine TA 2002 Rats 1mg/kg Male Frassetto 2010 Rats 1mg/kg Male Frassetto 2010 Rats 1mg/kg Male Fraine 1997 Rats 1mg/kg Male Faria 1997 Rats 1mg/kg Male Faria 1997 Rats 1mg/kg Male Fariebel G 1998 Rats 1mg/kg Male Fariebel G 1999 Rats 1mg/kg Male Ariebel G 1999 Rats 1mg/kg Male Painebel G 1999 Painebel G 1996	8 36.74 12.226 9 19.59 10.321 11 51.28 14.909 16 44.32 8.056 17 9.60 13.606	17 29.85 20.58 8 44.05 7.28 9 33.88 28.59 11 69.87 41.07 16 62.73 46.05 17 16.00 9.48 10 58 33 60 66	0 0 0 0 0	-0.69 [-1.70; -0.63 [-1.59; -0.58 [-1.44; -0.54 [-1.25; -0.53 [-1.22;	0.330] 0. 0.319] 0. 0.278] 0. 0.164] 0. 0.153] 0.
Firen 2004 Mile Img/kg Male Faria 1997 Rats 1mg/kg Male Griebel G 1998 Rats 1mg/kg Male Faiwo AE 2012 Rats 1mg/kg Female Griebel G 2002 Rats 1mg/kg Male	U 35.00 17.707 8 11.68 4.526 11 14.65 18.511 10 18.08 16.062 10 17.29 16.644 11 12.40 6.622	10 58.33 60.66 10 24.16 31.48 11 23.72 25.45 10 23.38 12.07 10 22.56 13.08 11 16.50 15.92		-0.50 [-1.39; -0.50 [-1.45; -0.39 [-1.24; -0.36 [-1.24; -0.34 [-1.22; -0.32 [-1.17;	0.393] 0. 0.448] 0. 0.453] 0. 0.528] 0. 0.547] 0. 0.519] 0.
Jaszberenyi M 2007 Rats 1mg/kg Male Griebel G 1999 Rats 1mg/kg Male Dkuyama S 1999 Rats 1mg/kg Male Wanasuntronwong 2012 Mice 1mg/kg Male Da Silva NL 1996 Rats 1mg/kg Male	7 4.08 2.622 10 18.18 15.245 14 12.10 5.543 9 4.94 2.910 12 39.61 27.444 12 12 00 10 446	7 4.96 2.44 10 23.00 14.81 14 13.70 7.85 9 8.28 21.00 12 43.71 6.86		-0.32 [-1.38; -0.31 [-1.19; -0.23 [-0.97; -0.21 [-1.14; -0.20 [-1.00;	0.734] 0. 0.576] 0. 0.514] 0. 0.715] 0. 0.605] 0.
Jaszberenyi 2009 Rats 1mg/kg Male Jaszberenyi 2009 Rats 1mg/kg Male Steiner MA 2012 Rats 1mg/kg Male Leggio 2011 Mice 1mg/kg Male Leggio 2011 Mice 1mg/kg Male Da Silva NL 1996 Rats 1mg/kg Female	12 13.20 10.446 10 22.63 4.993 12 22.73 8.998 7 0.98 0.108 7 1.63 0.216 12 44.50 10.978	$\begin{array}{cccccccccccccccccccccccccccccccccccc$		-0.14 [-0.95; -0.05 [-0.92; -0.03 [-0.83; 0.00 [-1.05; 0.00 [-1.05; 0.16 [-0.64;	0.829] 0 0.771] 0 1.048] 0 1.048] 0 0.959] 0
Ja Silva NL 1996 Rats 1mg/kg Male Da Silva NL 1996 Rats 1mg/kg Female Meyer L 2013 Rats 1.25mg/kg Male Dunn RW 1989 Rats 1.25mg/kg Male Klodzinska 2004 Rats 1.25mg/kg Male Klodzinska 2004 Rats 1.25mg/kg Male Tatarczynska E 2004 Rats 1.25mg/kg Male	12 8.99 5.738 8 16.40 5.091 12 19.33 7.420 7 10.75 4.267 7 3.61 1.368 6 10.90 3.429	12 15.86 3.45 8 24.13 5.75 12 25.68 5.14 7 20.70 15.65 7 6.72 4.92 7 20.60 15.19		-1.40 [-2.31; - -1.35 [-2.46; - -0.96 [-1.81; - -0.81 [-1.92; -0.80 [-1.91; -0.79 [-1.94;	0.231] 0 0.108] 0 0.293] 0 0.300] 0
Nesolowska A 2007 Rats 1.25mg/kg Male Jastrzebska–Wisek M 2014 Rats 1.25mg/kg Male Simpson J 2012 Rats 1.25mg/kg Male Volina–Hernendez M 2004 Rats 1.3mg/kg Male Zanoli 2002 Rats 1.5mg/kg Male	8 10.90 3.960 8 26.21 21.847 6 52.45 17.594 7 23.20 14.191 12 12.03 15.949	8 20.60 17.54 8 43.31 28.09 6 52.77 13.87 7 54.51 10.23 12 65.11 34.98	+0+ +0+ +0+ +0+ +0+		0.299] 0 0.370] 0 1.113] 0 0.904] 0 0.896] 0
Griebel G 1997 Rats 1.5mg/kg Male Dalvi A 2001 Mice 1.5mg/kg Male Rago L 1988 Mice 1.5mg/kg Male Sherif F 1994 Rats 1.5mg/kg Male Dalvi A 1999 Mice 1.5mg/kg Male Drapier D 2007 Rats 1.5mg/kg Male	11 15.03 15.549 13 8.50 7.572 18 22.50 12.374 24 5.42 6.532 10 9.85 10.904 11 29.45 11.209	11 55.45 30.03 13 23.60 11.90 8 39.17 15.91 8 19.58 24.49 10 19.21 9.35 11 47.23 25.80	<u>ې څ</u> څ څ څ څ	-1.63 [-2.61; - -1.47 [-2.35; - -1.20 [-2.10; - -1.05 [-1.90; - -0.88 [-1.81; -0.86 [-1.74;	0.585] 0. 0.292] 0. 0.205] 0. 0.045] 0. 0.021] 0.
Hago L 1988 Milce 1.5mg/kg Male Sherif F 1994 Rats 1.5mg/kg Male Dalvi A 1999 Mice 1.5mg/kg Male Drapier D 2007 Rats 1.5mg/kg Male Stankevicius D 2008 Mice 1.5mg/kg Not specified .aBuda 2001 Mice 1.5mg/kg Male Jones GH 1994 Rats 1.9mg/kg Male Gonzalez-Trujano ME 2012 Mice 2mg/kg Male Gonzalez-Trujano ME 2012 Mice 2mg/kg Male Gupta D 2014 Mice 2mg/kg Male Gupta D 2014 Mice 2mg/kg Male Gurhe YV 2014 Mice 2mg/kg Male Carro-Jurez M 2012 Rats 2mg/kg Male Carroer JUrez M 2012 Rats 2mg/kg Male Carroero LM 2005 Rats 2mg/kg Male Carroero LM 2005 Rats 2mg/kg </td <td>10 20.02 7.605 10 3.75 3.953 8 20.17 6.223 8 7.00 3.488 6 25.05 9.349 6 17 19 2.275</td> <td>10 34.23 44.18 10 15.47 42.11 7 43.53 14.61 8 21.67 28.53 6 81.89 2.26 6 55.85 7.19</td> <td></td> <td>-0.43 [-1.32; -0.38 [-1.26; -2.01 [-3.32; - -0.68 [-1.70; -7.71 [-11.60; - -6.69 [-10.11; -</td> <td>0.511] 0. 0.701] 0. 0.334] 0. 3.827] 0.</td>	10 20.02 7.605 10 3.75 3.953 8 20.17 6.223 8 7.00 3.488 6 25.05 9.349 6 17 19 2.275	10 34.23 44.18 10 15.47 42.11 7 43.53 14.61 8 21.67 28.53 6 81.89 2.26 6 55.85 7.19		-0.43 [-1.32; -0.38 [-1.26; -2.01 [-3.32; - -0.68 [-1.70; -7.71 [-11.60; - -6.69 [-10.11; -	0.511] 0. 0.701] 0. 0.334] 0. 3.827] 0.
Gupta D 2015 Mice 2mg/kg Female and Male Gupta D 2015 Mice 2mg/kg Male de Almeida 2012 Mice 2mg/kg Male Karim N 2011 Mice 2mg/kg Male Carro-Jurez M 2012 Rats 2mg/kg Male	6 16.59 2.370 6 2.33 0.416 7 38.90 3.122 8 16.84 5.380 7 11.49 5.242	6 52.12 7.11 6 8.24 1.24 7 68.95 6.31 8 55.48 7.84 7 46.76 7.86	+ 0 + + 0 + + 0 + + 0 + 0	-6.19 [-9.37; - -5.89 [-8.94; - -5.65 [-8.31; - -5.43 [-7.80; - -4.94 [-7.32; -	2.997] 0. 2.834] 0. 2.993] 0. 3.070] 0. 2.560] 0.
Swami SU 2014 Mice 2mg/kg Male Fortes AC 2013 Mice 2mg/kg Male Carneiro LM 2005 Rats 2mg/kg Female and Male Bhatt S 2013 Mice 2mg/kg Male Carro-Juarez M 2012 Rats 2mg/kg Male Han H 2009 Mice 2mg/kg Male	6 13.05 5.590 11 24.85 2.885 14 11.83 6.623 8 2.00 1.923 7 14.94 10.444 11 12.78 3.980	6 70.98 16.02 11 37.55 2.89 8 74.97 34.66 8 11.17 4.16 7 52.76 16.35 11 36.27 13.02	+ O + + + O + + + O + + + O + + + O + + + O + + + O + + + O + + + O + + + O + + + O + + + + + O +	-4.46 [-6.88; - -4.23 [-5.84; - -2.87 [-4.14; - -2.68 [-4.12; - -2.58 [-4.11; - -2.35 [-3.48; -	2.626] 0. 1.594] 0. 1.233] 0. 1.050] 0.
Lepicard EM 2000 Mice 2mg/kg Male Yao Y 2010 Mice 2mg/kg Male Rochford J 1997 Mice 2mg/kg Male Karim N 2011 Mice 2mg/kg Male Pellow S 1985 Rats 2mg/kg Male Faturi CB 2010 Rats 2mg/kg Male	15 16.42 26.013 10 22.35 6.966 8 3.27 3.081 8 13.68 16.683 7 18.88 4.371 10 8.55 10.724	20 65.30 15.90 10 40.91 9.05 8 10.24 3.08 8 50.07 16.18 7 29.97 5.93 10 47.03 25.64	+0+ +0+ +0+ +0+ +0+ +0+	-2.30 [-3.18; - -2.20 [-3.36; - -2.14 [-3.43; - -2.09 [-3.38; - -1.99 [-3.35; - -1.87 [-2.96; -	1.044] 0 0.844] 0 0.810] 0 0.636] 0
Chen SW 2005 Mice 2mg/kg Male Contreras CM 2011 Rats 2mg/kg Female	10 16.05 10.973 6 24.18 15.036 9 35.82 10.014 12 13.20 10.446 8 15.97 24.554	10 44.96 18.29 6 58.19 20.48 9 57.47 13.84 12 36.81 16.29 8 50.70 13.75	+0+ +0+ +0+ +0+ +0+	-1.84 [-2.92; - -1.84 [-2.92; - -1.75 [-3.16; - -1.71 [-2.82; - -1.67 [-2.62; - -1.65 [-2.83; -	0.756] 0 0.337] 0 0.591] 0 0.716] 0 0.472] 0
Farland SP 2008 Mice 2mg/kg Male Karim N 2011 Mice 2mg/kg Male Schmitt 2002 Rats 2mg/kg Male Schmitt 2002 Rats 2mg/kg Male Chen SW 2005 Mice 2mg/kg Male Wolander AC 2011 Rats 2mg/kg Male Schmitt 2001 Rats 2mg/kg Male Schmitt 2005 Mice 2mg/kg Male Contreras CM 2011 Rats 2mg/kg Male Contreras CM 2011 Rats 2mg/kg Male	12 5.53 4.704 9 12.88 10.815 10 12.97 8.749 8 37.43 11.240 10 3.62 5.010 10 0.17 0.129	9 43.36 33.61 9 35.02 14.94 10 42.67 24.45 8 56.46 12.03 8 40.46 33.95 10 0.37 0.13	+0+ +0+ +0+ +0+ +0+ +0+	-1.64 [-2.67; - -1.62 [-2.72; - -1.55 [-2.57; - -1.55 [-2.70; - -1.54 [-2.63; - -1.48 [-2.50; -	0.519] 0 0.523] 0 0.390] 0 0.452] 0
Zhang LM 2014 Mice 2mg/kg Male Zhang LM 2014 Mice 2mg/kg Male Zriebel G 1999 Rats 2mg/kg Male Colla AR 2015 Mice 2mg/kg Male	10 24.18 19.411 6 16.18 8.472 10 12.76 8.431 10 18.18 15.245 8 5.99 5.126 7 420 8 18.281	6 58.19 26.44 6 47.89 28.34 10 32.38 17.62 10 38.02 13.50 8 22.86 16.90 7 62.71 12.65		-1.45 [-2.61; - -1.40 [-2.72; - -1.36 [-2.35; - -1.32 [-2.31; - -1.28 [-2.38; - -1.28 [-2.38; - -1.22 [-2.340; -	0.082] 0 0.367] 0 0.332] 0 0.174] 0
Molander AC 2011 Rats 2mg/kg Male Griebel G 1998 Rats 2mg/kg Male Cha HY 2005 Mice 2mg/kg Male	7 42.08 18.381 11 14.65 18.511 10 1.99 1.571 10 2.53 1.600 10 3.99 2.894 10 4.94 3.502	7 62.71 12.66 11 43.26 26.03 10 29.55 30.63 10 29.60 30.66 10 29.53 30.52 10 29.81 30.44		-1.22 [-2.40; - -1.22 [-2.14; - -1.22 [-2.19; - -1.19 [-2.16; - -1.13 [-2.09; - -1.10 [-2.05; -	0.294] 0 0.246] 0 0.226] 0 0.170] 0
Fernandez F 2004 Rats 2mg/kg Male Cha HY 2005 Mice 2mg/kg Male Liu J 2015 Mice 2mg/kg Male Engin E 2009 Rats 2mg/kg Male Lepicard EM 2000 Mice 2mg/kg Male Manasuntronwong 2012 Mice 2mg/kg Male	10 31.94 23.231 10 5.07 3.472 15 0.22 0.086 10 18.30 15.832 18 17.54 9.498 9 4.94 2.910	10 56.37 20.03 10 29.65 30.71 15 0.31 0.08 10 44.16 34.77 20 30.60 19.00 9 10.35 8.36		-1.08 [-2.03; - -1.08 [-2.03; - -1.05 [-1.82; - -0.92 [-1.85; -0.84 [-1.50; - -0.82 [-1.80;	0.125] 0 0.280] 0 0.015] 0 0.171] 0
Cha HY 2005 Mice 2mg/kg Male Liu J 2015 Mice 2mg/kg Male Engin E 2009 Rats 2mg/kg Male Lepicard EM 2000 Mice 2mg/kg Male Vanasuntronwong 2012 Mice 2mg/kg Male Sakaue M1 2003 Mice 2mg/kg Male Fraser LM 2010 Mice 2mg/kg Female and Male Liu J 2015 Mice 2mg/kg Male Paine TA 2002 Rats 2mg/kg Male Paine TA 2002 Rats 2mg/kg Male Soerngen-Lacerda 2000 Mice 2mg/kg Male Karaka A 2011 Rats 2mg/kg Male	17 9.60 13.606 15 17.19 14.012 15 0.24 0.099 8 0.16 0.129 8 36.74 12.226	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	0 0 0 0 0 0	-0.79 [-1.49; - -0.72 [-1.46; -0.69 [-1.43; -0.44 [-1.43; -0.29 [-1.27;	0.091] 0 0.020] 0 0.047] 0 0.557] 0 0.701] 0
Soerngen-Lacerda 2000 Mice 2mg/kg Male Karaka A 2011 Rats 2mg/kg Male Iatarczynska E 2004 Rats 2.5mg/kg Male Nesolowska A 2007 Rats 2.5mg/kg Male Klodzinska A 2004 Rats 2.5mg/kg Male Klodzinska 2004 Rats 2.5mg/kg Male	12 6.48 7.918 5 0.17 0.046 6 10.90 3.429 8 10.90 3.960 7 10.75 4.267 7 3.61 1.368	8 12.76 32.66 5 0.18 0.03 6 47.20 11.02 8 47.20 12.73 7 47.31 13.51 7 15.71 5.20		-0.28 [-1.18; -0.28 [-1.53; -4.10 [-6.38;- -3.64 [-5.38;- -3.41 [-5.23;- -2.98 [-4.64;-	0.971] 0. 1.825] 0. 1.898] 0. 1.604] 0.
Karaka A 2011 Rats 2mg/kg Male Tatarczynska E 2004 Rats 2.5mg/kg Male Vesolowska A 2007 Rats 2.5mg/kg Male Klodzinska A 2004 Rats 2.5mg/kg Male Slodzinska A 2004 Rats 2.5mg/kg Male Dunn RW 1989 Rats 2.5mg/kg Male Dun RW 1989 Rats 2.5mg/kg Male Jastrzebska–Wiesek M 2014 Rats 2.5mg/kg Male Drapier D 2007 Rats 2.5mg/kg Male Tores JA 2006 Rats 2.5mg/kg Male Simpson J 2012 Rats 2.5mg/kg Male Popik P 2006 Rats 2.5mg/kg Male Popik P 2006 Rats 2.5mg/kg Male Popik P 2006 Rats 2.5mg/kg Male	8 16.40 5.091 10 41.00 8.433 8 26.21 21.847 11 29.45 11.209 20 6.07 3.737 7 36 60 13 229	8 33.70 7.17 10 73.00 24.24 8 57.38 24.97 11 57.78 29.48 20 12.62 7.87 7 52.20 18.52		-2.63 [-4.06; - -1.69 [-2.74; - -1.26 [-2.35; - -1.22 [-2.15; - -1.04 [-1.71; - -0.91 [-2.03;	0.637] 0. 0.157] 0. 0.297] 0. 0.378] 0.
Brioni 1994 Rats 2.99mg/kg Male	8 7.00 3.488	6 69.42 17.23 9 36.67 33.75 24 55.62 49.28 12 7.73 8.97 8 14.97 24.00	+ 0 + 0 - 0 - 0 - 0 - 0 - 0	-0.90 [-2.11; -0.74 [-1.70; -0.62 [-1.20; - -0.12 [-0.92; -0.44 [-1.43;	0.313] 0. 0.222] 0. 0.043] 0. 0.680] 0. 0.556] 0.
Dunn 1998 Rats 3mg/kg Male Stemmelin J 2008 Rats 3mg/kg Male Griebel G 2002 Rats 3mg/kg Male Griebel G 1999 Rats 3mg/kg Male Steiner MA 2012 Rats 3mg/kg Male Dkuyama S 1999 Rats 3mg/kg Male	6 8.21 4.012 13 1.45 2.653 11 12.40 6.633 11 15.20 16.915 12 22.73 8.998 14 12.10 5.543	6 24.54 11.29 13 11.95 8.90 11 32.90 20.89 11 47.80 31.51 12 35.06 14.49 14 19.88 9.70		-1.78 [-3.20; - -1.55 [-2.44; - -1.27 [-2.20; - -1.24 [-2.17; - -0.99 [-1.84; - -0.96 [-1.74; -	0.655] 0. 0.340] 0. 0.313] 0. 0.131] 0.
Stemmelin J 2008 Rats 3mg/kg Male Griebel G 2002 Rats 3mg/kg Male Steiner MA 2012 Rats 3mg/kg Male Dkuyama S 1999 Rats 3mg/kg Male Dkuyama S 1999 Rats 3mg/kg Male Jones GH 1994 Mice 3.13mg/kg Male Harada K 2006 Rats 3.2mg/kg Male Craser LM 2010 Mice 4mg/kg Male Moreira MR 2012 Rats 4mg/kg Male Moreira MR 2014 Rats 5mg/kg Male Itatarczynska E 2004 Rats 5mg/kg Male Klodzinska A 2004 Rats 5mg/kg Male Alodzinska A 2007 Rats 5mg/kg Male Larissa FdA 2013 Mice 5mg/kg Male Laris J 1990 Rats 5mg/kg Male	8 20.17 6.223 16 9.11 4.224 15 17.19 14.012 7 11.49 5.242 8 15.93 3.357	4 59.33 44.78 16 29.83 13.73 15 44.32 26.86 7 19.62 9.44 8 33.15 4.20 7 70.62 70		-1.44 [-2.83; - -1.99 [-2.85; - -1.23 [-2.02; - -1.00 [-2.13; -4.28 [-6.24; -	0.055] 0. 1.123] 0. 0.443] 0. 0.137] 0. 2.324] 0.
Klodzinska A 2004 Rats Smg/kg Male Klodzinska 2004 Rats Smg/kg Male Nesoowska A 2007 Rats Smg/kg Male Larissa FdA 2013 Mice Smg/kg Male Yasumatsu H 1994 Rats Smg/kg Male	7 10.75 4.267 7 3.61 1.368 8 10.90 3.960 9 32.37 16.189 20 6.07 3.737	7 70.40 26.70 7 70.43 28.45 7 23.46 10.12 8 70.40 30.83 9 72.84 16.19 20 15.48 7.28		-2.79 [-4.46; - -2.75 [-4.33; - -2.57 [-4.10; - -2.56 [-3.97; - -2.38 [-3.65; - -1.59 [-2.31; -	1.162] 0. 1.044] 0. 1.150] 0. 1.109] 0. 0.874] 0.
Jastrzebska-Wiesek M 2014 Rats 5mg/kg Male Harro J 1990 Rats 5mg/kg Male Nada T 1991 Rats 5mg/kg Male Simpson J 2012 Rats 5mg/kg Male Popik P 2006 Rats 5mg/kg Male	8 26.21 21.847 10 31.30 27.512 24 6.78 8.399 6 52.45 17.594 24 27.81 37.684 12 13.09 28.018	8 59.59 22.63 10 74.70 50.28 24 16.23 9.89 6 72.71 23.65 24 64.50 53.63 12 28.38 50.94		-1.42 [-2.55; - -1.03 [-1.97; - -1.01 [-1.62; - -0.90 [-2.11; -0.78 [-1.37; - -0.36 [-1.17;	0.081] 0 0.409] 0 0.315] 0 0.190] 0
Harro J 1990 Rats 5mg/kg Male Wada T 1991 Rats 5mg/kg Male Simpson J 2012 Rats 5mg/kg Male Popik P 2006 Rats 5mg/kg Male Langen B 2005 Rats 6mg/kg Male Jones GH 1994 Mice 6.25mg/kg Male Tanaka M 2013 Mice 10mg/kg Male Hazim Al 2014 Rats 10mg/kg Male Hazim Al 2014 Rats 10mg/kg Male Dkuyama S 1999 Rats 10mg/kg Male	8 20.17 6.223 5 12.69 3.153 8 7.88 7.800 8 14.30 8.932 14 12.10 5.543	4 44.00 47.52 5 45.97 12.41 8 46.61 14.85 8 38.51 8.93 14 40.00 18.02	+ 0 + + 0 + + 0 + + 0 + + 0 + + 0 +	-0.83 [-2.09; -3.32 [-5.53; - -3.09 [-4.65; - -2.56 [-3.97; - -2.03 [-2.97; -	0.436] 0 1.104] 0 1.520] 0 1.152] 0 1.096] 0
Mada T 1991 Rats 10mg/kg Male Nada T 1991 Rats 10mg/kg Male Yasumatsu H 1994 Rats 10mg/kg Male Steiner MA 2012 Rats 10mg/kg Male Jarada K 2006 Rats 10mg/kg Male	11 12.40 6.633 12 6.78 5.939 10 3.75 5.558 20 6.07 3.737 12 22.73 8.998 16 9.11 4.224	11 49.10 23.88 12 19.66 10.49 10 21.21 17.40 20 12.27 8.06 12 32.14 13.50 16 28.51 34.32		-2.01 [-3.08; - -1.46 [-2.38; - -1.29 [-2.28; - -0.97 [-1.63; - -0.79 [-1.63; - -0.77 [-1.50; -	0.541] 0 0.312] 0 0.309] 0 0.044] 0
Popik P 2006 Rats 10mg/kg Male Jones GH 1994 Mice 12.5mg/kg Male Rex 2002 Rats 15mg/kg Male Popik P 2006 Rats 20mg/kg Male Random effects model	24 27.81 37.684 8 20.17 6.223 10 8.43 5.376 24 27.81 37.684	24 58.88 52.18 3 33.77 56.10 10 18.57 16.34 24 77.22 62.32 2763		-0.67 [-1.25; - -0.46 [-1.81; -0.80 [-1.72; -0.94 [-1.54; - -1.33 [-1.44; -	0.089] 0 0.888] 0 0.121] 0 0.345] 0
Heterogeneity: I-squared=67.8%, tau-squared=0.5613, p<0.0001 Assay = LD Nikinski SI 2001 Rats 0.03mg/kg Male Costall 1990 Mice 0.063mg/kg Male Barnes NM 1990 Mice 0.063mg/kg Male Nikinski SI 2001 Rats 0.1mg/kg Male	10 11.50 5.493 5 47.81 10.795 10 43.51 18.244	10 18.28 17.79 5 49.84 11.66 10 44.47 13.68		-0.49 [-1.39; -0.16 [-1.41; -0.06 [-0.93;	1.080] 0
Costall 1990 Mice 0.125mg/kg Male	10 11.50 5.493 5 47.81 10.795	10 22.33 8.11 5 67.09 15.29		-1.50 [-2.51; - -1.31 [-2.75; -1.08 [-2.03; - -1.55 [-3.06; - -1.39 [-2.39; -	0.482] 0 0.122] 0 0.129] 0 0.046] 0 0.395] 0
Arkinski Si 2001 Pars 0.3mg/kg Male Birkett 2011 Mice 0.32mg/kg Male Harada K 2006 Mice 0.32mg/kg Male Alahendra P 2011 Mice 0.5mg/kg Male Costall 1990 Mice 0.5mg/kg Male Barnes NM 1990 Mice 0.5mg/kg Male	14 11.70 5.332 19 0.90 1.744 6 20.17 3.707 5 47.81 10.795 10 43.51 18.244	10 16.13 9.94 10 13.04 22.48 20 1.40 2.91 6 43.33 5.06 5 70.14 15.22 10 78.85 27.37		-0.55 [-1.45; -0.09 [-0.90; -0.20 [-0.83; -4.82 [-7.40; - -1.53 [-3.03; - -1.46 [-2.46; -	0.726] 0. 0.427] 0. 2.236] 0. 0.028] 0. 0.446] 0.
Zheng 2009 Mice 0.5mg/kg Female and Male Sugiyama A 2012 Rats 1mg/kg Male Yadav AV 2008 Mice 1mg/kg Male Pires LF 2013 Mice 1mg/kg Male Saitoh A 2013 Rats 1mg/kg Male Faiemirove JO 2014 Mice 1mg/kg Male	10 11.77 13.377 6 16.89 10.343 6 26.67 6.859 10 20.64 5.084 6 24.12 13.634 9 30.17 3.500	7 24.24 6.33 6 75.32 13.51 6 55.83 7.02 10 46.95 7.76 6 78.91 14.85 9 45.50 6.00		-1.07 [-2.11; - -4.48 [-6.92; - -3.88 [-6.06; - -3.84 [-5.43; - -3.55 [-5.60; - -2.97 [-4.40; -	2.043] 0. 1.693] 0. 2.256] 0. 1.498] 0.
shaq H ² 014 Rats 1mg/kg Male Galeotti N 2013 Mice 1mg/kg Male Fhippeswamy BS 2011 Mice 1mg/kg Male Fernandez SP 2008 Mice 1mg/kg Male Costall 1990 Mice 1mg/kg Male	6 9.30 3.466 6 33.61 6.579 6 39.73 10.476 12 26.46 9.388 5 47.81 10.795	6 24.58 6.83 6 50.21 6.16 6 66.07 10.32 12 42.87 12.38 5 68.30 16.19	+ O + + O + + O + + O + + O + + O + + O +	-2.60 [-4.29; - -2.40 [-4.02; - -2.34 [-3.93; - -1.44 [-2.36; - -1.34 [-2.79;	0.914] 0 0.784] 0 0.740] 0 0.527] 0 0.102] 0
Jarnes NM 1990 Mice 0.125mg/kg Male Costall 1990 Mice 0.25mg/kg Male Sarnes NM 1990 Mice 0.25mg/kg Male Wikinski SI 2001 Rats 0.3mg/kg Male Vikinski SI 2001 Rats 0.3mg/kg Male Harada K 2006 Mice 0.32mg/kg Male Costall 1990 Mice 0.5mg/kg Male Pres LF 2013 Mice 1mg/kg Male Saitoh A 2013 Rats 1mg/kg Male Saitoh A 2013 Rats 1mg/kg Male Saitoh A 2013 Mice 1mg/kg Male Saitoh C 1013 Mice 1mg/kg Male Costall 1990 Mice 1mg/kg Male Costall 1990 Mice 1mg/kg Male Saitone EXP 2008 Mice 1mg/kg Male Saitoh 2013 Rats 1mg/kg Male Costall 1990 Mice 1mg/kg Male Saitoh 2013 Rats 1mg/kg Male Saitoh 2013 Rats 1mg/kg Male Costal 1990 Mice 1mg/kg Male Saitoh 2013 Rats 1mg/kg Male Saitoh 2014 Rats 1mg/kg Male Saitoh 2001 Rats 1mg/kg Male Saitoh 2001 Rats 1mg/kg Male Saitoh 2001 Rats 1mg/kg Male Nikinski SI 2001 Rats 1mg/kg Male Saitoh 2005 Mice 1mg/kg Male Saitoh 2006 Mice 1mg/kg Male Saitoh 2007	9 32.61 10.916 10 24.74 6.766 12 12.96 10.264 10 11.50 5.493 9 29.29 9.520	10 71.15 27.37 9 47.45 14.18 10 36.24 13.11 12 22.41 15.40 10 17.29 12.29 9 39.62 24.00		-1.14 [-2.10; - -1.12 [-2.13; - -1.06 [-2.00; - -0.70 [-1.53; -0.58 [-1.48; -0.54 [-1.48;	0.106] 0 0.107] 0 0.132] 0 0.317] 0 0.406] 0
Solianto i Eori inido inigita inalo	11 10.00 10.010	20 5.27 11.04 12 11.83 11.71 7 29.77 27.71 69 19.91 11.71 13 17.70 24.08 6 7.06 1.16		-0.53 [-1.17; -0.44 [-1.26; -0.40 [-1.46; -0.35 [-0.68; - -0.34 [-1.10; -5.69 [-8.65; -	0.367] 0. 0.663] 0. 0.012] 0. 0.421] 0.
Sirkett 2011 Mice 1mg/kg Male Kurhe YV 2014 Mice 2mg/kg Male Gupta D 2014 Mice 2mg/kg Male Swami SU 2014 Mice 2mg/kg Male Shatt S 2013 Mice 2mg/kg Male Gupta D 2015 Mice 2mg/kg Female and Male Karim N 2011 Mice 2mg/kg Male		8 57.59 8.82 6 61.64 5.19 8 33.94 4.79 6 58.47 13.72 8 47.39 2.97	+ 0 + + 0 + + 0 + + 0 + 0	-5.18 [-7.45; - -3.79 [-5.95; - -3.49 [-5.19; - -3.23 [-5.16; - -2.82 [-4.30; -	2.906] 0. 1.645] 0. 1.799] 0. 1.308] 0. 1.334] 0.
arim N 2011 Mice 2mg/kg Male hi XJ 2005 Mice 2mg/kg Male wansumrohy dqu: 2019 Sm/ce Angol 10,1101/02070 ngd Gerthigd Dy Deer raview 1s the author/fun	11 26.21 3.471 8 25.04 10.990 1; 10 30.00 8.854 1; thysyersigg bo der 5 who has gra ble ungersactor f	11 33.90 1.34 8 50.18 5.08 10 42.93 8.12 sted Decembe inted bio R xiv a 4 0 4.05 r 13.90 10 4.05 r 13.90 10 4.05 r 13.90	r 18, 2015, The cc license to display nal license		1.304] 0. 0.449] 0. dis4preprint etuity. It is0
iadulovi NS 2013 Mice 2mg/kg Male ernandez F 2004 Rats 2mg/kg Male iu J 2015 Mice 2mg/kg Male olla AR 2015 Mice 2mg/kg Male ernandez SP 2008 Mice 2mg/kg Male	6 38.30 4.821 8 29.25 16.213 15 28.68 10.294 8 31.71 5.560 12 26.46 9.388	6 50.18 14.59 8 46.84 19.29 15 37.39 8.10 8 48.00 29.90 12 35.21 17.64		-1.08 [-2.03; - -1.01 [-2.24; -0.93 [-1.98; -0.91 [-1.67; - -0.72 [-1.74; -0.60 [-1.42; -0.46 [-1.62]	0.222] 0. 0.114] 0. 0.158] 0. 0.304] 0. 0.223] 0.
antos Rosa D 2012 Rats 2mg/kg Male lores JA 2006 Rats 2.5mg/kg Male iriebel G 2002 Rats 2.5mg/kg Male kuyama S 1999 Mice 3mg/kg Male iriebel G 2002 Rats 3mg/kg Male	6 9.39 6.604 7 10.33 8.819 13 8.38 13.821 12 12.96 10.264 13 6.92 15.023 14 11.70 5.332	6 13.34 8.93 7 29.33 10.58 13 34.83 22.08 12 40.00 15.40 13 52.42 34.40 14 17.65 28.61		-0.46 [-1.62; -1.83 [-3.14; - -1.39 [-2.26; - -2.00 [-3.00; - -1.66 [-2.57; - -0.28 [-1.03;	0.514 0. 0.520 0. 0.986 0. 0.750 0. 0.465 0.
Sirkett 2011 Mice 3mg/kg Male Iarada K 2006 Mice 3.2mg/kg Male Jnusic 2002 Rats 4mg/kg Male Aoreira MR 2014 Rats 5mg/kg Male Sorsini F 1993 Mice 5mg/kg Male Sukuyama S 1999 Mice 10mg/kg Male Tanaka M 2013 Mice 10mg/kg Male	19 0.90 1.744 9 4.77 4.462 8 36.66 7.822	19 4.73 7.41 9 20.27 28.02 8 73.33 6.31 8 74.43 27.17 12 47.22 15.40		-0.70 [-1.35; - -0.74 [-1.70; -4.88 [-7.04; - -1.64 [-2.82; - -2.53 [-3.64; -	0.040 0. 0.227 0. 2.714 0. 0.465 0. 1.412 0.
Borsini F 1993 Mice 5mg/kg Male Okuyama S 1999 Mice 10mg/kg Male Ganaka M 2013 Mice 10mg/kg Male Sostall 1990 Mice 10mg/kg Male Harada K 2006 Mice 10mg/kg Male Bandom effects model Heterogeneity: I-squared=67.9%, tau-squared=0.5407, p<0.0001	5 41.28 3.887 5 47.81 10.795 19 0.90 1.744 698	5 56.04 7.33 5 70.31 16.12 20 16.43 21.94 691	+ 0 +	-2.27 [-4.03; - -1.48 [-2.97; -0.96 [-1.63; - -1.37 [-1.60; -	0.506] 0. 0.005] 0. 0.298] 0.
Assay = OF Stefanski R 1992 Rats 0.05mg/kg Male Plaznik A 1994 Rats 0.05mg/kg Male Jessa M 1996 Rats 0.05mg/kg Male Jessa M 1996 Rats 0.05mg/kg Male	8 1.43 0.613 8 19.32 6.850 8 1.67 1.886 8 1.67 1.886 8 1.43 0.613	8 2.73 1.56 8 24.22 5.38 8 1.70 1.27 8 0.83 0.80 8 2.37 0.94	+0 +0 +0 +0 +0 +0	-1.04 [-2.10; -0.75 [-1.78; -0.02 [-1.00; 0.54 [-0.46; -1.11 [-2.18]	0.272] 0. 0.960] 0. 1.547] 0.
Stefaski R 1992 Rats 0.1mg/kg Male Plaznik A 1994 Rats 0.1mg/kg Male Ene HM 2015 Mice 0.25mg/kg Male Mesfin M 2014 Mice 0.5mg/kg Male Ennaceur A 2010 Mice 0.5mg/kg Male Stefaski R 1992 Rats 0.5mg/kg Male	8 1.43 0.613 8 19.32 6.850 6 17.83 4.899 6 0.72 1.086 9 18.30 13.481 8 1.43 0.613	8 2.37 0.94 8 16.55 6.52 7 15.56 5.58 6 3.11 4.34 9 21.73 11.52 8 1.48 1.37	+0- +0+ +0+ -0+	-1.11 [-2.18; - 0.39 [-0.60; 0.40 [-0.70; -0.70 [-1.88; -0.26 [-1.19; -0.04 [-1.02;	0.036] 0. 1.384] 0. 1.507] 0. 0.485] 0. 0.668] 0.
Ene HM 2015 Mice 0.5mg/kg Male 3ahi A 2014 Mice 1mg/kg Male Saitoh A 2013 Rats 1mg/kg Male Sugiyama A 2012 Rats 1mg/kg Male Kebebew Z 2013 Mice 1mg/kg Male	6 17.83 4.899 9 12.11 6.470 8 8.02 8.642 6 5.81 4.522 8 2.71 0.933	$\begin{array}{cccccc} 7 & 16.28 & 3.81 \\ 8 & 44.66 & 12.32 \\ 8 & 56.22 & 36.04 \\ 6 & 20.50 & 10.11 \\ 8 & 6.00 & 3.00 \end{array}$		0.33 [-0.77; -3.20 [-4.75; - -1.74 [-2.94; - -1.73 [-3.14; - -1.40 [-2.53; -	1.434] 0. 1.654] 0. 0.541] 0. 0.326] 0. 0.276] 0.
Ennaceur A 2010 Mice 1mg/kg Male Wanasuntronwong 2012 Mice 1mg/kg Male Thompson T 2015 Mice 1mg/kg Male Birkett 2011 Mice 1mg/kg Male Bellavite P 2011 Mice 1mg/kg Male Bellavite P 2011 Mice 1mg/kg Male	9 18.30 13.481 9 9.04 4.120 8 9.02 3.003 14 8.09 5.277 69 69.32 66.122 69 6.34 3.406	8 29.17 24.51 9 14.75 13.90 8 9.73 5.65 13 7.83 32.23 69 67.04 93.30 69 6.21 3.99		-0.53 [-1.50; -0.53 [-1.47; -0.15 [-1.13; 0.01 [-0.74; 0.03 [-0.31; 0.03 [-0.30;	0.443] 0. 0.414] 0. 0.834] 0. 0.766] 0. 0.362] 0. 0.369] 0.
Dchoa–Sanchez R 2012 Rats 1mg/kg Male Guilloux JP 2013 Mice 1.5mg/kg Male Choleris 2001 Mice 1.5mg/kg Male Kurhe YV 2014 Mice 2mg/kg Male Kong WX 2006 Rats 2mg/kg Male	1316.107.638114.643.177120.330.22261.220.83392.102.302	8 15.23 9.79 11 8.78 3.51 12 0.47 2.19 6 8.72 1.34 9 7.37 3.77		0.10 [-0.78; -1.19 [-2.11; - -0.09 [-0.89; -6.21 [-9.40; - -1.60 [-2.70; -	0.980] 0. 0.271] 0. 0.712] 0. 3.009] 0. 0.509] 0.
Colla AR 2015 Mice 2mg/kg Male (araka A 2011 Rats 2mg/kg Male Liu J 2015 Mice 2mg/kg Male Liu J 2015 Mice 2mg/kg Male Fernandez F 2004 Rats 2mg/kg Male Wanasuntronwong 2012 Mice 2mg/kg Male Fernance J 2015 Mice 2mg/kg Male	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	8 28.25 13.23 5 0.07 0.02 15 8.43 3.69 15 8.43 3.58 8 2.98 2.43 9 15.29 16.33		-1.38 [-2.50; - -1.22 [-2.63; -0.97 [-1.74; - -0.97 [-1.73; - -0.74 [-1.76; -0.50 [-1.44; -0.50 [-1.44;	0.189 0. 0.211 0. 0.208 0. 0.286 0. 0.442 0.
Thompson T 2015 Mice 2mg/kg Male Ennaceur A 2010 Mice 3mg/kg Male Birkett 2011 Mice 3mg/kg Male Gonzlez-Pardo 2006 Rats 5mg/kg Male a.ngen B 2005 Rats 6mg/kg Male	8 11.82 5.009 9 18.30 13.481 14 8.09 5.277 10 6.16 4.235 12 0.47 0.520	8 2.80 1.84 8 37.09 34.80 14 8.78 37.48 10 3.88 21.43 12 0.85 0.69	+O+ +O+ +O+ +O+ +O+	2.26 [0.93; -0.69 [-1.68; -0.03 [-0.77; 0.14 [-0.74; -0.60 [-1.43;	3.587 0. 0.295 0. 0.716 0. 1.019 0. 0.217 0.
Hazim Al 2014 Rats 10mg/kg Male Birkett 2011 Mice 10mg/kg Male Random effects model	8 2.55 1.380 14 8.09 5.277 478	8 11.77 4.87 10 15.08 47.00 467		-2.44 [-3.81; - -0.22 [-1.04; -0.53 [-0.77; -	0.592] 0.

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 NC and NT respectively.

A Ht1a Knockout	N	KO Mean SD	N	WT Mean SD	-2 -1 0 1 2 3	SMD 95%-CI	W(random)
Assay = EPM Groenink, 2003 Mice Female and Male Gross, 2002 Mice Female and Male Piszczek, 2013 Mice Female and Male Ramboz, 1998 Mice Female and Male Ferres-Coy, 2013 Mice Male Freeman-Daniels, 2011 Mice Male Gleason, 2010 Mice Male Gleason, 2010 Mice Male Random effects model	8 16 27 20 12 43 9 11 7 153	4.10 3.1 46.39 14.7 19.67 12.6 28.53 18.3 29.48 12.3 21.30 15.1 16.61 8.4 22.53 10.7 29.35 12.3	0 16 30 30 1 20 6 12 5 39 7 9 12 11	17.17 13.93 14.93 12.03 13.23 13.22 5.97 10.89 10.31 10.14 16.96 11.79		-0.81 [-1.84; 0.22] 0.73 [0.02; 1.45] 0.83 [0.29; 1.38] 0.68 [0.05; 1.32] 1.15 [0.28; 2.03] 0.56 [0.12; 1.00] 1.04 [0.04; 2.04] 1.13 [0.22; 2.04] 0.96 [-0.17; 2.09] 0.71 [0.33; 1.09]	5.3% 6.9% 6.0% 4.2% 7.9% 3.6% 4.0% 3.0%
Heterogeneity: I^2=29.7%, Tau^2=0.1777, p=0.10 Assay = LD Groenink, 2003 Mice Female and Male Klemenhagen, 2006 Mice Male Random effects model Heterogeneity: I^2=79.9%, Tau^2=0.7019, p=0.02	12 10 22	24.16 29.3 43.69 33.4				-0.34 [-1.14; 0.47] 0.99 [0.15; 1.83] 0.32 [-0.98; 1.62]	4.4%
Assay = OF Ramboz, 1998 Mice Female Groenink, 2003 Mice Female and Male Gross, 2002 Mice Female and Male Piszczek, 2013 Mice Female and Male Gleason, 2010 Mice Male Jain, 2012 Mice Male Parks, 1998 Mice Male Ramboz, 1998 Mice Male Vinkers, 2010 Mice Male Random effects model	20 16 28 9 14 15 20 11 149	0.83 0.9 9.69 11.6 4.72 3.6 14.75 8.8 8.22 2.8 11.07 7.3 5.43 5.0 3.02 4.5 10.09 6.8	6 16 6 16 1 34 4 9 8 14 5 15 6 20	0.47 0.72 6.52 4.46 1.20 2.15 8.93 4.23 3.34 3.93 4.83 4.33 1.41 1.81 1.03 2.88 1.29 6.00		0.41 [-0.22; 1.04] 0.35 [-0.35; 1.05] 1.14 [0.39; 1.90] 0.86 [0.34; 1.38] 1.35 [0.30; 2.40] 1.00 [0.21; 1.79] 1.03 [0.26; 1.80] 0.51 [-0.12; 1.14] 1.31 [0.37; 2.25] 0.80 [0.56; 1.04]	5.5% 5.0% 7.1% 3.3% 4.8% 4.9% 6.1% 3.9%
Heterogeneity: 1/2=0%, Tau/2=0, p=0.4802 Random effects model Heterogeneity: 1/2=28%, Tau/2=0.1188, p=0.12	324		335			0.73 [0.50; 0.96]	100%

Anxiolytic

Anxiogenic

Anxiogenic

В							-4 L	-2	0	2 4	1 I		
Ht1a Over-expression	N	OE Mean	SD	N	WT Mean	SD	Stan	dardise	ed mear	n differenc	^e SMD	95%-CI	W(random)
Assay = EPM									-				
Kusserow, 2004 Mouse Female EPM	13	6.95	6.20	12	18.64	4.65		-0+	÷		-2.049	[-3.05; -1.05]	11.8%
Audero, 2013 Mouse Male EPM	12	29.28	7.42	15	29.37	17.15		•••••	-0-1		-0.006	[-0.77; 0.75]	13.0%
Bert, 2006 Mouse Male EPM	11	14.93	8.58	11	17.29	8.96			0 -1		-0.258	[-1.10; 0.58]	12.6%
Bert, 2006 Mouse Male EPM	13	9.08	5.96	13	27.34	10.59		-01	÷		-2.058	[-3.04; -1.08]	11.9%
Kusserow, 2004 Mouse Male EPM	13	6.69	6.99	12	16.48	6.53		-0-	∳ -		-1.398	[-2.29; -0.51]	12.3%
Random effects model	62			63					⊨		-1.117	[-1.97; -0.26]	61.6%
Heterogeneity: I^2=79%, Tau^2=0.7397, p=0.000	18												
Assay = OF													
Kusserow, 2004 Mouse Female OF		11.64		12		28.17				•		[-0.73; 0.84]	
Audero, 2013 Mouse Male OF	12	33.28		11	28.81					••••••		[-0.25; 1.43]	
Kusserow, 2004 Mouse Male OF	13	11.94	29.87	12	9.29	28.17			-0-1		0.088	[-0.70; 0.87]	12.9%
Random effects model	38			35					÷+		0.231	[-0.23; 0.69]	38.4%
Heterogeneity: I^2=0%, Tau^2=0, p=0.6015													
Random effects model	100			98			·····		▶		-0.599	[-1.32; 0.13]	100%
Heterogeneity: I^2=80.2%, Tau^2=0.8989, p<0.0	001												

Figure 4. Meta-analyses of serotonin receptor 1A interventions on rodent anxietyrelated 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.

Anxiolytic

		ко			w	-				
Htt Knockout	Ν	Mean	SD	Ν	Mean	SD	Standardised mean difference	SMD	95%-CI	W(rando
Assay = EPM										
Holmes, 2003 Mice Female	11	16.26	14.21	13	5.16	5.830	H-0-1		[0.158; 1.88]	3.4%
Holmes, 2003 Mice Female	14	6.03	9.98	11	4.96	8.915		0.11	[-0.682; 0.90]	
Li, 2004 Mice Female	11	48.38	25.83	11	14.87	21.915	⊢−○−	1.35	[0.403; 2.29]	3.1%
Carroll, 2007 Mice Female and Male	30	49.68	27.69	29	41.51	34.514	HOH	0.26	[-0.254; 0.77]	5.0%
Line, 2011 Mice Female and Male	9	10.82	6.62	7	2.94	5.601	 0	1.20	[0.105; 2.30]	2.6%
Holmes, 2003 Mice Male	15	45.86	34.16	15	19.76	21.859		0.89	[0.131; 1.64]	3.9%
Holmes, 2003 Mice Male	11	12.30	7.61	10	1.40	2.833	F	1.79	[0.744; 2.83]	2.8%
Holmes, 2003 Mice Male	13	5.75	8.51	12	0.04	0.001		0.90	[0.068; 1.73]	3.6%
Kalueff, 2007 Mice Male	11	292.00	126.03	8	168.00	138.593			[-0.064: 1.87]	
Kalueff, 2007 Mice Male	11	48.67	21.01	8	28.00	23.099			[-0.064; 1.87]	
Lira, 2003 Mice Male	20	6.79	5.92	17	7.67	7.999			[-0.771; 0.52]	
Schipper, 2011 Rats Female	5	38.45	10.52	5	18.58	5.521			[0.424; 3.85]	1.4%
Olivier, 2008 Rats Male	12	10.14	8.25	12	5.56	5.498			[-0.192; 1.46]	
Random effects model	173	10.14	0.25	158	5.50	3.430			[0.450: 1.10]	
Heterogeneity: I^2=45.3%, Tau^2=0.1572, p=0				150				0.70	[0.450, 1.10]	40.77
Assay = LD										
Holmes, 2003 Mice Female	11	23.01	13.93	13	7.57	7.769		1.35	[0.450; 2.26]	3.3%
Holmes, 2003 Mice Female	14	5.33	6.51	11	6.46	11.361	—— 0		[-0.913; 0.67]	3.7%
Carroll. 2007 Mice Female and Male	30	47.98	14.17	29	32.93	20.900			[0.301; 1.37]	4.9%
Holmes, 2003 Mice Male	15	29.28	23.68	15	18.70	12.297			[-0.185; 1.28]	
Holmes, 2003 Mice Male	11	19.06	7.51	10	4.59	4.106			[1.122; 3.41]	2.5%
Holmes, 2003 Mice Male	13	3.76	7.47	12	1.05	2.967			[-0.343; 1.25]	
Random effects model	94	0.70	1.41	90	1.00	2.507			[0.171; 1.49]	
Heterogeneity: I^2=64.4%, Tau^2=0.5054, p=0	• •			50				0.00	[0.17 1, 1.40]	22.1
Assay = OF										
Kalueff, 2007 Mice Female	8	42.92	22.95	7	6.82	13.067			[0.531; 3.04]	2.2%
Carroll, 2007 Mice Female and Male	30	20.11	13.52	29	12.82	13.296	-0-1	0.54	[0.016; 1.06]	5.0%
Zhao, 2006 Mice Female and Male	12	36.69	25.05	12	18.96	12.526		0.86	[0.021; 1.71]	3.5%
Zhao, 2006 Mice Female and Male	12	34.14	22.92	12	16.93	9.471	 	0.95	[0.096; 1.80]	3.5%
Holmes, 2003 Mice Male	14	39.63	11.88	14	17.17	15.401	——— ——	1.59	[0.721; 2.45]	3.49
Kalueff, 2007 Mice Male	11	64.00	19.90	8	37.00	22.627		1.22	[0.215; 2.23]	2.9%
Kalueff, 2007 Mice Male	11	42.07	9.04	8	25.16	11.333			[0.533; 2.68]	2.79
Moya, 2011 Mice Male	10	55.97	18.96	10	22.70	10.410			[0.952; 3.22]	2.5%
Pang, 2011 Mice Male	25	54.68	31.75	28	48.68	28.003			[-0.343; 0.74]	
Olivier, 2008 Rats Male	12		15.89	12		16.191			[0.008; 1.69]	3.5%
Random effects model	145	23.12	15.09	140	13.01	10.191			[0.658; 1.36]	
Heterogeneity: I^2=52.3%, Tau^2=0.1309, p=0				140				1.01	[0.050, 1.50]	34.2
Random effects model	412			388				0.88	[0.648; 1.10]	100%
Heterogeneity: I^2=50.3%, Tau^2=0.2011, p=0	0.0012									
						An	kiolytic Anxio	aenic		

B Htt Overexpression	N	OE Mean SD	N	WT Mean	SD	-2 -1 0 1 2 3 L L L L L L L L L L L L L L L L L L L	SMD	95%-CI	W(random)
Jennings, 2006 Mouse Male EPM Jennings, 2006 Mouse Male EPM Line, 2011 Mouse Male and Female EPM Jennings, 2006 Mouse Male OF	8 8 9 8	15.52 14.63 1.59 2.74 18.34 14.29 38.69 29.27	8 8 7 8	30.69 28.84 39.16 36.34	21.70 15.63		-1.67 -1.32	[-2.13; 0.005] [-2.85; -0.484] [-2.44; -0.205] [-0.89; 1.067]	25.4% 22.7% 24.2% 27.7%
Random effects model Heterogeneity: I^2=51.1%, Tau^2=0.2678, p=0.1053	33		31		An	kiolytic Anxiog		[–1.69; –0.202]	100%

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.

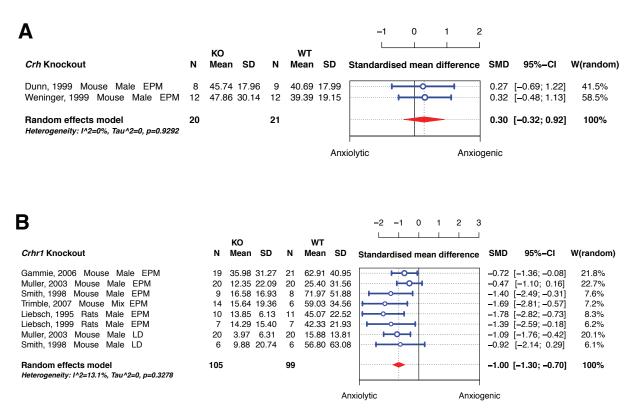


Figure 6. Meta-analyses of the effects of stress signaling genes on anxiety-related behaviors. Meta-analysis of effect sizes of stress signaling genes, shown as a forest plot of standard-ized effect sizes (Hedges' g). Error bars indicate the 95% confidence intervals of g. The weighted average mean effect 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.

Α							-2 0 2 4			
		Control			Treatm	ent				
Pain	Ν	Mean	SD	Ν	Mean	SD	Standardised mean difference	SMD	95%-CI	W(random)
Assay = EPM	_									
Shang, 2015 1 days Mice Male EPM	9	8.72		10 7		8.23 16.67			[-0.30; 1.56]	4.9%
Shang, 2015 3 days Mice Male EPM Shang, 2015 7 days Mice Male EPM	9	9.73 15.62		15		24.67			[-0.92; 1.05] [-0.41; 1.08]	4.7% 5.5%
Shang, 2015 14 days Mice Male EPM Shang, 2015 14 days Mice Male EPM		17.98			11.03				[-0.41; 1.08] [-0.40; 1.07]	5.6%
Liu, 2015 12 days Mice Male EPM	8	84.64			92.57				[-0.40, 1.07]	4.9%
Liu, 2015 12 days Mice Male EPM	-	75.43			71.36				[-0.65: 0.92]	5.4%
Parent, 2012 28 days Rats Male EPM	9	40.81		9	20.71	14.85			[0.05; 2.05]	4.7%
Leite-Almeida, 2009 30 days Rats Male EPM	12	26.43	21.91	12	5.97	4.92			[0.36; 2.13]	5.0%
Benbouzid, 2008 30 days Mice Male EPM	6	33.94	11.33	8	17.56	4.60		1.89	[0.55; 3.23]	3.7%
Matsuzawa-Yanagida, 2008 28 days Mice Male EPM	18	35.91	5.47	18	22.41	6.40			[1.37; 3.07]	5.2%
Leite-Almeida, 2009 30 days Rats Male EPM		26.43			25.79				[-0.77; 0.83]	
Schellinck, 2003 0 days Mice Female and Male EPM	6	48.80	8.64	6	28.93	13.01			[0.27; 3.05]	3.6%
Random effects model	126			139			· · · · · · · · · · · · · · · · · · ·	0.72	[0.27; 1.17]	58.5%
Heterogeneity: I^2=64.2%, Tau^2=0.4142, p=0.0012										
Assay = LD										
Parent, 2012 28 days Rats Male LD	5	23.10	10.69	5	31.65	5.42		-0.91	[-2.25; 0.43]	3.7%
Matsuzawa-Yanagida, 2008 28 days Mice Male LD	18	24.55	6.30	18	14.90	2.43			[1.16; 2.79]	5.3%
Random effects model	23			23				0.58	[-2.24; 3.41]	9.0%
Heterogeneity: I^2=92.3%, Tau^2=3.849, p=0.0003										
Assay = OF Shang, 2015 1 days Mice Male OF	7	4.51	3.92	8	4.96	8.82		_0.06	[-1.08; 0.95]	4.6%
Shang, 2015 3 days Mice Male OF	6		2.38	8	3.65	7.38			[-0.93; 1.19]	4.5%
Shang, 2015 7 days Mice Male OF	10	4.11	1.71	10	1.98	4.40	-		[-0.29; 1.51]	5.0%
Shang, 2015 14 days Mice Male OF	13	3.60		12	1.69	3.64			[-0.22; 1.38]	5.3%
Liu, 2015 12 days Mice Male OF	7	30.68		10	23.46	7.99			[-0.28; 1.72]	4.7%
Liu, 2015 12 days Mice Male OF	8	12.45	4.08	8	21.53	11.23			[-2.08; 0.04]	4.5%
Parent, 2012 28 days Rats Male OF	5	4.99	2.71	5	2.04	11.14		0.33	[-0.92; 1.58]	3.9%
Random effects model	56			61				0.22	[-0.21; 0.66]	32.5%
Heterogeneity: I^2=25.1%, Tau^2=0.0867, p=0.2372										
Random effects model	205			223				0.56	[0.19; 0.93]	100%
Heterogeneity: I/2=65.7%, Tau/2=0.4967, p<0.0001	200			220				0.50	[0.10, 0.93]	100 /0
							<u>├</u> ───┤			
						Anx	iolytic Anxio	genic		

:	Anxiogen

R							-4 -2 0 2 4 6			
Physical Restraint	N	Inrestra Mean		N	Restra Mean		Standardised mean difference	SMD	95%–Cl	W(random)
Assay = EPM										
Harris, 2001 12 mins Mice Male	14	15.67	9.35	14	25.67	6.24	HOH	-1.22	[-2.038; -0.40]	
Lunga, 2004 60 mins Rats Female	7	28.72		7	13.78				[0.749; 3.55]	4.4%
Carvajal, 2004 60 mins Rats Female and Male	12	23.92	3.59	12	8.95	3.28		4.20	[2.680; 5.72]	4.3%
Rylkova, 2009 15 mins Rats Male	9		25.40	9		31.75			[-1.022; 0.83]	5.0%
Walf, 2012 20 mins Rats Male	24	8.47	9.80	24	4.40	7.35	••••	0.46	[-0.112; 1.04]	5.3%
Liu, 2013 30 mins Rats Male	9	41.86	25.40	10	9.53	12.64	₩	1.57	[0.509; 2.63]	4.8%
Locchi, 2007 45 mins Rats Male	9	15.44	5.48	9	5.01	2.17		2.38	[1.110; 3.65]	4.6%
Anand, 2012 60 mins Rats Male	6	19.30	6.86	6	4.10	2.94		2.66	[0.949; 4.37]	4.0%
Busnardo, 2013 60 mins Rats Male	10	14.24	6.99	7	6.13	5.03	⊢⊙ →	1.22	[0.152; 2.30]	4.8%
Estanislau, 2005 60 mins Rats Male	11	29.37	13.16	12	13.05	16.50	►○+	1.05	[0.166; 1.93]	5.0%
Granjeiro, 2011 60 mins Rats Male	6	11.43	5.65	7	5.11	5.74	1 O1	1.03	[-0.155; 2.22]	4.7%
Joshi JC, 2014 60 mins Rats Male	6	11.88	4.57	6	1.54	2.14		2.67	[0.959; 4.39]	4.0%
Nosek, 2008 60 mins Rats Male	7	9.66	6.45	7	6.87	5.00	HO-	0.45	[-0.613; 1.52]	4.8%
Nosek, 2008 60 mins Rats Male	7	6.87	4.16	7	16.37	6.83		-1.57	[-2.821; -0.32]	4.6%
Reis, 2011 60 mins Rats Male	6	56.56	32.55	6	19.05	15.84		1.35	[0.047; 2.66]	4.5%
Random effects model	143			143					[0.376: 1.90]	70.0%
Heterogeneity: I^2=83.3%, Tau^2=1.904, p<0.0001										
Assay = LD										
Ouagazzal, 2003 5 mins Mice Female	15	4.98	7.68	15	4.47	4.65		0.08	[-0.637; 0.80]	5.2%
Harris, 2001 12 mins Mice Male	14	1.67	0.62	14	2.50	1.25		-0.82	[-1.596; -0.04]	5.1%
Random effects model	29			29				-0.36	[-1.239; 0.52]	10.3%
Heterogeneity: I/2=64.1%, Tau/2=0.2596, p=0.0949									,	
Assay = OF										
Chesworth, 2012 15 mins Mice Male	11	25.71	9.67	11	19.34	9.19		0.65	[-0.213; 1.51]	5.0%
Chesworth, 2012 15 mins Mice Male	17	26.70	10.45	17	29.18	11.68	HCH	-0.22	[-0.893; 0.46]	5.2%
Nosek, 2008 60 mins Rats Male	7	3.44	1.85	7	29.37	23.26		-1.47	[-2.696; -0.25]	4.6%
Nosek, 2008 60 mins Rats Male	7	5.35	1.86	7	10.70	5.97		-1.13	[-2.290; 0.02]	4.7%
Random effects model	42			42					[-1.392; 0.47]	
Heterogeneity: 1^2=70.5%, Tau^2=0.6481, p=0.0171									,	
Random effects model	214			214				0.66	[0.005; 1.32]	100%
Heterogeneity: I^2=83.3%, Tau^2=2.048, p<0.0001										
						Anx	kiolytic Anxiog	jenic		

Figure 7. Part 1. 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.

1	1	
		J

							-2 0 2	4		
Social Isolation		Group-I Mean			lsola Mean		Standardised mean difference	SMD	95%–CI	W(random)
Assay = EPM Abramov, 2004 21 days Mice Female	10	5.40	18 / 1	10	3.03	18.96		10-01	[-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 Majercsik, 2003 7 days Mice Male	10	14.95 22.12		10 10	17.50 15.40				[-1.11; 0.65] [-0.30; 1.50]	
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 Rodgers, 1993 7 days Mice Male	10 14	15.47 4.67	6.96 2.99	10 14	13.42 4.83				[-0.71; 1.05] [-0.80; 0.68]	
Estelles J, 2007 14 days Mice Male	97			94		63.27	0		[-0.05; 0.52]	
Rodgers, 1993 14 days Mice Male Abramov, 2004 21 days Mice Male	14 10	4.67 2.71	2.99	14 10		4.24 37.53			[-0.97; 0.51]	
Rodgers, 1993 21 days Mice Male	14	4.67	2.99	14	6.00	3.12		_4e_01	[-1.05; 0.71] [-1.17; 0.33]	
Wei XY, 2007 21 days Mice Male Koike H, 2009 28 days Mice Male	10 10	29.25 13.41		10 11	11.07 8.20				[0.48; 2.51]	0.9%
Koike H, 2009 28 days Mice Male		41.80		12		5.56			[-0.15; 1.64] [0.35; 2.23]	1.0%
Moragrega, 2003 30 days Mice Male	32			33		14.36		-8e-02	[-0.57; 0.40]	
Liu X, 2013 42 days Mice Male Workman JL, 2011 42 days Mice Male	10 10	8.09 31.46		10 10	17.50	49.05 8.92			[-0.86; 0.89] [-0.07; 1.78]	
Voikar V, 2005 49 days Mice Male	15	2.43	17.04		15.97		-0	-3e-01	[-1.02; 0.42]	1.4%
Imanaka A, 2006 7 days Rats Female Leussis MP, 2008 7 days Rats Female		24.37 16.17		12	22.50	20.37 28.27			[-0.69; 0.82] [-0.71; 1.27]	
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 12	19.24 25.80		26 13		19.73 15.86			[-0.11; 0.99]	
Imanaka A, 2009 35 days Rats Female Doremus–Fitzwater TL, 2009 49 days Rats Female			23.70	8		24.07			[-0.14; 1.48] [-1.04; 0.92]	
Knuth ED, 2007 7 days Rats Female and Male	18	0.06		18		46.90		3e-04	[-0.65; 0.65]	1.6%
Reis FM, 2012 0.02 days Rats Male Reis FM, 2012 0.08 days Rats Male	14 14	27.94 27.94			22.18 12.33				[-0.48; 1.04] [-0.05; 1.55]	
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 Cheeta S, 2001 7 days Rats Male	9 7	13.83 36.57		9 8	2.70	5.29 11.89			[-0.27; 1.64] [-0.59; 1.48]	
Cheeta S, 2001 7 days Rats Male	7	33.62		8	31.66			2e-01	[-0.83; 1.21]	0.9%
Cheeta S, 2001 7 days Rats Male	7			8		23.23			[-1.48; 0.58]	
Haller J, 2000 7 days Rats Male Haller J, 2000 7 days Rats Male	9 12	7.06 14.46		9 12	11.88	10.23 8.55			[-1.56; 0.34] [-0.46; 1.15]	
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 Majercsik, 2003 7 days Rats Male	9 9	14.36 12.66		9 9	8.62 4.81	8.61 6.60			[-0.42; 1.46] [-0.24; 1.68]	
Majercsik, 2003 7 days Rats Male	9	9.75	8.10	9	6.82				[-0.52; 1.35]	
Reis FM, 2012 7 days Rats Male		27.94 10.57		14		15.84			[0.03; 1.58]	1.3%
Wright, 2001 7 days Rats Male Das SK, 2015 14 days Rats Male	5 12		6.32	5 8	5.53 23.24	4.99 4.29			[-0.78; 1.76] [-0.05; 1.85]	
dos Santos L, 2010 14 days Rats Male	12	9.08	7.33	12					[0.35; 2.12]	1.1%
Lapiz MD, 2001 14 days Rats Male Lapiz MD, 2001 14 days Rats Male	6 6	29.75 17.19	14.40 7.79	6 6	17.17 9.82				[-0.19; 2.28] [-0.10; 2.43]	
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 Brenes JC, 2009 28 days Rats Male	12 12			12 12		45.16 1.12	H-0		[-0.66; 0.94] [1.30; 3.47]	1.3% 0.8%
Da Silva NL, 1996 28 days Rats Male		38.92			18.18				[-0.53; 1.25]	
Pisu MG, 2011 28 days Rats Male		19.89		18	1.18		+0-+	4e-01	[-0.29; 1.03]	1.6%
Pisu MG, 2014 28 days Rats Male Serra M, 2000 28 days Rats Male	20	12.77 30.92		20 13	12.68 13.96			2e-03 6e-01	[-0.62; 0.62] [-0.23; 1.34]	1.7% 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 Fone KC, 1996 35 days Rats Male	5 5	10.58 12.47		5 5		26.87 22.81			[-1.38; 1.10] [-1.63; 0.89]	
Imanaka A, 2008 35 days Rats Male		18.57		16	16.13				[-0.62; 0.87]	
Chappell AM, 2013 42 days Rats Male	16 8	23.62 18.47		17 8		28.26 6.15	+0-1		[-0.26; 1.12]	1.5% 0.8%
Hirani K, 2005 42 days Rats Male Kokare DM, 2010 42 days Rats Male	7	13.55		7		14.90		4e-01	[0.26; 2.50] [-0.68; 1.44]	
McCool BA, 2009 42 days Rats Male	28	21.50		31		51.09	+0+	1e-01	[-0.40; 0.62]	2.0%
Pritchard LM, 2008 42 days Rats Male Ryu V, 2009 42 days Rats Male	6 36	23.88 12.83		6 36		13.82 42.25	-0-H		[-1.89; 0.47] [-0.34; 0.58]	
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 Yorgason JT, 2013 49 days Rats Male	10 7	27.34 20.77		10 7		33.19 9.33			[-0.96; 0.79] [0.02; 2.36]	1.1% 0.8%
Zhang Y, 2012 56 days Rats Male	10			10	12.10				[-0.65; 1.11]	
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 Heterogeneity: I^2=31%, Tau^2=0.1354, p=0.0103	889			906				3e-01	[0.18; 0.45]	79.5%
Assay = LD Wei XY, 2007 21 days Mice Male		47.62			26.22				[0.14; 2.11]	1.0%
Voikar V, 2005 49 days Mice Male Voikar V, 2005 49 days Mice Male	15 15	43.10 46.40			42.20 31.00				[-0.67; 0.76] [0.05; 1.54]	1.5% 1.4%
Carrier N, 2012 21 days Rats Female	9	31.61	27.47		17.35			6e-01	[-0.34; 1.56]	
Carrier N, 2012 21 days Rats Male McCool BA, 2009 42 days Rats Male	9	29.45 40.47	21.16		14.01			7e-01	[-0.21; 1.71] [-0.56; 0.46]	1.0%
Random effects model	20 85	40.47	47.02	89	42.40	31.10			[-0.56, 0.46]	
Heterogeneity: I^2=35.1%, Tau^2=0.0301, p=0.1735										
Assay = OF Linge R, 2013 28 days Mice Male		13.65		10		11.15			[-0.43; 1.35]	
Liu X, 2013 42 days Mice Male Conrad KL, 2011 49 days Mice Male		22.13 23.43		10 10	5.93 12.04	17.84 22.24		5e-01	[-0.35; 1.45] [-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 Knuth ED, 2007 7 days Rats Male	8 9	43.63 6.77	20.29 15.00		19.52 4.57				[0.13; 2.32] [-0.78; 1.07]	
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 Djordjevic J, 2012 21 days Rats Male		20.48 16.97			16.84 11.38				[-0.06; 1.83] [-0.49; 0.82]	
Lukkes JL, 2009 21 days Rats Male		48.09			34.92				[-0.49; 0.82]	
Thorsell A, 2006 84 days Rats Male	10	11.31		9	20.04			_4e_01	[-1.35; 0.47]	1.1%
Random effects model Heterogeneity: I^2=0%, Tau^2=0.0157, p=0.4675	140			114				3e-01	[0.06; 0.59]	12.7%
Random effects model Heterogeneity: I^2=26.9%, Tau^2=0.1091, p=0.0151	1114			1109				3e-01	[0.21; 0.44]	100%
						Anx	iolytic Anxio	1 ogenic		

Figure 7. Part 2. 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.

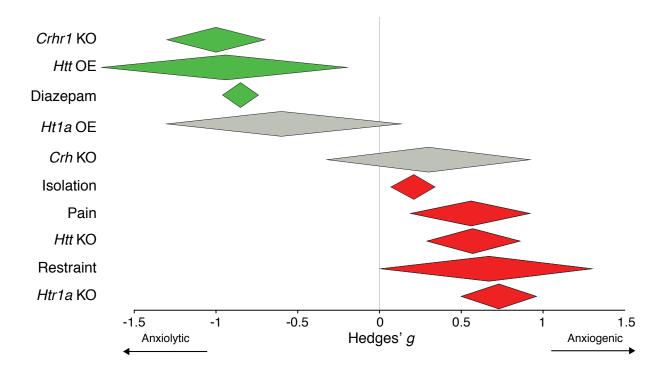


Figure 8. Summary effect sizes of all meta-analyses. The weighted mean effect sizes of all 10 interventions are shown here. Each mean effect size is represented by the central vertices of a diamond; the outer vertices indicate the 95% confidence intervals. The horizontal axis is Hedges' g, the standard deviation change relative to control animals. 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.

Table 1. Summary of systematic reviews of anxiety-related interventions in mouse and rat. The PubMed and EMBASE query phrases below were used to identify articles that might contain data relevant to the interventions and assays of interest. Of the hits, title, abstract and full-text study and experiment selections were performed to yield the articles meeting 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
<i>Ht1a</i> 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
<i>Htt</i> 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 <i>Mus</i>)	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 <i>Mus</i>)	65	2
<i>Crh</i> 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
<i>Crhr1</i> 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 <i>Mus</i>)	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 <i>Mus</i>)	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 <i>Mus</i>)	167	50

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, the corresponding 95% confidence interval for the intercept, and the P-values of Egger's test are listed.

	No. of Experiments	No. of Studies	Hedges' <i>g</i> [95% Cl]	p-value	l² [95% Cl]	Egger's Test p-value	No. of Experiments Added After Trim-and-Fill	Adjusted Hedges' g [95% Cl]	p-value Adjusted Hedges' <i>g</i>
Diazepam	386	172	-1.3 [-1.4; -1.2]	1.36e-144	70.4 [67.2; 73.3]	2.35e-56	102	-0.85 [-0.96; -0.74]	4.72e-56
Isolation	83	50	0.33 [0.21; 0.44]	3.37e-08	26.9 [3.5; 44.6]	0.0146	12	0.21 [0.069; 0.34]	0.00316
Htt KO	29	13	0.88 [0.65; 1.1]	5.18e-14	50.3 [23.7; 67.6]	6.72e-06	10	0.57 [0.29; 0.86]	9.71e-05
Restraint	21	16	0.67 [0.0062; 1.3]	0.0479	83.4 [75.8; 88.7]	0.0305	0	0.67 [0.0062; 1.3]	0.0479
Pain	21	7	0.56 [0.19; 0.92]	0.00283	65.1 [44.6; 78]	0.763	NA	NA	NA
Ht1a KO	20	11	0.73 [0.5; 0.96]	3.15e-10	28.0 [0; 58.2]	0.545	NA	NA	NA
Ht1a OE	8	3	-0.6 [-1.3; 0.13]	0.105	80.2 [61.8; 89.8]	NA	NA	NA	NA
Crhr1 KO	8	6	-1 [-1.3; -0.7]	6.64e-11	13.1 [0; 55.7]	NA	NA	NA	NA
Htt OE	4	2	-0.94 [-1.7; -0.2]	0.0127	51.1 [0; 83.8]	NA	NA	NA	NA
Crh KO	2	2	0.3 [-0.32; 0.92]	0.34	0 [NA; NA]	NA	NA	NA	NA